# Diversité génétique et phénotypique des levures du raisin et du vin

Warren Albertin

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ECOLE DOCTORALE SCIENCES DE LA VIE ET DE LA SANTE
SPECIALITE OENOLOGIE

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MEMOIRE PRESENTE EN VUE DE L'OBTENTION DE
L'HABILITATION A DIRIGER DES RECHERCHES

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# Diversité génétique et phénotypique des levures du raisin et du vin

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WARREN ALBERTIN
MAITRE DE CONFERENCES ENSCBP, BORDEAUX INP
UNITE DE RECHERCHE ŒNOLOGIE, EA 4577, USC 1366 INRA, BORDEAUX INP


Date de soutenance : 3 juillet 2020

MEMBRES DU JURY

| | |
|---|---|
| Anne Friedrich, MdC, Univ Strasbourg | Rapporteur |
| Cécile Fairhead, Professeur UPSud | Rapporteur |
| François Delmotte, DR INRAE | Rapporteur |
| Christophe d'Enfert, Professeur Institut Pasteur | Examinateur |
| Dominique de Vienne, Professeur émérite UPSud | Examinateur |
| Jean-Marie Sablayrolles, DR INRAE | Examinateur |
| Isabelle Masneuf-Pomarède, Professeur BSA | Garante |

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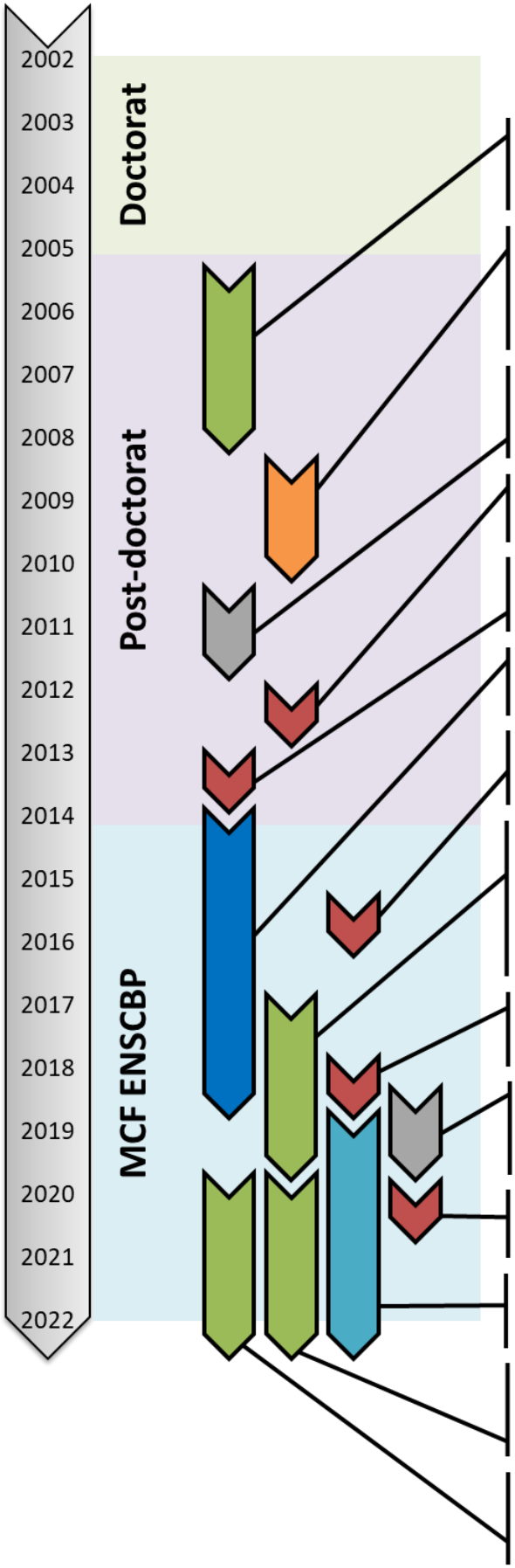
**FIGURE 1. CHRONOLOGIE DES PRINCIPAUX PROJETS DE RECHERCHE MENES.**




**ADAPTALEVURE** (ANR programme blanc) 2006-2008
- UMR Génétique Végétale, Gif-sur-Yvette
- UR Œnologie, Univ Bordeaux
- UMR5122 Microbiol. & Génétique, Univ Lyon

**HETEROSYEAST** (ANR ALIA) 2009-2010
- UMR Génétique Végétale, Gif-sur-Yvette
- UR Œnologie, Univ Bordeaux
- SARCO, Bordeaux
- INRA, Jouy-en-Josas

**PREFERMENT** (Etat (DGAL) – Région) 2011-2012
- UR Œnologie, Univ Bordeaux
- Laffort, Bordeaux
- Pernod-Ricard, Creteil

**WILDWINE** (Union Européenne) 2013
- 5 partenaires académiques et 10 privés

**YIELD** (Biolaffort) 2014
- UR Œnologie, Univ Bordeaux
- Biolaffort, Bordeaux

**TYPEBrett** (AST) 2014-2018
- UR Œnologie, Univ Bordeaux

**MAEL** (FR BIE) 2016-2017
- UR Œnologie, Univ Bordeaux
- UMR1332, INRA Bordeaux

**Chitowine** (ANR AAPG2017) 2018-2020
- UR Œnologie, Univ Bordeaux
- Institut Pascal, Clermont-Ferrand
- Biolafort, Bordeaux
- Microflora, Bordeaux
- UMR SPO, Montpellier

**Brett&cie** (Lallemand) 2018-2019
- UR Œnologie, Univ Bordeaux
- Lallemand, Toulouse

**SoLt** (Biolaffort) 2018-2020
- UR Œnologie, Univ Bordeaux
- Univ. Adélaïde
- Biolafort, Bordeaux

**GCbase** (BordeauxINP) 2020
- UR Œnologie, Univ Bordeaux

**BrettAdapt** (ANR AAPG2018) 2019-2022
- UR Œnologie, Univ Bordeaux
- Univ. Strasbourg

**MousyWine**(Région - BAG) 2020-2022
- UR Œnologie, Univ Bordeaux
- Geisenheim Univ.
- Univ. Adélaïde

**BRETTADHERE** (CIFFRE) 2020-2022
- UR Œnologie, Univ Bordeaux
- UMR GMPA, AgroParisTech, INRA
- Biolaffort/Excell

# PREAMBULE

Ce manuscrit présente une synthèse de mes activités de recherche menées depuis mon doctorat. J'ai centré mes travaux autour de l'étude de la biodiversité et des mécanismes à l'origine de celle-ci, en passant d'un modèle de plante (lors de ma thèse) à un modèle 'levure' ces 15 dernières années.

La rédaction d'un manuscrit de HDR est l'occasion de faire un bilan, de prendre du recul sur le chemin parcouru et de réfléchir aux travaux à venir ainsi qu'aux questions de recherche que l'on souhaite prioriser. Et comme la recherche ne se fait jamais seul, cela permet aussi de mettre en lumière le rôle des personnes qui m'ont accompagnée et qui m'accompagnent encore : mes collègues actuels, mes encadrants passés et les étudiants que j'encadre aujourd'hui à mon tour, sans qui ces travaux n'auraient pas été possibles et que je ne remercierai jamais assez.

Mon aventure scientifique commence quand, en 2002, j'ai un « coup de cœur » pour un projet de thèse proposé par Hervé Thiellement. Le projet porte sur l'étude des génomes complexes (hybrides et polyploïdes), appliquée à un modèle de plante (les Brassicacées) à l'aide d'une approche protéomique – originale à l'époque. La rencontre avec Hervé fut déterminante dans mon parcours, nos conversations initiales prenant rapidement la tournure de débats (parfois passionnés) sur des sujets très variés. Hervé m'a transmis une vision de la science un peu utopique, et je garde précieusement le souvenir de ses opinions sans concession et de son humour mordant. Ma thèse fut aussi l'opportunité d'intégrer l'UMR de Génétique Végétale du Moulon (Gif-sur-Yvette), qui reste encore aujourd'hui pour moi un exemple de collaboration inter-équipes, de dynamisme, de foisonnement d'idées et d'entraide généralisée.

En 2006, j'intègre l'Unité de Recherche Œnologie (EA 4577, USC 1366 INRA, Bordeaux INP-ISVV), à Bordeaux pour un contrat postdoctoral, le premier d'une série de cinq (sans compter les avenants !). L'œnologie est une science particulière de par sa pluridisciplinarité mais aussi sa dimension finalisée, qui favorise un contact étroit avec la profession vitivinicole et qui se traduit (de temps à autre) par du transfert de connaissance ou des innovations, immensément gratifiants. De 2006 à 2014, les différents projets menés ont porté sur l'étude de la diversité appliquée aux levures d'intérêt en œnologie, en combinant plusieurs niveaux d'intégration cellulaire (capacité fermentaire, croissance cellulaire, abondance protéique, métabolisme, marqueurs aromatiques, diversité génétique). Ce fut l'opportunité pour moi de découvrir le potentiel des approches intégratives, mais aussi le monde du vin, les fermentations, le programme R, les statistiques et les manipes de nuit.

En septembre 2014, je suis recrutée en tant que Maitre de Conférences au sein de l'ENSCBP (Ecole Nationale Supérieure de Chimie, de Biologie et de Physique). Mes enseignements incluent la microbiologie alimentaire, l'hygiène et la sécurité des aliments et les statistiques pour de futurs ingénieurs en agroalimentaire. Côté recherche, je poursuis les travaux entamés au sein de l'UR Œnologie sur l'étude de la biodiversité des levures d'œnologie, et je me prends d'affection pour une espèce en particulier, la levure d'altération des vins *Brettanomyces bruxellensis.* Je m'intéresse également à un thème de recherche supplémentaire, qui concerne les interactions entre microorganismes du vin au cours du processus de vinification.

Ce manuscrit se découpe en trois grandes parties. Après le traditionnel CV, je détaillerai mes travaux antérieurs. J'ai choisi de décrire mes activités par thème plutôt que par ordre chronologique, en abordant quelques approches fondamentales et certains résultats plus appliqués. Ce découpage reste toutefois un peu artificiel tant les modèles biologiques, les approches et les questions de recherche se recouvrent. Il en va de même pour la présentation des projets à venir, plus ou moins imbriqués les uns dans les autres. Néanmoins, tous ces projets ont des objectifs communs : sur le plan fondamental, il s'agit de mieux décrire la diversité génétique et phénotypique des levures du raisin et du vin (des auxiliaires technologiques aux microorganismes d'altération), et d'identifier les mécanismes et les facteurs biotiques ou abiotiques sous-tendant cette diversité. Et, sur le plan finalisé, l'objectif est d'utiliser ces connaissances pour mieux maitriser la qualité et l'identité des vins, mais aussi pour accompagner la filière vitivinicole dans les grands défis qui l'attendent, qu'ils soient environnementaux (eg. réchauffement climatique) ou sociétaux (réduction des intrants d'origine chimiques, évolution des consignes de santé publique pour une consommation modérée, etc.).

# I – CURRICULUM VITAE

# ETAT CIVIL

Warren ALBERTIN

40 ans, née le 16 novembre 1979 à Bondy (93).

Mariée, 2 enfants (Timothé, 12 ans ½ et Evy, 11 ans).

Adresse personnelle : 27 rue Pierre Vincent – 33 720 Podensac, Tel : 06.13.68.12.98

# SITUATION ACTUELLE

Depuis 2014 : Maître de Conférences (CNU 65) à l'ENSCBP – Bordeaux INP

16 avenue Pey-Berland

33 600 Pessac

Unité de recherche de rattachement :

Unité de Recherche Œnologie, EA 4577, USC 1366 INRAE, Bordeaux INP

Institut des Sciences de la Vigne et du Vin

210 chemin de leysotte – 33 140 Villenave d'Ornon

warren.albertin@u-bordeaux.fr ; https://www.researchgate.net/profile/Warren_Albertin/

https://orcid.org/0000-0002-7385-9882

# CURSUS UNIVERSITAIRE

2005 • Doctorat en Sciences de la Vie, Université Paris-Sud XI, mention Très Honorable

2002 • DEA de Génétique Multifactorielle, Université Paris-Sud XI, mention Bien

• Magistère de Génétique, Université Paris VII, mention Assez Bien

2001 • Maîtrise de Génétique, Université Paris VII, mention Bien

2000 • Licence de Génétique, Université Paris VII, mention Assez Bien

1999 • DEUG de Biologie cellulaire & physiologie, Université d'Orléans, mention Assez Bien

1997 • Baccalauréat Scientifique, lycée Marceau de Chartres (28), mention Assez Bien

# EXPERIENCES DE RECHERCHE

## Post-doctorat, 2006 – 2014

Unité de Recherche Œnologie, EA 4577, USC 1366 INRA, ISVV, Bordeaux INP

Axe Microbiologie

Plusieurs projets successifs :

* *Adaptalevure* puis *HeterosYeast*, financements ANR, 2006-2008 et 2009-2010

* *Preferment*, financement région & partenaires privés (Pernod-Ricard et Laffort), 2011-12

* *WildWine*, financement européen, 2013

* *Yield*, financement privé Biolaffort, 2014

## Doctorat, 2002 - 2005

UMR de Génétique Végétale du Moulon – 91190 Gif-sur-Yvette

Régulation de l'expression des gènes dupliqués chez les polyploïdes : approche protéomique appliquée à l'analyse de Brassicacées autopolyploïdes et allopolyploïdes

**Allocation MENRT, école doctorale Gènes, Génomes, Cellules (Univ Paris-Sud XI)**

*Thèse soutenue le 27 octobre 2005 devant le jury composé* d'Anne-Marie Chèvre, DR INRA, rapporteur ; Michel Delseny, DR CNRS, examinateur ; Marc Ghesquière, CR INRA, examinateur ; Serge Hamon, DR IRD, rapporteur ; Hervé Thiellement, DR INRA, directeur de thèse ; Dominique de Vienne, professeur Université Paris XI, président.

## Autres expériences de recherche (stages)

| | |
|---|---|
| 2002 – 6 mois<br>DEA | • Institut Cochin, Paris, sous la direction d'Isabelle Tardieux<br>Une nouvelle phosphatase du parasite *Toxoplasma gondii* : fonctions biologiques et approches thérapeutiques |
| 2002 – 4 mois<br>DEA | • Institut National Agronomique, Paris, sous la direction de Martine Boccara<br>Analyse d'une famille multigénique : les Chitines Synthases de *B. cinerea* |
| 2001 – 7 mois<br>Maîtrise | • Institut Jacques Monod, Paris, sous la direction de Pierre Netter<br>Etude des modifications génétiques affectant les répétitions directes chez la levure *Saccharomyces cerevisiae* |
| 2000 – 2 mois<br>Licence | • Institut Gustave Roussy, Villejuif, sous la direction de Thierry Magnaldo<br>Etude d'un gène suppresseur de tumeur humain, human patched *hptc* |

# ACTIVITE D'EXPERTISE ET RESPONSABILITES COLLECTIVES

**Membre du comité scientifique** et du comité d'organisation des congrès joints Oeno-iVAS2019 (25-28 juin 2019, Pessac).

**Examinatrice de thèses de doctorat :**

- **2018** : Dispersion, sélection et rôle des espèces microbiennes des levains en boulangerie française à faible intrant, présentée par Elisa Michel, Université de Montpellier.
- **2017** : Comparative study of the proteome of S. coelicolor M145 and S. lividans TK24, two phylogenetically closely related strains with very different abilities to accumulate TAG and produce antibiotics, présentée par Aarón Millán Oropeza, Université Paris Saclay.

**Rapporteur d'une thèse de Master, Université de Stellenbosch, 2018** : Investigating the influence of a wine yeast consortium on population dynamics, alcoholic and malolactic fermentation, présentée par PJ Janse van Rensburg.

**Activité d'expertise :** bourse CIFRE, 2018 ; Programme de coopération ECOS Sud-CONICYT (Chili) 2018 ; INACH, 2018 (Chilean Antarctic Institute) ; ANR, 2013 : Programme Jeunes Chercheuses et Jeunes Chercheurs (JCJC) ; UEFISCDI, 2012 (Executive Agency for Higher Education, Research, Development and Innovation Funding, Roumanie) : the Romanian-EEA Research Programme.

***Referee* pour une vingtaine de revues internationales** : Biological J. of the Linnean Society, BMC Genomics, Food Microbiol, Frontiers in Microbiology, Functional Plant Biology, Genetica, Heredity, Intern. J. of Molecular Sciences, Int. J. of Food Microbiology, J. Applied Microbiol, J. of Chromatography B, J. of Genetics and Genomics, J. of Proteome Research, J. of Proteomics, Journal of the Science of Food and Agriculture, Molecular Phylogenetics & Evolution, Plant Journal, Plant Molecular Biology Reporter, Planta, PLoS ONE, Proteomics, Proteome Science, World J. of Microbiology & Biotechnology, Yeast.

**Depuis 2012 : Formation du personnel de l'Unité Œnologie** (chercheur, technicien, étudiant) à l'analyse statistique, programme R et Rcmdr, environ 5H/an.
**Depuis 2017 :** mise à jour de la **revue technique de l'unité**, publication sur le site internet.
**Depuis 2018 :** participation au **comité de projets Bio-Informatique**, CBiB (Centre de Bio-informatique de Bordeaux)

# ACTIVITE D'ENCADREMENT

| Nom | Niveau | Sujet, communications éventuelles | Devenir |
|---|---|---|---|
| Maroula DIMOPOULOU 2018 | Post-doctorat Univ Thessaloniki | Diversity and bioadhesion of *Brettanomyces bruxellensis*<br>2 articles [1, 2] ; 1 article soumis [3]<br>1 communication orale [4] ; 3 posters [5-7] | Lecturer (Univ Athens) |
| Jules HARROUARD 2019-2022 | Doctorat, Univ Bordeaux Encadrement à 100% | Adaptation de la levure *Brettanomyces bruxellensis* aux procédés fermentaires | |
| Ana HRANILOVIC 2015-2018 | Doctorat, Univ Adélaide Encadrement à 100% (6 mois en 2016), puis à 30% en 2017. Dir. de thèse : Pr. Vladimir Jiranek (Univ Adélaïde) | Managing ethanol and sensory compounds by non-*Saccharomyces* yeasts<br>2 articles [8, 9]<br>2 communications orales [10, 11]<br>1 poster [12] | Post-doctorante (Univ Adélaïde) |
| Marine TRUJILLO 2015-2018 | Doctorat, Univ Bordeaux Encadrement (10%). Dir. de thèse : Dr. JC Barbe (MCF Bordeaux Sciences Agro) | Approche multiparamétrique de l'arôme fruité des vins rouges<br>1 publication en préparation [13]<br>1 communication orale [14]<br>2 posters [15, 16] | |
| Marta AVRAMOVA 2014-2017 | Doctorat, Univ Bordeaux + Adélaide Encadrement (30%). Dir. de thèse : Pr. I. Masneuf-Pomarède (BSA) et P. Grbin (AWRI) | Comparative and functional genomics of *Dekkera (Brettanomyces) bruxellensis*<br>3 articles [17-19]<br>1 publication soumise [3]<br>3 communications orales [20-22]<br>6 posters [23-28]<br>9 communications techniques [29-37] | Post-doctorante (INRA Montpellier) |
| Laura CHASSERIAUD 2012-2015 | Doctorat, Univ Bordeaux Encadrement (40%). Dir. de thèse : Dr. Marina Bely (MCF Univ de Bordeaux) | Interactions entre levures Saccharomyces cerevisiae et non-Saccharomyces en vinification<br>3 articles [38-40]<br>11 posters [41-51]<br>2 communications techniques [52, 53] | Post-doctorante (Univ Bordeaux) |
| Telma da SILVA 2010-2014 | Doctorat, Univ Paris-Sud Encadrement (15%). Directeur de thèse : Pr. Dominique de Vienne (Prof. Université Paris-Sud) | Exploration du phénomène d'heterosis chez deux espèces de levure d'oenologie : *Saccharomyces cerevisiae* et *S. uvarum*<br>4 articles [54-57]<br>3 communications avec acte [58-60]<br>6 communications orales [61-66]<br>9 posters [67-75] | Biostatisticienne (Ariana Pharma) |
| Maura MAGANI 2019 (6 mois) | Master Food Safety, Wageningen University Encadrement à 100% | The spoilage yeast *Brettanomyces bruxellensis*: genetic and phenotypic characterization<br>2 publications soumises [3, 76] | Food Control (Ikea) |

| | | | |
|---|---|---|---|
| Ulises GUTIERREZ REYNOSO 2018/19 (6 mois) | Stage Ingénieur, UTC Compiègne<br>Encadrement à 100%, Collaboration avec Lallemand | Brett&cie (*Saccharomyces cerevisiae* – Non-*Saccharomyces* – *Brettanomyces bruxellensis*) : interactions et impact des pratiques œnologiques | |
| Rémy VIANNAIS 2018 (6 mois) | Master 2 Bioinformatique, Univ Bordeaux<br>Encadrement avec Dr. Philippe Marullo | Étude de l'impact des mutations des gènes impliqués dans la production des esters chez *Saccharomyces cerevisiae* à l'aide d'une approche de transcriptomique<br>1 manuscrit en préparation [13]<br>1 poster [16] | Développeur web, DTA Ingénierie |
| Lydia JIMENEZ GADEA 2017 (6 mois) | Master 2 Microbiologie-Immunologie, Univ Bordeaux<br>Encadrement à 100% | Etude des interactions de type 'cell-cell contact' entre microorganismes du vin | Food Safety auditor (Mérieux) |
| Sara WINDHOLTZ 2015 (6 mois) | Master 2 Œnologie, Univ Bordeaux<br>Encadrement à 100% | Diversité génétique et phénotypique de la levure *Torulaspora delbrueckii*<br>2 communications avec acte [77, 78] | Doctorante (Univ Bordeaux) |
| Elodie JUQUIN 2014 (6 mois) | Master 2 Génétique et gestion de la biodiversité, Univ Paris VI<br>Co-encadrement avec Pr. I.Masneuf-Pomarède | Développement de marqueurs microsatellites pour le typage de levures non-*Saccharomyces*<br>1 article [79] | Professeur des Ecoles |
| Emilien PELTIER 2014 (6 mois) | Master 2 Génétique Moléculaire et Cellulaire, Univ Bordeaux<br>Co-encadrement avec Pr. Isabelle Masneuf-Pomarède | Étude de la diversité génétique de *Brettanomyces bruxellensis*<br>1 article [17]<br>1 titre de propriété intellectuel [80]<br>1 communication avec acte [77]<br>1 communication orale [21]<br>5 posters [23-25, 81, 82] | Post-doctorant (Univ Strasbourg) |
| Guillaume COMTE 2013 (6 mois) | Master 2 Viticulture et Œnologie, Univ Bordeaux<br>Encadrement avec Dr. Marina Bely et Dr. Philippe Marullo | Développement d'une méthode de génotypage pour l'étude de *Torulaspora delbrueckii*<br>1 article [38]<br>2 posters [41, 42] | Responsable Oenologie/Hygiène France (Soufflet Vigne) |
| Gwenaelle MENTEC 2012-2013 (6 mois) | Stage Ingénieur, UTC Compiègne<br>Co-encadrement avec Patrick Girard (Pernod Ricard) | Passage à l'échelle pilote de fermentations par cultures mixtes sur Sauvignon Blanc | Manager (Saint Michel Biscuits) |
| Ana-Sofia LANDOLT 2019 (4 mois) | BTS Bioanalyses et Contrôles, Lycée Saint Louis - Bordeaux<br>Encadrement avec Dr. Laura Chasseriaud | Développement de marqueurs moléculaires permettant le génotypage de l'espèce de levure *Metschnikowia pulcherrima* | Poursuite d'étude (BTS) |
| Léa CEMBELLIN 2019 (2 mois) | Licence Sciences et tech., Univ Bordeaux<br>Encadrement à 100% | Développement d'un nouvel outil de typage génétique pour la levure d'altération des vins *Schizosaccharomyces pombe* | Poursuite d'étude (Licence) |

| | | | |
|---|---|---|---|
| Paloma PLATA<br>2018 (4 mois) | BTS Bioanalyses et Contrôles, Lycée Saint Louis - Bordeaux<br>Encadrement à 100% | Etude de la levure *Brettanomyces bruxellensis* : recherche de marqueurs de prédiction de la résistance aux sulfites | Poursuite d'étude (BTS) |
| Léa CEMBELLIN<br>2017 (4 mois) | BTS Bioanalyses et Contrôles, Lycée Saint Louis – Bordeaux<br>Encadrement avec Dr. Laura Chasseriaud | Etude de l'impact de produits commerciaux dérivés sur le développement de *Saccharomyces cerevisiae* pendant la fermentation alcoolique | Poursuite d'étude (Licence) |
| Aurélie PANFILI<br>2013 (2 mois) | IUT Génie Biologie, Université de Pau et des Pays de l'Adour<br>Encadrement à 100% | Développement d'outil moléculaire pour le génotypage de *Brettanomyces bruxellensis*<br>2 articles [38, 83]<br>3 posters [41, 42, 81] | Responsable production (Prunidor) |
| Laure GUILLOTEAU<br>2008 (2 mois) | Licence Biologie-Chimie, Univ Paris-Sud<br>Encadrement à 100% | Etude des capacités fermentaires des levures industrielles et de leurs hybrides | Œnologue (Maison JC Boisset) |
| Vanessa MARTIN<br>2003 (2 mois) | BTS biotechnologie, Lycée Vallée - Chevreuse<br>Encadrement à 100% | Analyse phénotypique & génétique d'*Arabidospis thaliana* et de *Zea mays* aux niveaux diploïde et tétraploïde | |

# COLLABORATIONS

Collaborations internationales

| Début | Collaborateurs | Laboratoire | Projet / Thème de recherche | Financement | Publications |
|---|---|---|---|---|---|
| 2011 | Dr. Luca Cocolin | Food Microbiology Laboratory, Univ. of Turin, Italie | Biodiversité des levures non-*Saccharomyces* | Union européenne (WILDWINE 2007-2013) | 1 article [79]<br>2 posters [43, 84] |
| 2011 | Dr A. Nisiotou | Wine Institute, Athènes, Grèce | Biodiversité des levures non-*Saccharomyces* | Union européenne (WILDWINE 2007-2013) | 1 article [79]<br>2 posters [43, 84] |
| 2011 | Prof. A. Mas, Dr. Gemma Beltran | Universidad Rovira i Virgili, Tarragone, Espagne | Biodiversité des levures non-*Saccharomyces* | Union européenne (WILDWINE 2007-2013); fonds propres | 1 article [79]<br>2 articles soumis [3, 76]<br>3 posters [43, 84, 85] |
| 2012 | Dr. C. Curtin | Australian Wine Research Institute, Adelaide, Australie | Diversité génétique et génomique de *B. bruxellensis* | Alliance BAG (2014-2017); fonds propres | 2 articles [17, 83]<br>2 comm. orales [20, 21]<br>4 posters [24, 25, 81, 86]<br>3 comm. techniques [29, 35, 36] |
| 2012 | Prof. G. Spano, Dr Vittorio Capozzi | Department of Agriculture, Food & Environment Sciences, Univ. Foggia, Italie | Biodiversité des levures non-*Saccharomyces* | Union européenne (WILDWINE 2007-2013); fonds propres | 3 articles [17, 79, 87]<br>2 posters [43, 84] |
| 2013 | Prof. M. Sipiczki | Genetics & Applied Microbiology, Univ. of Debrecen, Hongrie | Biodiversité des levures non-*Saccharomyces* | Fonds propres | 2 articles [79, 88]<br>2 posters [43, 84] |
| 2013 | Prof. J. Sampaio | Universidade Nova de Lisboa, Portugal | Génomique comparative des *Saccharomyces* | Fonds propres | 1 article [89]<br>1 poster [90] |
| 2014 | Dr. B. Divol | Institute for Wine Biotechnology, Stellenbosch Univ., Afrique du Sud | Diversite génétique et phenotypique de *Hansenisapora uvarum* | Fonds propres | 1 article [91]<br>1 comm. avec acte [78] |
| 2014 | Pr. A. Rosa | Universidad Católica de Córdoba, Argentine | Diversité métabolique des levures non-*Saccharomyces* | Financement ISVV et Bordeaux INP, 2018 | 2 articles [88, 92]<br>1 poster [93] |
| 2014 | Dr. A. Aranda | Instituto Agroquimica y Tecnologia Alimentos, Valence, Espagne | Hybrides de levures du genre *Saccharomyces* | Fonds propres | 1 article [94]<br>1 poster [82] |
| 2015 | Pr. V. Jiranek | School of Agriculture, Food and Wine, University of Adélaide, Australie | Diversite génétique et phenotypique de *Lachancea thermotolerans* | Fonds propres | 2 articles [8, 9]<br>2 comm. orales [10, 11]<br>1 poster [12] |
| 2016 | Pr. G. Blaiotta | Dipartimento Agraria, Universita degli Studi di Napoli, Italie | Caractérisation des levures *Brettanomyces* en Œnologie | Fonds propres | 1 article [17]<br>1 poster [26] |
| 2017 | Drs. Anne C Gschaedler Mathis, Manuel Reinhart Kirchmayer | CIATEJ, Guadalajara, Mexico | Caractérisation des levures de Tequila ou Mezqual | Fonds propres | |

Collaborations nationales

| Début | Collaborateurs | Laboratoire | Projet / Thème de recherche | Financement | Publications |
|---|---|---|---|---|---|
| 2006 | Prof. D. de Vienne<br>Pr. C. Dillmann<br>Dr. D. Sicard | Univ. Paris Sud , UMR de Génétique Végétale, Gif sur Yvette | Projets Adaptalevure, HeterosYeast | ANR (Adaptalevure); ANR (HeterosYeast) | 7 articles [54-57, 95-97]<br>3 comm. avec acte [58-60]<br>8 comm. orales [61-66, 98, 99]<br>16 posters [67-75, 82, 100-105] |
| 2009 | Drs. D. Sherman<br>P. Durrens | INRIA & CNRS, MAGNOME, Talence | Génomique comparative des *Saccharomyces* | | 2 articles [89, 106]<br>1 poster [90] |
| 2009 | Dr. M. Zivy<br>Dr. Mélisande Blein-Nicolas | Univ. Paris Sud, PAPPSO, Gif sur Yvette | Protéomique des levures | ANR (HeterosYeast), financement privé | 3 articles [56, 97, 107]<br>1 comm. avec acte [59]<br>2 comm. orales [61, 64]<br>6 posters [47, 48, 71, 103-105] |
| 2011 | Dr. F. Salin | INRA, Plateforme Génome-Transcriptome, Pierroton | Génotypage ; Séquencage *de novo* de génome de non-*Saccharomyces* | Fonds propres | 8 articles [17, 38, 54, 79, 83, 88, 91, 108]<br>1 comm. avec acte [78]<br>2 comm. orales [21, 109]<br>7 posters [23-25, 41, 42, 67, 81]<br>2 comm. techniques [35, 36] |
| 2012 | Prof. M. Rigoulet | Univ. Bordeaux, IBGC UMR 5095, Bordeaux | Etude des capacités respiratoires des hybrides interspécifiques | Fonds propres | 1 article [54]<br>2 posters [54] |
| 2013 | Prof. JC. Batsale<br>Prof. A. Colin<br>Dr. P. Guillot | Institut de Mécanique et d'ingénierie, TREFLE I2M LOF, Laboratoire mixte Rhodia/CNRS/Universités de Bordeaux, Pessac | Mesure du flux de chaleur chez la levure | Financement région Aquitaine | 1 poster [51] |
| 2014 | Drs. Y. Gibon<br>B. Beauvois<br>S. Colombié | INRA, Univ. Bordeaux, UMR1332 Biologie du fruit et pathologie, Villenave d'Ornon | Quantification haut-débit de metabolites et d'activités enzymatiques | Financement FR BIE 2014 et 2016 | 1 poster [110] |
| 2016 | Dr J. Schacherer<br>Dr A. Friedrich | Univ. Strasbourg, UMR 7156 Genetique Moléculaire Genomique Microbiologie | Approche multi-échelle de l'adaptation de la levure *B. bruxellensis* aux procédés fermentaires | Financement ANR 2019-2022 | 1 article [17]<br>1 comm. orale [20] |
| 2017 | Dr MN Bellon-Fontaine<br>Dr M. Renault | INRA, Bioadhésion et hygiène des matériaux , Jouy en Josas | Capacité de bioadhesion de l'espèce *B. bruxellensis* | Fonds propres | 1 article [1]<br>2 posters [5, 111] |

Partenaires privés

| Début | Collaborateurs | Entreprise | Projet / Thème de recherche | Financement | Publications |
|---|---|---|---|---|---|
| 2006 | Dr. J. Coulon<br>Dr. V. Moine<br>Dr. S. La Guerche | Biolaffort, Bordeaux | Biodiversité des levures d'œnologie | Fonds propres, fonds privés | 5 articles [39, 40, 91, 112, 113]<br>1 manuscrit en préparation [114]<br>1 comm. avec acte [58]<br>2 comm. orales [10, 115]<br>7 posters [44-50]<br>1 comm. technique [53] |
| 2010 | Dr. B. Colonna-Ceccaldi | CRPR, Créteil | Etude et utilisation des levures non-conventionnelles | Fonds propres, fonds privés Pernod-Ricard | 4 articles [79, 91, 112, 113]<br>1 article en préparation [13]<br>1 comm. orale [14]<br>3 posters [15, 16, 43] |
| 2018 | Dr. Anne Ortiz-Julien<br>Dr. Magali Deleris-Bou | Lallemand Œnologie, Blagnac | Interactions entre microorganismes, lutte contre *B. bruxellensis* | Financement privé Lallemand | |
| 2019 | Dr. Vincent Renouf | Excell/Sarco, Bordeaux | BrettAdhere : Capacité de bioadhesion de l'espèce *B. bruxellensis* | Financement privé Excell/ Sarco | |

# CONTRATS DE RECHERCHE ET FINANCEMENTS

Depuis mon recrutement en 2014, plusieurs financements ont été obtenus ou sollicités.

| Date | Intitulé | Origine du financement | Rôle | Partenaires | Budget |
|---|---|---|---|---|---|
| 2014-2018 | TYPE\Brett: Test moléculaire pour détecter les souches d'altération des vins *B. bruxellensis* résistantes au dioxyde de soufre | Aquitaine Science Transfert, en réponse à l'appel à résultats de recherche Technovin 2014 | Porteur du projet | UR Œnologie | 134k€ |
| 2016-2017 | MAEL : Mesure des Activités Enzymatiques des Levures | Fédération de Recherche Biologie Integrative et Ecologie, en réponse à l'appel à projets transversaux 2016 | Porteur du projet | UR Œnologie | 3,5k€ |
| | | | | UMR1332 | 1,5k€ |
| 2017 | CCC : Etude des interactions de type cell-cell contact entre microorganismes du vin | Bordeaux INP, en réponse à l'AAP 'Accompagnement des MCF' | Porteur du projet | UR Œnologie | 2,6k€ |
| 2018-2020 | Chitowine : Recherches sur l'origine et les effets secondaires des propriétés stabilisantes du chitosane fongique dans le vin | ANR, AAPG2017 | Participation (porteur du projet : Marguerite Dols, UR Oenologie) | UR Œnologie | 559K€ pour la totalité du projet, dont 182k€ pour l'UR Œnologie |
| | | | | Institut Pascal | |
| | | | | Biolaffort | |
| | | | | Microflora | |
| | | | | UMR 1083 SPO | |
| 2018-2020 | SoLt : Selection of *Lachancea thermotolerans* strain for ethanol reduction and acidity modulation | Financement Biolaffort | Participation (porteur du projet : Marina Bely, UR Oeno) | UR Œnologie | 106k€ |
| | | | | Université d'Adélaide | |
| | | | | Biolaffort | |
| 2018-2019 | Brett&cie : Saccharomyces cerevisiae – Non-Saccharomyces – Brettanomyces bruxellensis : interactions et impact des pratiques œnologiques | Financement Lallemand | Porteur du projet | UR Œnologie | 10k€ |
| | | | | Lallemand | |
| 2019-2022 | BrettAdapt : Approche multi-échelle de l'adaptation de la levure *B. bruxellensis* aux procédés fermentaires | ANR, AAPG2018 | Porteur du projet | UR Œnologie | 520k€ pour la totalité du projet, 260K€ par équipe |
| | | | | Université Strasbourg | |
| 2020 | GCbase : Etablissement d'une base de données spectrales en GCxGC TOF MS des composés volatils des boissons fermentées | Bordeaux INP, en réponse à l'AAP 'Accompagnement des MCF' | Porteur du projet | UR Œnologie | 2,6k€ |
| 2020-2022 | MousyWine : Exploration des phénomènes liés à la production des dérivés de pyrrole et de pyridine responsables de déviations organoleptiques dans les vins | Région Nouvelle Aquitaine – BAG (Bordeaux-Adélaïde-Geisenheim) | Participation (porteur du projet : Patricia Ballestra, UR Oeno) | UR Œnologie | 149k€ |
| | | | | Geisenheim University | |
| | | | | AWRI | |

| | | | | | |
|---|---|---|---|---|---|
| 2020-2022 | BRETTADHERE : Recherches sur les propriétés de bioadhésion de l'espèce Brettanomyces bruxellensis ; applications à l'hygiène des caves et à la prévention de la contamination des vins | CIFRE Biolaffort/Excell | Participation (porteur du projet : Isabelle Masneuf-Pomarède, UR oeno) | UR Œnologie<br>Biolaffort/ Excell | |
| Demande de financement de 4 ans | METASIMFOOD: Anticipating the impact of food societal transitions and climate change on the quality and diversity of vegetable fermented food through synthetic ecology approaches | ANR, AAPG2020 | Participation (porteur du projet : Stéphane Chaillou, MICALIS) | UMR1319 MICALIS<br>UMR0782 GMPA<br>UR1404 MaIAGE<br>ITAI Aerial<br>UR Œnologie<br>MNHM UMR7196<br>UMR1253 STLO | Demande de financement de 830k€ pour la totalité du projet |

# ACTIVITE D'ENSEIGNEMENT

- ***ENSCBP - Bordeaux INP***. Depuis 2014, j'effectue un service complet :
  - 185H en 2014-2015 (137H attribuées, incluant une décharge de 30%)
  - Entre 219-250H depuis 2015 (192H attribuées).

Cours magistraux (CM), travaux dirigés (TD) et travaux pratiques (TP).

Spécialités : Microbiologie alimentaire, Statistiques, Hygiène et Sécurité des Aliments.

1ère et 2ème année d'école d'ingénieur, formations Agroalimentaire - Génie biologique, Chimie – Génie physique, formation par alternance Agroalimentaire - Génie industriel.

- ***SupAgro Montpellier, Vinifera EuroMaster***, depuis 2012, 3H/an.

UE 'Project Management in Science', encadrement d'étudiants pour l'analyse statistique d'un jeu de données, la bibliographie et la rédaction d'un manuscrit scientifique.

- ***Université de Bordeaux***, 2018, 3H

CM et TD, Génétique des populations.

Master 2, UE « Génétique et omiques appliquées à la vigne et au vin »

- ***ENSTBB- Bordeaux INP***, 2014 à 2016, 20H puis 36H

CM et TD, Statistiques appliquées

1ère et 2ème année d'école d'ingénieur en Biotechnologies

- ***Bordeaux Sciences Agro****, 2013, 2H de CM*

2ème année d'école d'ingénieur, UE « Génétique Moléculaire Appliquée à la Production »

- ***Université Bordeaux Segalen****, 2012 à 2014, 9H/an de CM*

Master Recherche et Master Pro 'Oenologie et Environnement Vitivinicole', UE 'Communication et Conception projet de recherche', *Statistiques appliquées à l'expérimentation*.

- ***Université Bordeaux Segalen****, 2012, 10H de CM*

Licence Pro Oenocontroleur, UE « Traitements de l'information »

- ***Université Paris-Sud****, de 2003 à 2005, 2H/an de CM*

Master Recherche 'Science du Végétal', UE 'Métabolisme, Métabolome et protéome'.

## *Responsabilités administratives*

- **Depuis 2018** : responsable des visites d'entreprise pour le département AGB (Agroalimentaire - Génie biologique) de l'ENSCBP.
- **2017** : Membre de la section disciplinaire compétente à l'égard des usagers, élue par le conseil d'administration de **Bordeaux INP.**

## *Participaction à des Jurys (soutenance, recrutement, distinction)*

- **2018** : membre du jury de l'édition 2018 du prix Jean-Marc GEY, délivré par l'Association pour le Développement du management Qualité-Sécurité-Environnement (ADQSE) et l'ENSCBP
- **Depuis 2014** : participation régulière à des jurys de soutenance de stages, présidence de soutenance. ***ENSCBP – Bordeaux INP, Univ de Bordeaux***.
- **2013** : Evaluation (contrôle continu) des synthèses bibliographiques, UE 'Génétique Moléculaire Appliquée à la Production', ***Bordeaux Sciences Agro***.
- **2012** : Jury de soutenance Master Européen Vinifera, UE « Project Management in Science » (en anglais), ***Montpellier SupAgro***.
- **2003-2005** : Jury de soutenance des stages de DEUG Sciences de la Vie, ***U-PSud***.

## *Tutorat*

- 2016-2017 : encadrement d'un projet tutoré, étudiants du ***Cycle préparatoire de Bordeaux*** (CPBx)
- Depuis 2014 : tutorat de 6 à 12 étudiants en école d'ingénieur par an, ***ENSCBP – Bordeaux INP***
- Depuis 2014 : encadrement de projets tutorés, étudiants en école d'ingénieur, ***ENSCBP – Bordeaux INP***
- Depuis 2012 : encadrement d'étudiants du Master Européen Vinifera, ***Montpellier SupAgro*****,** pour l'UE « Project Management in Science » (en anglais).

# LISTE COMPLETE DES PUBLICATIONS & COMMUNICATIONS

- 45 publications, dont 16 en premier auteur, 7 en dernier auteur et 16 en 'corresponding author'.
- 6 manuscrits soumis ou en préparation
- 1 Titre de Propriété Intellectuelle
- 1 chapitre de livre
- 6 communications avec acte
- 27 communications orales
- 57 communications affichées
- 14 communications techniques

H index = 21[a] ; IF min-max = 1.6-13.7[b] ; 1414 citations cumulées[a].

[a] source Google Scholar, nov. 2019;

[b] IF : facteur d'impact 2014 (Journal Citation Reports, Thomson Reuters)

## REVUES INTERNATIONALES AVEC COMITE DE LECTURE (45)


Cibrario, A., C. Miot-Sertier, M. Paulin, B. Bullier, L. Riquier et al., 2020 Brettanomyces bruxellensis phenotypic diversity, tolerance to wine stress and spoilage ability. Food Microbiology 87.

Lebleux, M., H. Abdo, C. Coelho, L. Basmaciyan, W. Albertin et al., 2020 New advances on the Brettanomyces bruxellensis biofilm mode of life. International Journal of Food Microbiology 318.

Avramova, M., C. Varela, P. Grbin, A. Borneman, W. Albertin et al., 2019 Competition experiments between Brettanomyces bruxellensis strains reveal specific adaptation to SO2. FEMS Yeast Res 19.

Cibrario, A., M. Avramova, M. Dimopoulou, M. Magani, C. Miot-Sertier et al., 2019 Brettanomyces bruxellensis wine isolates show high geographical dispersal and long persistence in cellars Plos One.

Dimopoulou, M., M. Hatzikamari, I. Masneuf-Pomarede and W. Albertin, 2019a Sulfur dioxide response of Brettanomyces bruxellensis strains isolated from Greek wine. Food Microbiol 78: 155:163.

Dimopoulou, M., M. Renault, M. Dols-Lafargue, W. Albertin, J. M. Herry et al., 2019b Microbiological, biochemical, physicochemical surface properties and biofilm forming ability of Brettanomyces bruxellensis. Annals of Microbiology In press.

Feghali, N., W. Albertin, E. Tabet, Z. Rizk, A. Bianco et al., 2019 Genetic and Phenotypic Characterisation of a Saccharomyces cerevisiae Population of 'Merwah' White Wine. Microorganisms 7: 492.

Raymond Eder, M. L., F. Conti, M. Bely, I. Masneuf-Pomarede, W. Albertin et al., 2019 Vitis species, vintage, and alcoholic fermentation do not drive population structure in Starmerella bacillaris (synonym Candida zemplinina) species. Yeast 36: 411-420.

Roudil, L., P. Russo, C. Berbegal, W. Albertin, G. Spano et al., 2019 Non-Saccharomyces Commercial Starter Cultures: Scientific Trends, Recent Patents and Innovation in the Wine Sector. Recent Pat Food Nutr Agric.

Albertin, W., M. Chernova, P. Durrens, E. Guichoux, D. J. Sherman et al., 2018 Many interspecific chromosomal introgressions are highly prevalent in Holarctic Saccharomyces uvarum strains found in human-related fermentations. Yeast 35: 141-156.

Avramova, M., A. Vallet-Courbin, J. Maupeu, I. Masneuf-Pomarède and W. Albertin, 2018a Molecular Diagnosis of Brettanomyces bruxellensis' Sulfur Dioxide Sensitivity Through Genotype Specific Method. Frontiers in Microbiology 9.

Avramova, M., A. Cibrario, E. Peltier, M. Coton, E. Coton et al., 2018b Brettanomyces bruxellensis population survey reveals a diploid-triploid complex structured according to substrate of isolation and geographical distribution. Scientific reports 8.

Chasseriaud, L., J. Coulon, P. Marullo, W. Albertin and M. Bely, 2018 New oenological practice to promote non-Saccharomyces species of interest: saturating grape juice with carbon dioxide. Applied Microbiology and Biotechnology 102: 3779-3791.

Hranilovic, A., J. M. Gambetta, L. Schmidtke, P. K. Boss, P. R. Grbin et al., 2018 Oenological traits of Lachancea thermotolerans show signs of domestication and allopatric differentiation. Sci Rep 8: 14812.

Rosa, A. L., C. Miot-Sertier, Y. h. Laizet, F. Salin, M. Sipiczki et al., 2018 Draft Genome Sequence of the Candida zemplinina (syn., Starmerella bacillaris ) Type Strain CBS 9494. Microbiology Resource Announcements 7.

Tempère, S., A. Marchal, J.-C. Barbe, M. Bely, I. Masneuf-Pomarede et al., 2018 The complexity of wine: clarifying the role of microorganisms. Applied Microbiology and Biotechnology.

Albertin, W., A. Zimmer, C. Miot-Sertier, M. Bernard, J. Coulon et al., 2017 Combined effect of the Saccharomyces cerevisiae lag phase and the non-Saccharomyces consortium to enhance wine fruitiness and complexity. Appl Microbiol Biotechnol 101: 7603-7620.

Dequin, S., J. L. Escudier, M. Bely, J. Noble, W. Albertin et al., 2017 How to adapt winemaking practices to modified grape composition under climate change conditions. OENO One 51.

Hranilovic, A., M. Bely, I. Masneuf-Pomarede, V. Jiranek and W. Albertin, 2017 The evolution of Lachancea thermotolerans is driven by geographical determination, anthropisation and flux between different ecosystems. PLoS One 12: e0184652.

Albertin, W., C. Miot-Sertier, M. Bely, T. T. Mostert, B. Colonna-Ceccaldi et al., 2016 Hanseniaspora uvarum from winemaking environments show spatial and temporal genetic clustering. Frontiers in Microbiology 6.

Masneuf-Pomarede, I., M. Bely, P. Marullo and W. Albertin, 2016 The genetics of non-conventional yeasts in winemaking: current knowledge and future challenges. Frontiers in Microbiology 6: 1563.

Michel, J., W. Albertin, M. Jourdes, A. Le Floch, T. Giordanengo et al., 2016 Variations in oxygen and ellagitannins, and organoleptic properties of red wine aged in French oak barrels classified by a near infrared system. Food Chem 204: 381-390.

Blein-Nicolas, M., W. Albertin, T. da Silva, B. Valot, T. Balliau et al., 2015 A Systems Approach to Elucidate Heterosis of Protein Abundances in Yeast. Mol Cell Proteomics 14: 2056-2071.

Chasseriaud, L., C. Miot-Sertier, J. Coulon, N. Iturmendi, V. Moine et al., 2015 A new method for monitoring the extracellular proteolytic activity of wine yeasts during alcoholic fermentation of grape must. J Microbiol Methods 119: 176-179.

da Silva, T., W. Albertin, C. Dillmann, M. Bely, S. la Guerche et al., 2015 Hybridization within Saccharomyces Genus Results in Homoeostasis and Phenotypic Novelty in Winemaking Conditions. PLoS One 10: e0123834.

Masneuf-Pomarede, I., E. Juquin, C. Miot-Sertier, P. Renault, Y. Laizet et al., 2015 The yeast Starmerella bacillaris (synonym Candida zemplinina) shows high genetic diversity in winemaking environments. FEMS Yeast Res 15: fov045.

Picazo, C., E. Gamero-Sandemetrio, H. Orozco, W. Albertin, P. Marullo et al., 2015 Mitochondria inheritance is a key factor for tolerance to dehydration in wine yeast production. Lett Appl Microbiol 60: 217-222.

Albertin, W., L. Chasseriaud, G. Comte, A. Panfili, A. Delcamp et al., 2014a Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast Torulaspora delbrueckii. PLoS One 9: e94246.

Albertin, W., A. Panfili, C. Miot-Sertier, A. Goulielmakis, A. Delcamp et al., 2014b Development of microsatellite markers for the rapid and reliable genotyping of Brettanomyces bruxellensis at strain level. Food Microbiol 42: 188-195.

Albertin, W., C. Miot-Sertier, M. Bely, P. Marullo, J. Coulon et al., 2014c Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. Int J Food Microbiol 178: 87-97.

Almeida, P., C. Goncalves, S. Teixeira, D. Libkind, M. Bontrager et al., 2014 A Gondwanan imprint on global diversity and domestication of wine and cider yeast Saccharomyces uvarum. Nat Commun 5: 4044.

Gammacurta, M., S. Marchand, W. Albertin, V. Moine and G. de Revel, 2014 Impact of yeast strain on ester levels and fruity aroma persistence during aging of Bordeaux red wines. J Agric Food Chem 62: 5378-5389.

Martins, G., J. Vallance, A. Mercier, W. Albertin, P. Stamatopoulos et al., 2014 Influence of the farming system on the epiphytic yeasts and yeast-like fungi colonizing grape berries during the ripening process. Int J Food Microbiol 177: 21-28.

Albertin, W., P. Marullo, M. Bely, M. Aigle, A. Bourgais et al., 2013a Linking post-translational modifications and variation of phenotypic traits. Mol Cell Proteomics 12: 720-735.

Albertin, W., T. da Silva, M. Rigoulet, B. Salin, I. Masneuf-Pomarede et al., 2013b The mitochondrial genome impacts respiration but not fermentation in interspecific Saccharomyces hybrids. PLoS One 8: e75121.

Blein-Nicolas, M., W. Albertin, B. Valot, P. Marullo, D. Sicard et al., 2013 Yeast proteome variations reveal different adaptive responses to grape must fermentation. Mol Biol Evol 30: 1368-1383.

Renault, P. E., W. Albertin and M. Bely, 2013 An innovative tool reveals interaction mechanisms among yeast populations under oenological conditions. Appl Microbiol Biotechnol 97: 4105-4119.

Albertin, W., and P. Marullo, 2012 Polyploidy in fungi: evolution after whole-genome duplication. Proc Biol Sci 279: 2497-2509.

Albertin, W., P. Marullo, M. Aigle, C. Dillmann, D. de Vienne et al., 2011 Population size drives industrial Saccharomyces cerevisiae alcoholic fermentation and is under genetic control. Appl Environ Microbiol 77: 2772-2784.

Albertin, W., P. Marullo, M. Aigle, A. Bourgais, M. Bely et al., 2009a Evidence for autotetraploidy associated with reproductive isolation in Saccharomyces cerevisiae: towards a new domesticated species. J Evol Biol 22: 2157-2170.

Albertin, W., O. Langella, J. Joets, L. Negroni, M. Zivy et al., 2009b Comparative proteomics of leaf, stem, and root tissues of synthetic Brassica napus. Proteomics 9: 793-799.

Marullo, P., C. Mansour, M. Dufour, W. Albertin, D. Sicard et al., 2009 Genetic improvement of thermo-tolerance in wine Saccharomyces cerevisiae strains by a backcross approach. FEMS Yeast Res 9: 1148-1160.

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Albertin, W., T. Balliau, P. Brabant, A. M. Chevre, F. Eber et al., 2006 Numerous and rapid nonstochastic modifications of gene products in newly synthesized Brassica napus allotetraploids. Genetics 173: 1101-1113.

Albertin, W., P. Brabant, O. Catrice, F. Eber, E. Jenczewski et al., 2005 Autopolyploidy in cabbage (Brassica oleracea L.) does not alter significantly the proteomes of green tissues. Proteomics 5: 2131-2139.

## ARTICLES SOUMIS/EN PRÉPARATION (6)

Borlin, M., O. Claisse, W. Albertin, F. Salin, J.L. Legras, and I. Masneuf-Pomarede, Quantifying anthropogenic effect on S. cerevisiae vineyard metapopulation diversity. Submitted to Evolutionary Applications.

Borlin M, Miot-Sertier C, Vinsonneau E, Becquet S, Salin F, Bely M, Lucas P, Albertin W, Legras JL, Masneuf-Pomarede I. The “Pied de Cuve” as an alternative way to manage indigenous fermentation: impact on the fermentative process and the Saccharomyces cerevisiae diversity. Submitted to OENO One

Chasseriaud, L., W. Albertin, J. Coulon, and M. Bely, Interactions between Saccharomyces cerevisiae and Torulaspora delbrueckii in winemaking: mixed cultures with and without physical contact reveals different behaviours. In preparation.

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# II – RAPPORT D'ACTIVITES SCIENTIFIQUES

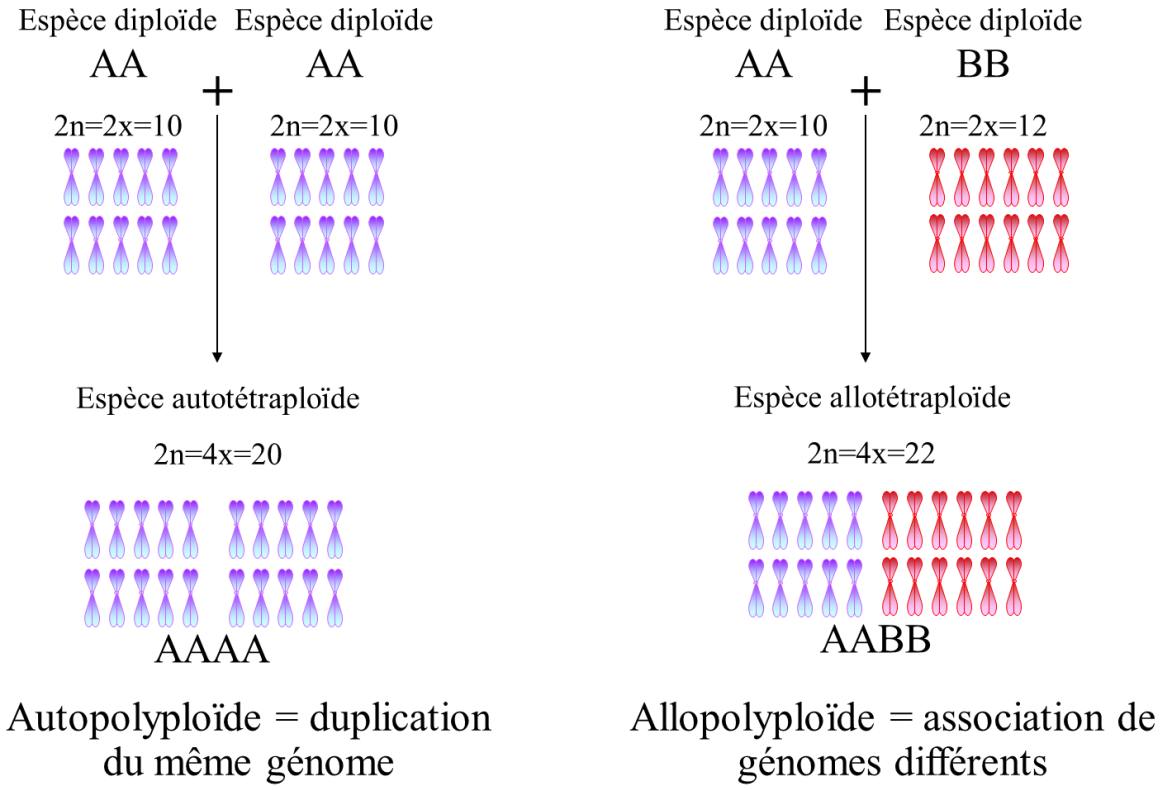



**FIGURE 2. AUTOPOLYPLOÏDIE VERSUS ALLOPOLYPLOÏDIE.** UNE ESPECE (OU UN ORGANISME) AUTOPOLYPLOÏDE CONTIENT PLUS DE DEUX JEUX COMPLETS DE CHROMOSOMES RESULTANT DE LA DUPLICATION DU MEME GENOME. UN ALLOPOLYPLOÏDE COMBINE DES JEUX DE CHROMOSOMES PROVENANT DE DIFFERENTS GENOMES.

# II-A- TRAVAUX DE THESE

Mes travaux de thèse portaient sur les organismes à génome complexe, polyploïdes, c'est à dire possédant plus de deux génomes haploïdes (Fig. 2). La polyploïdie est un phénomène majeur dans l'évolution des eucaryotes : omniprésente chez les plantes [206], elle est également bien décrite chez certaines familles d'animaux [207] et de champignons [208]. L'objectif de ma thèse était de mieux comprendre les mécanismes moléculaires impliqués dans les toutes premières générations après polyploïdisation, aussi bien dans un contexte allopolyploïde (plus de deux génomes haploïdes provenant d'espèces différentes, Fig. 2) que dans un contexte autopolyploïde (plus de deux génomes haploïdes provenant de la même espèce), peu étudié [209]. Nous avons choisi une approche globale originale au regard des approches transcriptomiques traditionnellement utilisées : la protéomique quantitative qui permet de mesurer avec précision l'abondance de plusieurs centaines de protéines. Nous avons appliqué cette méthode à un allotétraploïde important en agronomie, le colza *Brassica napus*, qui combine les génomes diploïdes du chou *B. oleracea* et du navet *B. rapa*. Une série autopolyploïde de chou *B. oleracea* a également été étudiée aux niveaux haploïde, diploïde et tétraploïde.

Nos travaux montrent que le doublement du génome *per se* n'a que peu d'effet sur le protéome : les proportions relatives des protéines sont en effet bien conservées, aussi bien dans un contexte autopolyploïde (pas de différence entre les protéomes de choux haploïdes, diploïdes et tétraploïdes) [123] que dans un contexte allopolyploïde (peu de différences entre hybrides et hybrides doublés) [120]. L'hybridation interspécifique, quant à elle, est associée à un remodelage drastique de l'abondance des protéines : 25 à 38% des polypeptides sont sur- ou sous-exprimés par rapport à la moyenne de leurs parents [120]. L'hybridation génère, à partir de génomes préexistants, de nouveaux profils protéiques et permet de 'faire du neuf avec du vieux'.

L'identification des protéines concernées par spectrométrie de masse [107] montrent que la modification de l'expression génique est organe-spécifique et isoforme-spécifique, puisqu'une même protéine peut être la cible de variation d'expression différente en fonction de l'organe considéré et en fonction de ses modifications post-traductionnelles [119]. Une analyse *in silico* des propriétés des protéines identifiées montre qu'il n'y a pas de fonction, de voie métabolique ou de localisation cellulaire particulièrement ciblées par le remodelage de l'expression génique, indiquant que les mécanismes en jeu sont immédiats, non-stochastiques mais ne sont pas liés aux propriétés fonctionnelles des protéines concernées [119].

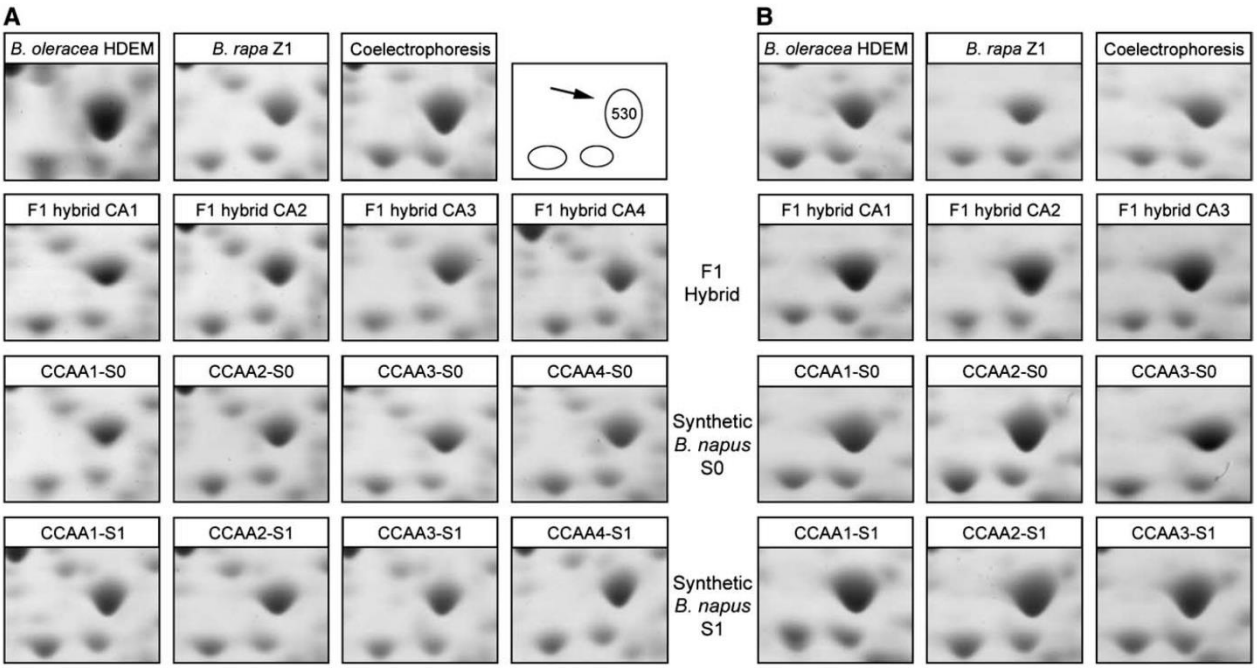



**FIGURE 3. GELS D'ELECTROPHORESE BIDIMENSIONNELLE.** *B. OLERACEA*, *B. RAPA*, LEURS HYBRIDES F1 (CA1-CA4) ET LEURS HYBRIDES DOUBLES (*B. NAPUS* SYNTHETIQUES CCAA1-CCAA4) INITIAUX (S0) OU APRES UNE GENERATION D'AUTOFECONDATION (S1). PROTEINES EXTRAITES DE TIGE (A) OU DE RACINE (B). LA CO-ELECTROPHORESE EST REPRESENTATIVE DE L'HYPOTHESE D'ADDITIVITE DES PROTEOMES PARENTAUX. LE SPOT 530 EST SOUS-EXPRIME DANS LA TIGE, ET SUR-EXPRIME DANS LA RACINE

Mes travaux de thèse m'ont donc permis d'acquérir de bonnes connaissances du fonctionnement des organismes au génome 'complexe' (hybride et/ou polyploïde), ainsi que de solides compétences dans les approches dites 'omics' : protéomique, qui repose nécessairement sur des bases de données génomiques, mais aussi sur l'analyse statistique de large jeu de données et la programmation informatique pour leur traitement automatisé ('pipeline'). Les quatre articles [107, 119, 120, 123] résultant de ce travail ont été bien accueillis par la communauté scientifique, deux d'entre eux étant particulièrement cités (>100 citations, source Google Scholar).

Sur le plan scientifique, ces travaux montrent que l'hybridation interspécifique est associée à un remodelage drastique, mais non stochastique, de l'abondance des protéines. La question qui reste en suspens est celle de l'impact au niveau phénotypique, non traitée au cours de ma thèse. Ces travaux m'ont donc conduite à développer par la suite des approches plus intégratives, mais aussi plus finalisées, appliquées à d'autres modèles.

**FIGURE 4. L'ELABORATION DES VINS ROUGES.** LA VINIFICATION EST LE PROCESSUS DE TRANSFORMATION DU RAISIN EN VIN. LES ETAPES DIFFERENT EN FONCTION DU TYPE DE VIN (ROUGE, BLANC, ROSE OU EFFERVESCENT). SOURCE : CNIV (WWW.INTERVIN.FR/)

# II-B- DIVERSITE GENETIQUE ET PHENOTYPIQUE DES LEVURES DU RAISIN ET DU VIN

Les levures sont des modèles qui se prêtent bien au développement d'approches intégratives et appliquées. Leur manipulation est généralement simple et économique, et de très nombreux outils et méthodes d'analyse sont disponibles. Ainsi, la levure modèle *Saccharomyces cerevisiae* est le premier eucaryote dont le génome a été entièrement séquencé, dès 1996 [210]. Sur le plan appliqué, elles sont exploitées pour leur capacité fermentaire depuis des siècles, voire des millénaires dans différents procédés alimentaires, en boulangerie, distillerie, brasserie, œnologie, etc. [211]. Des applications en ingénierie métabolique ou en bio-remédiation ont vu le jour plus récemment [212].

En œnologie, les levures jouent un rôle clé : elles sont responsables de l'étape de fermentation alcoolique (FA), qui permet la transformation du sucre contenu dans le moût de raisin en éthanol et $CO_2$ (Fig. 3). Seules deux espèces de levure sont capables de 'terminer' la FA en conditions œnologiques (*i.e.* consommer la totalité du sucre présente dans le moût) : *S. cerevisiae* et dans une moindre mesure *S. uvarum*. Outre leur capacité fermentaire, les levures sont aussi responsables de la révélation d'une partie des arômes du vin. Les arômes du vin sont généralement catégorisés en trois groupes [213] :

- les arômes primaires (ou variétaux), souvent typiques des cépages (≈ variétés de vigne), sont associés aux notes fruitées et florales. Ces arômes primaires sont généralement présents à l'état de précurseurs inodores dans les moûts (car conjugués ou liés à d'autres molécules), ils sont libérés en molécules volatiles (et odorantes) par l'action enzymatique des levures. Ces arômes provenant du raisin, ils forment le « potentiel aromatique du raisin », et comptent quelques grandes familles bien connues comme les thiols volatils ou certains terpénols.
- les arômes secondaires (ou fermentaires), libérés par l'activité métabolique des levures durant la FA. Parmi les grandes familles d'arômes fermentaires, on retrouve les alcools supérieurs et les esters.
- Enfin les arômes tertiaires, associés à l'élevage et au vieillissement des vins, issus de l'évolution temporelle des vins au contact du bois et/ou de l'oxygène. Ils apportent (entre autre) les traditionnelles notes boisées.

La littérature comporte de très nombreux exemples d'études portant sur l'impact des levures sur l'arôme des vins (pour revue récente voir Lambrechts et Pretorius [214]), à laquelle l'UR Œnologie a largement contribué [215-221]. Dans la plupart de ces travaux, l'espèce modèle est l'espèce de levure la plus employée en œnologie : *S. cerevisiae*.

| PRODUCT | COMPANY | SPECIES | ADVANTAGES (Features of interest in winemaking) |
|---|---|---|---|
| Atecream 11H | BioEnologia | *Candida zemplinina* | Indicated for high alcohol content wines. Increases the production of glycerine and has a low alcohol rate. |
| Atecream 12H | BioEnologia | *Schizosaccharomyces pombe* | Used for the MLF. High production of glycerol. Low production of volatile acidity, sulfites and acetic acid. |
| FROOTZEN® | Christian Hansen | *Pichia kluyveri* | Secure the fermentation and enhance fruit flavors. |
| Biodiva | Lallemand | *Torulaspora delbrueckii* | Increases the perception of some esters without masking the typicity. Produces low volatile acidity. |
| CONCERTO™ | Christian Hansen | *Kluyveromyces thermotolerans* | Ideal for hot climate. Increases total acidity, Produces fresh strawberry aroma. |
| ENARTIS FERM BRETT OUT K | Enartis | *K. wickerhamii* | Produces a mycotoxin inhibiting the growth of spoilage microorganisms. |
| ENARTIS FERM BRETT OUT W | Enartis | *Wickerhamomyces anomalus* | Produces a mycotoxin inhibiting the growth of spoilage microorganisms. |
| Excellence® BIO-NATURE | Lamothe-Abiet | *Metschnikowia pulcherrima* | Protects and control the indigenous microbiota |
| Flavia | Lallemand | *M. pulcherrima* | Releases thiols and terpenic compounds during AF, favorising the expression of red and white wines. |
| Gaïa | Lallemand | *M. fructicola* | Protects from spoilage yeasts. Reduces the risk of early pre-fermentation. |
| LAKTIA™ | Lallemand | *Lachancea thermotol erans* | Produces high amount of lactic acids during AF. Produces complex aromas at the beginning of the AF. |
| Level²TD | Lallemand | *S. cerevisiae* and *T. delbrueckii* | Increases the aromatic complexity thanks to terpenes and esters production and reduces the volatile acidity. |
| Levulia® Alcomeno | AEB | *K. thermotolerans* | High production of lactic acid. Indicated for hot climates or overripe grapes |
| VINIFLORA RYTHM | Christian Hansen | *K. thermotolerans* and *S. cerevisiae* | Produces aromatic red wines rich in blackberry and blackcurrant flavors, and preserves the acidity in wine. |
| VINIFLORA HARMONY | Christian Hansen | *K. thermotolerans, T. delbrueckii* & *S. cerevisiae* | Enhances mouthfeel & palate weight. Generates sweet fruit intense aromas. |
| VINIFLORA SYMPHONY | Christian Hansen | *K. thermotolerans* and *S. cerevisiae* | Clear floral aroma and bright, tropical fruity notes in white wines. complex and round flavors in red wines. |
| MELODY™ | Christian Hansen | *K. thermotolerans, T. delbrueckii* and *S. cerevisiae* | For red and white wines. Generates fruity and spicy aromas. |
| Oenoferm®wild & pure | Erbslòh | *T. delbrueckii* | Brings a creamy texture with a pleasant lasting mouthfeel |
| Oenovin Torulaspora Bio | Oeno | *T. delbrueckii* | Increases the olfactory notes of red fruit and improves the softness and roundness of wines. |
| PRELUDE™ | Christian Hansen | *T. delbrueckii* | Guarantees flavor complexity by producing medium-chain (stable) fatty acid esters and promotes MLF. Produces a high concentration of mannoproteins. |
| PRIMAFLORA® | AEB GROUP | *T. delbrueckii* | Protects the must from spoilage microorganisms by competitive selection. Brings some aromatic complexity and improves mouthfeeling. |
| ProMalic® | Proenol | *Schizo. pombe* | Allows maloalcoholic deacidification. |
| Qτ | Enartis | *T. delbrueckii* | Produces high amounts of esters & terpenoids that create fresh, red fruits aromas. Produces low volatile acidity. Increases smoothness & volume |
| viniferm NSTD | AGRO VIN | *T. delbrueckii* | Intensifies the perception of floral aromas by producing β-phényl éthanol. Produces high amounts of mannoproteins. |
| ZYMAFLORE® Alpha TD n.sacc | Laffort | *T. delbrueckii* | Produces varietal thiols. Low production of volatile acidity. |
| ZYMAFLORE®É GIDE$^{TDMP}$ | Laffort | *T. delbrueckii* & *M. pulcherrima* | Pre-fermentative control and bioprotection. |

**TABLEAU 1. LISTE (NON-EXHAUSTIVE) DE PREPARATIONS COMMERCIALES DE LEVURE INCLUANT DES NON-*SACCHAROMYCES* POUR L'OENOLOGIE.** D'APRES ROUDIL ET AL., 2019 [87].

Toutefois, d'autres genres et espèces de levure sont présents en vinification et sont même majoritaires lors des phases pré-fermentaires (*i.e.* avant le démarrage de la fermentation alcoolique). Ainsi, les levures présentes à la surface de la baie de raisin vont être retrouvées dans les moûts, comme par exemple les espèces des genres *Hanseniaspora, Starmerella*, etc. Ces levures, dites 'non-*Saccharomyces*' (NS), ont longuement été considérées comme indésirables par les praticiens, mais sont très étudiées depuis une vingtaine d'années car certaines présentent des caractéristiques d'intérêt pour le vinificateur (impact aromatique, acidification des moûts, etc.). En l'absence d'intervention humaine, ce sont les levures dites 'indigènes', naturellement présentes dans les moûts, qui démarrent la FA. Ces fermentations spontanées peuvent toutefois conduire à des fermentations languissantes, voire à des arrêts précoces de fermentation, associées à des défauts organoleptiques. Ainsi, afin de mieux maitriser la FA, la pratique du levurage (*i.e.* apport exogène de levure) s'est généralisée ces dernières décennies (environ 80% des FA sont levurées aujourd'hui). *S. cerevisiae* reste la principale espèce commercialisée, généralement sous forme LSA (Levures Sèches Actives). Toutefois, de plus en plus de NS sont disponibles sous forme de préparations commerciales destinées à des levurages mixtes en combinaison avec une *Saccharomyces* sp. pour sécuriser la FA (Tableau 1).

En plus de ces levures d'intérêt œnologique, des levures d'altération peuvent aussi se développer dans les vins, généralement lors d'étapes post-fermentaires (Fig. 3). La plus fameuse des levures d'altération est *Brettanomyces bruxellensis*. Le développement de l'espèce *B. bruxellensis* dans les vins est lié à l'apparition de déviations aromatiques regroupées sous le terme 'goût de brett', caractérisées par des notes désagréables rappelant l'écurie, le phénol, le cuir, la gouache, etc. *B. bruxellensis* est en effet capable de décarboxyler les acides cinnamiques naturellement présents dans le raisin et le vin (acide *p*-coumarique, férulique et caféique) en vinyl-phénols et de les réduire ensuite en éthyl-phénols (4-éthyl-phénol, 4-éthyl-guaiacol et 4-éthyl-catéchol), responsables du caractère 'brett' [222]. Cette altération concerne particulièrement les vins rouges et cause d'importantes pertes économiques pour la filière viti-vinicole : si seulement 1% des vins sont définitivement rejetés à cause d'une déviation de ce type [223], un tiers des bouteilles sont potentiellement affectées par un défaut détectable à la dégustation [222], avec des inégalités géographiques importantes (certaines régions étant plus touchées que d'autres).

Au sein de l'UR Œnologie, mes travaux visent à approfondir nos connaissances de la diversité de ces levures, aussi bien au niveau génétique que phénotypique. Dans cette partie, je détaillerai plus spécifiquement des projets d'étude du genre *Saccharomyces*, de certaines levures non-conventionnelles et de quelques mécanismes moléculaires qui permettent de générer de la diversité chez ces microorganismes.

## DIVERSITE GENETIQUE ET PHENOTYPIQUE DES *SACCHAROMYCES* SP.

Malgré une utilisation presque séculaire de *Saccharomyces cerevisiae* comme organisme modèle en biologie moléculaire et génétique, l'étude de son écologie et l'histoire des populations en lien avec la sélection humaine étaient mal connues au démarrage du projet *Adaptalevure* (2006-2008). Recrutée comme post-doctorante au sein de l'UR Œnologie (responsable : Marina Bely), en collaboration avec l'UMR de Génétique Végétale du Moulon, nous souhaitions mettre en œuvre une méthodologie originale pour l'étude de la diversité d'une espèce domestiquée, en utilisant les levures utilisées par l'homme dans différents procédés alimentaires. L'objectif du projet était de préciser si les souches étaient adaptées à leur environnement d'origine, et si cette adaptation était visible à différents niveaux d'intégration cellulaire : génétique et phénotypique (dont protéomique).

Nos travaux montrent un lien étroit entre diversité génétique et origine industrielle des souches de *S. cerevisiae* [95], en accord avec de nombreuses études [224-226]. Nous avons analysé la diversité phénotypique de plusieurs souches dans des milieux mimant les conditions en œnologie, brasserie et boulangerie. La vitesse de fermentation des souches de levure industrielles est positivement corrélée à la taille maximale de la population [96]. Nous avons d'ailleurs montré par des approches de rétrocroisements que l'amélioration génétique de souches industrielles modifiait significativement la taille maximale de la population [151].

Concernant l'expression des protéines, nous mettons en évidence que le pool enzymatique dédié au protéome fermentaire est invariant (Fig. 4), quels que soient la souche et le milieu testés, suggérant de fortes contraintes évolutives [55]. Nous avons tiré parti de l'approche de protéomique quantitative pour différencier les modifications post-traductionnelles (MPT). La domestication a eu un impact significatif sur le protéome fermentaire, à travers la sélection (directe ou indirecte) de certaines MPT pour chaque origine alimentaire. Certaines MPT spécifiques sont d'ailleurs significativement corrélées à des caractères phénotypiques. Ces données mettent en évidence le rôle majeur des MPT dans le contrôle phénotypique [55], rarement étudié.

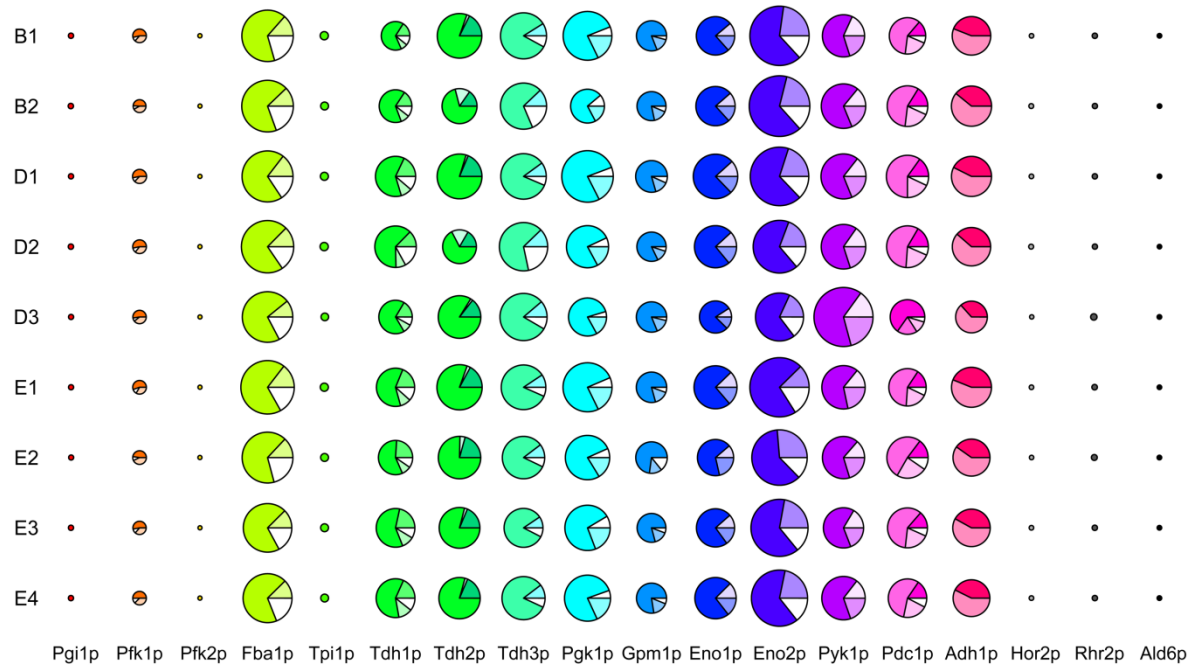



**FIGURE 5. DISTRIBUTION DE L'ABONDANCE DE 18 PROTEINES IMPLIQUEES DANS LA GLYCOLYSE ET LA FERMENTATION ALCOOLIQUE CHEZ 9 SOUCHES DE *S. CEREVISIAE*.** B1 ET B2 DESIGNENT DES SOUCHES DE BOULANGERIE, D1-D3 DES SOUCHES DE DISTILLERIE, E1-E4 DES SOUCHES D'ŒNOLOGIE. POUR CHAQUE PROTEINE, LES DIFFERENTS SECTEURS REPRESENTENT LES DIFFERENTES FORMES POST-TRADUCTIONNELLES.

Nous avons ensuite appliqué ces mêmes approches (génétique, protéomique, phénotypique) à une autre espèce du genre *Saccharomyces*, *S. uvarum*, la seule autre espèce capable de compléter la FA en conditions œnologiques, mais qui est associée à des températures de fermentation plus basses [227]. *S. cerevisiae* et *S. uvarum* présentent des niches écologiques partiellement recouvrantes : toutes deux sont naturellement associées à des environnements fermentaires, dont l'œnologie. Les souches appartenant à ces deux espèces présentent de la convergence phénotypique et il est impossible de les différencier sur la base de traits liés à la capacité fermentaire et à la dynamique des populations en conditions œnologiques [227]. Nous souhaitions savoir si le protéome reflétait cette convergence phénotypique, dans le cadre du projet *HeterosYeast*. Nos résultats montrent que *S. cerevisiae* et *S. uvarum* présentent des profils protéiques divergents, notamment pour certaines voies métaboliques dont la voie de la glycolyse et de la production d'éthanol. En particulier, la plupart des gènes dupliqués communs aux deux espèces présentent des profils d'expression opposés, indiquant un recrutement différentiel des différents paralogues (Fig. 5). Des fonctions similaires sont présentes chez ces deux espèces par le biais de l'expression de paralogues différents, ce qui pourrait expliquer le chevauchement partiel de leur niche écologique [103].

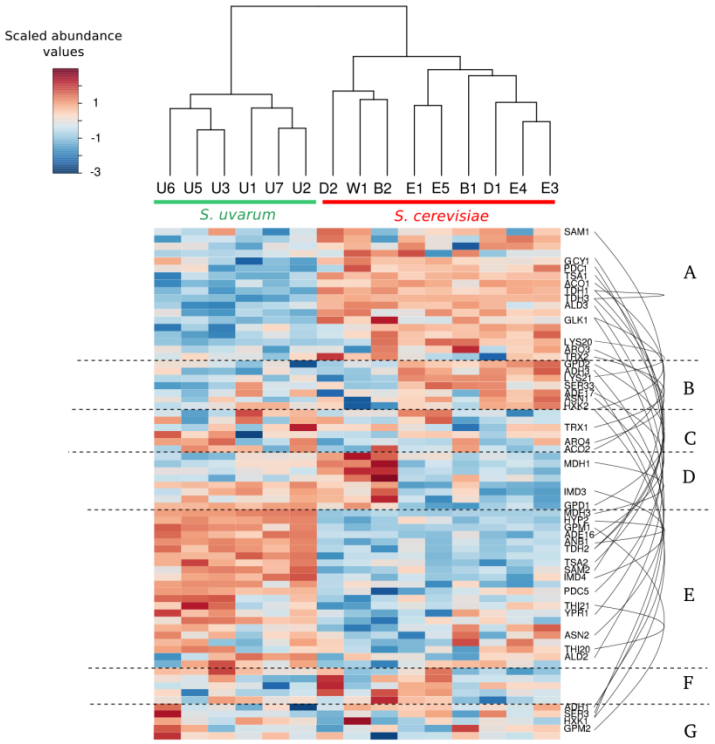



**FIGURE 6. HEATMAP DE L'ABONDANCE DES PROTEINES CHEZ 9 SOUCHES DE *S. CEREVISIAE* ET 6 SOUCHES DE *S. UVARUM*.** LES LETTRES (A-G) INDIQUENT LES 8 GROUPES DE PROTEINES DETECTES. SEULS LES GROUPES DE PARALOGUES SONT REPRESENTES ICI ET SONT RELIES LES UNS AUX AUTRES. U1-U7 DESIGNENT LES 6 SOUCHES DE *S. UVARUM* ; B1-B2, D1-D3, E1-E4 DESIGNENT DES SOUCHES DE *S. CEREVISIAE* DE BOULANGERIE, DISTILLERIE ET ŒNOLOGIE RESPECTIVEMENT [97].

Ces projets soulignent l'intérêt des approches multi-échelles, intégratives : appliquées à *S. cerevisiae*, elles montrent que les caractères métaboliques et de croissance peuvent être reliés au protéome. Appliquées à la comparaison d'espèces (*S. cerevisiae* vs. *S. uvarum*), elles soulignent que des scénarii d'évolution indépendants peuvent conduire à l'apparition ou au maintien de fonctions similaires.

Les approches multi-échelles permettent une meilleure compréhension du mode de fonctionnement des systèmes complexes pris dans leur ensemble. Au fil des projets, nous avons ainsi importé/développé de nouvelles approches afin de compléter l'éventail des outils déjà disponibles au laboratoire (voir Encadré 1) telles que la transcriptomique, la volatolomique ou la mesure des activités enzymatiques. Ces approches haut-débit nous permettent l'accès à des niveaux d'intégration cellulaire supplémentaires, complémentaires des niveaux protéomiques et génétiques qui étaient déjà accessibles en routine au laboratoire. La plupart de ces approches, bien que développées et/ou validées pour l'espèce modèle *S. cerevisiae*, sont aussi applicables à d'autres espèces de levure, dites 'non-conventionnelles'.

**ENCADRE 1 :** EXEMPLES DE DEPLOIEMENT/DEVELOPPEMENT DE NOUVELLES APPROCHES

Approche transcriptomique : nous avons utilisé une approche transcriptomique pour l'étude de souches de *S. cerevisiae* sauvage et mutante pour des gènes codant des estérases. En effet, des travaux récents ont montré que certains esters étaient impliqués, au moins en partie, dans l'expression de la perception fruitée des vins rouges [221, 228, 229]. Afin de préciser le rôle de ces esters, une souche de levure mutée pour 4 gènes codant des estérases a été construite. Cette souche présente de drastiques modifications de son transcriptome, y compris pour des processus biologiques inattendus (organisation de la chromatine, transcription, etc.) [13, 15, 16]. La comparaison des données transcriptomiques avec des données phénotypiques (quantification des esters, analyse sensorielle) est en cours de finalisation et fera l'objet d'une publication conjointe avec nos collègues de l'axe 'Qualité et identité du vin' (JC Barbe) mais également avec les industriels Pernod-Ricard et Biolaffort [13].

Développement de la mesure des activités enzymatiques chez la levure (projet MAEL): nous avons obtenu en 2016 un financement de la Fédération de Recherche Biologie Intégrative et Ecologie pour mettre au point la mesure d'activités enzymatiques d'intérêt, en collaboration avec l'UMR1332 (Yves Gibon et al.). Cette approche a été validée sur un petit nombre de souches [40], et reste à valoriser par une publication.

Quantification du *volatolome* : je participe au développement de méthodes haut-débit permettant la quantification de plusieurs centaines de composés volatils par échantillon, en collaboration avec mes collègues chimistes (Alexandre Pons, Philippe Darriet). L'un des objectifs clé de l'UR Œnologie est d'identifier les facteurs physico-chimiques, biochimiques et microbiologiques qui contribuent à la genèse des composés aromatiques, intrinsèquement liés à la qualité et à l'identité des vins. Les outils les plus récents de chromatographie en phase gazeuse (GC bidimensionnelle) permettent de générer plusieurs dizaines de milliers de spectres par échantillon, la limite de ces approches restant le traitement automatique des données. J'ai ainsi développé des algorithmes informatiques permettant d'automatiser la quantification des composés volatils. Ces outils ont été appliqués avec succès à l'étude d'un vin de merlot fermenté par deux souches de *S. cerevisiae* [133]. 676 composés ont été quantifiés de façon reproductible, dont 61 variaient significativement avec la souche de levure utilisée. Le dernier obstacle à surmonter est l'identification automatique des composés d'intérêt. Pour cela, nous projetons de créer une base de données interne, projet pour lequel j'ai obtenu le soutien financier de Bordeaux INP (gratification de stage M2 pour 2020, projet GCbase). Cette base de données spectrales sera construite à partir de nombreux échantillons de vins (rouges, blancs, issus de cépages variés, etc.) mais aussi d'autres boissons fermentées (bière, kombucha, cidre, etc.).

## DIVERSITE DES LEVURES NON-CONVENTIONNELLES

Depuis le début des années 2000, le marché des levures d'œnologie a connu une diversification importante avec l'arrivée de nombreuses préparations incluant des 'non-Sacch' (cf Tableau 1), seules ou en mélange. Ces préparations ne représentent qu'environ 1% des quantités de levures vendues, mais ce chiffre est en constante augmentation. Les avantages apportés par ces préparations varient d'une espèce à l'autre (parfois d'une souche à l'autre), mais parmi les bénéfices supposés l'on peut citer l'impact sur l'arôme des vins (*Pichia kluyveri* FROOTZEN, *Torulaspora delbrueckii* ZYMAFLORE Alpha TD ou Biodiva, etc.), la modification de l'acidité des vins (production d'acide lactique par *Lachancea* (syn. *Kluyveromyces*) *thermotolerans* CONCERTO ou LAKTIA, consommation d'acide malique par *Schizosaccharomyces pombe* ProMalic, etc.) ou encore des applications de type bio-contrôle (*Metschnikowia* sp. Excellence BIO-NATURE ou Gaïa, etc.). La bibliographie concernant ces espèces non-conventionnelles en œnologie a elle aussi connu un essor important sur cette même période, la plupart des publications proposant une étude des propriétés d'intérêt œnologique de quelques souches, généralement peu ou pas caractérisées génétiquement. Difficile, dans ces conditions, de savoir si les caractéristiques d'intérêt sont spécifiques de l'espèce, d'une sous-population, d'une souche, ou bien liées à l'environnement.

Afin d'étudier au mieux la diversité des levures non-conventionnelles, nous avons développé des outils de marquage moléculaire. Nous avons fait le choix de développer des marqueurs microsatellites, particulièrement puissants pour l'analyse des relations génétiques entre individus. Ce travail nécessite d'avoir à disposition la séquence d'un génome de référence pour identifier les microsatellites (à l'aide d'un algorithme développé au laboratoire). Pour certaines espèces (*T. delbrueckii*, *B. bruxellensis*, *L. thermotolerans*, *Schizo. pombe*), nous nous sommes appuyés sur la publication de génomes de référence [230-234], tandis que pour d'autres (*Starm. bacillaris*, *H. uvarum*, *M. pulcherrima* ou *Pichia kluyveri*), nous avons assemblé *de novo* des génomes avec l'appui de la plateforme de Génomique fonctionnelle de Bordeaux/Pierroton [88]. Pour certaines espèces, l'étude de la diversité a été couplée à l'étude de phénotypes d'intérêt œnologique. Nous avons également assemblé des collections aussi représentatives que possible de chaque espèce, en variant les origines géographiques, et les origines de substrat. Les collections assemblées comportent quelques dizaines de souches (eg *P. kluyveri*) à quelques milliers d'individus (*B. bruxellensis*). Ce lourd travail de collecte a été rendu possible grâce à la confiance de nos collaborateurs nationaux et internationaux qui ont accepté de nous confier leurs souches, parmi lesquels les universités de Dijon, Toulouse, Strasbourg, Brest, Turin, Foggia, Naples, Tarragone, Stellenbosch, Debrecen, Cordoba, Adélaïde, des équipes ou instituts de recherche dédiés au vin (SPO-INRA à Montpellier, AWRI en Australie, DEMETER en Grèce) ou encore des industriels (Biolaffort, Pernod-Ricard, Lesaffre, Lallemand, AEB).

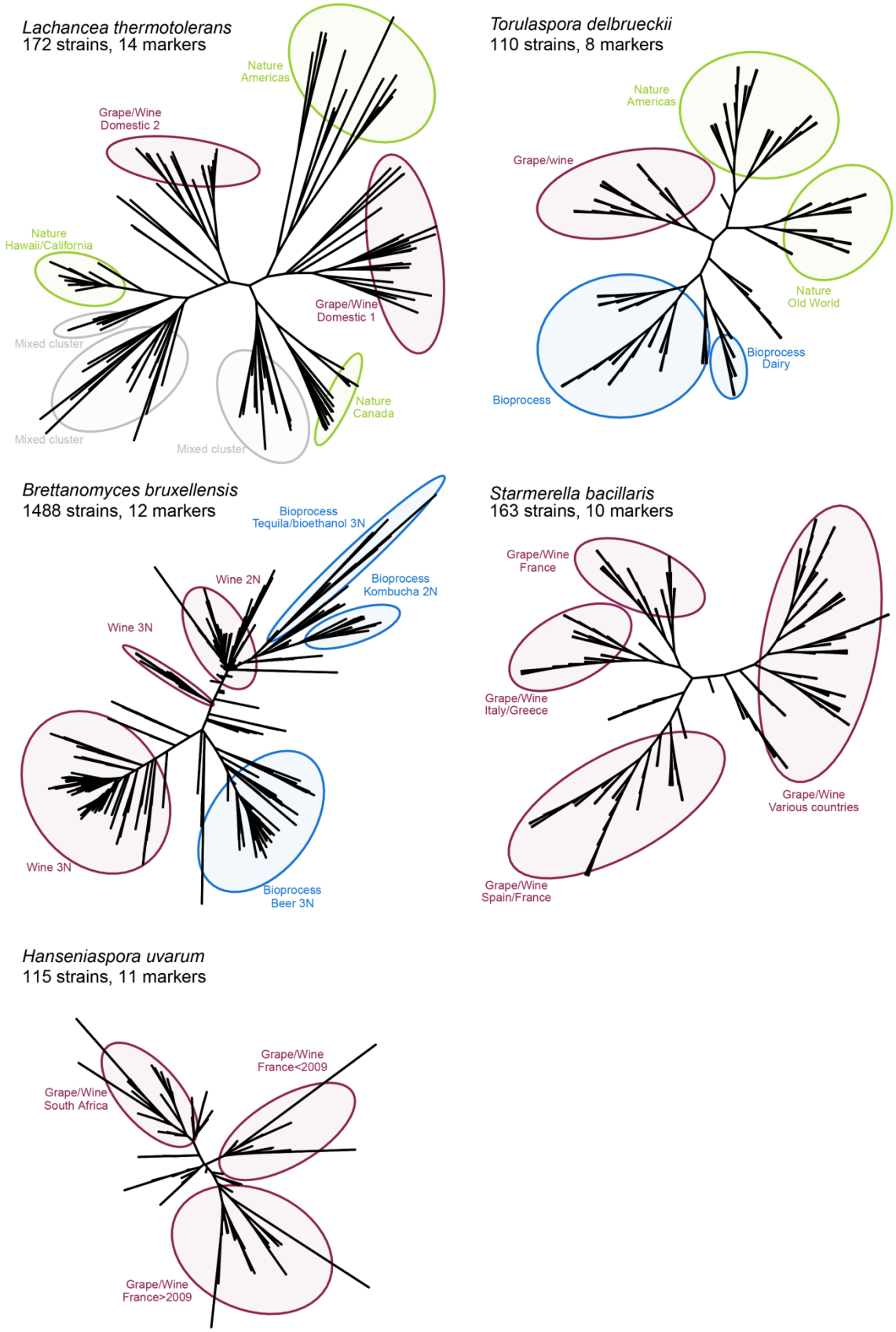


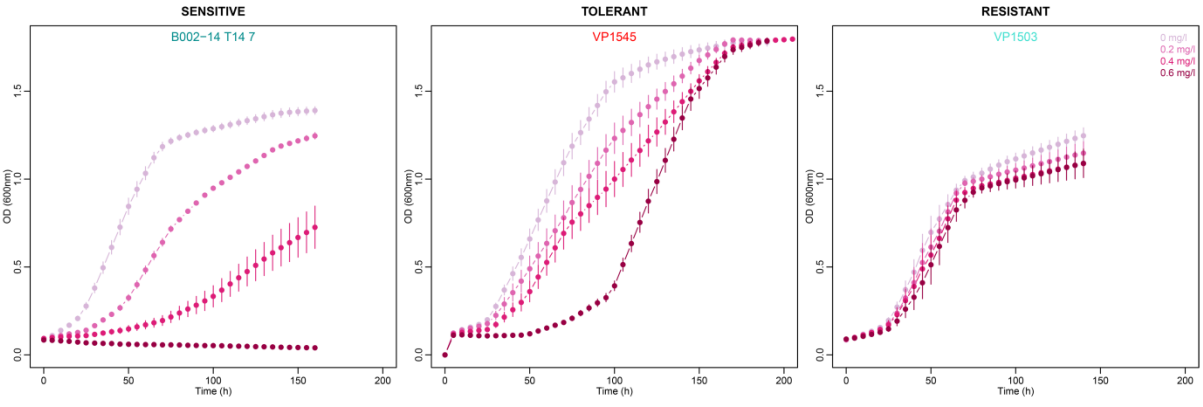

**FIGURE 7. ETUDE DE LA DIVERSITE GENETIQUE DE CINQ ESPECES DE LEVURE A L'AIDE DE MARQUEURS MICROSATELLITES.** D'APRES ALBERTIN ET AL., 2019 [202].

L'étude de la diversité des NS donne des résultats contrastés en fonction de l'espèce considérée (Fig. 7). Ainsi, pour *T. delbrueckii,* le génotypage de 110 souches montre que les activités humaines ont significativement modelé la diversité génétique de l'espèce, avec un groupe associés à la vigne/vin, deux groupes associés aux procédés alimentaires et deux groupes contenant essentiellement des souches isolées à partir d'environnements non-anthropisés ('Nature'), des continents américains et européens respectivement [38]. Cette différenciation génétique est associée à de la différenciation phénotypique : l'analyse des capacités fermentaires d'un petit nombre de souches (une 20aine) suggère que le groupe vigne/vin est capable de consommer significativement plus de sucre que les autres groupes – et ont donc une meilleure capacité fermentaire – en conditions œnologiques [78]. Ces résultats indiquent que, à l'instar de *S. cerevisiae*, l'espèce *T. delbrueckii* a été domestiquée, consciemment ou inconsciemment, par l'homme [38]. De la même façon, le génotypage de 172 souches de *L. thermotolerans* révèle l'existence de différentes sous-populations, certaines associées à la vigne ou au vin, d'autres à des environnements naturels, ainsi que des groupes mélangés [9]. Sur le plan phénotypique, 114 caractères liés à la capacité fermentaire, la croissance de la population ou la production/consommation de métabolites ont été mesurés pour 94 souches représentatives des différentes sous-populations. Nous montrons que certains phénotypes varient considérablement d'un groupe à l'autre : ainsi, la capacité à produire de l'acide lactique est d'environ 3g/L pour le groupe 'domestic 1', et 10g/L pour le groupe 'domestic 2'. Or, cette capacité à produire de l'acide lactique est l'une des propriétés d'intérêt de l'espèce pour l'œnologie : l'acidification qui en résulte permettrait une meilleure stabilité microbiologique des vins, tout en réduisant l'utilisation de certains intrants (e.g. l'acide tartrique, couramment utilisé pour abaisser le pH des vins produits dans des vignobles au climat chaud comme en Australie). Cette différenciation génétique et phénotypique en fonction du degré d'anthropisation des milieux suggère une adaptation aux activités humaines de *L. thermotolerans*, qui rejoint ainsi *S. cerevisiae* et *T. delbrueckii* sur la liste des espèces de levure domestiquées.

Quant aux espèces *H. uvarum* et *C. zemplinina*, majoritaires durant les phases préfermentaires, toutes les sous-populations identifiées sont associées à la vigne ou au vin, avec des facteurs de différenciation géographique [79, 91, 92], temporel pour *H. uvarum* ('effet millésime' [91]), et sans lien avec les pratiques œnologiques pour *C. zemplinina* [92]. Une des hypothèses pouvant expliquer l'absence de groupe 'sauvage' ou 'naturel' serait que ces espèces sont entièrement inféodées et adaptées à l'environnement vigne (et plus particulièrement à la baie de raisin), hypothèse qui reste à tester formellement.

La distribution génétique de la levure d'altération des vins *B. bruxellensis* est encore différente : on retrouve l'existence de six sous-populations associées à des procédés fermentaires (vin, bière, kombucha, tequila, bioéthanol), mais sans la présence de groupe

'sauvage' ou 'naturel' (pas d'isolats correspondants dans les différentes collections) [17]. En œnologie, le moyen de lutte le plus courant contre 'Brett' est l'ajout de sulfites. Nous montrons que deux sous-populations ('Wine 3N'), associées au vin, sont résistantes ou tolérantes aux sulfites [17, 18] (Fig. 7). Des expériences de compétition entre souches sensibles et résistantes confirment que les souches sensibles sont mieux adaptées aux environnements sans sulfite qu'elles colonisent rapidement au détriment des souches résistantes [19]. A l'inverse, en présence de sulfite, les souches sensibles disparaissent rapidement au profit des souches tolérantes. La production de phénols volatils (les composés responsables du défaut 'Brett') semble être une caractéristique générale de l'espèce : toutes les souches testées ont la capacité à produire des phénols volatils à des concentrations délétères pour le vin [137]. Certains clones (présentant des génotypes identiques) ont été isolés de façon répétée au cours des décennies dans plusieurs chais, indiquant une capacité de rémanence remarquable [3]. Compte-tenu de l'importance de cette espèce en œnologie (*B. bruxellensis* est la principale levure d'altération des vins), d'autres caractères phénotypiques sont en cours d'analyse. Des résultats préliminaires sur un petit nombre de souches montrent des capacités de formation de biofilm importantes, qui pourraient expliquer leur rémanence en chai sur de longues périodes en dépit des pratiques modernes de nettoyage/désinfection [1].




**B002-14 T14 7**

| mSO2 (mg/L) | lag phase (h) | max. growth rate (div/h) | ODmax (600nm) |
|---|---|---|---|
| **0** | 22.4 ± 3.2 | 0.09 ± 0.01 | 1.42 ± 0.04 |
| **0.2** | 39.7 ± 4.51 | 0.06 ± 0 | 1.27 ± 0.06 |
| **0.4** | 99.25 ± 36.59 | 0.02 ± 0 | 0.77 ± 0.33 |
| **0.6** | 173.37 ± 8.3 | 0.01 ± 0 | 0.09 ± 0.01 |

**VP1545**

| mSO2 (mg/L) | lag phase (h) | max. growth rate (div/h) | ODmax (600nm) |
|---|---|---|---|
| **0** | 36.91 ± 7.87 | 0.07 ± 0.01 | 1.82 ± 0.1 |
| **0.2** | 55.67 ± 12.6 | 0.06 ± 0.01 | 1.85 ± 0.04 |
| **0.4** | 63.43 ± 14 | 0.09 ± 0.02 | 1.83 ± 0.04 |
| **0.6** | 94.42 ± 11.62 | 0.06 ± 0.01 | 1.85 ± 0.08 |

**VP1503**

| mSO2 (mg/L) | lag phase (h) | max. growth rate (div/h) | ODmax (600nm) |
|---|---|---|---|
| **0** | 27.24 ± 3.46 | 0.08 ± 0.01 | 1.31 ± 0.08 |
| **0.2** | 30.59 ± 5.45 | 0.09 ± 0 | 1.19 ± 0.12 |
| **0.4** | 32.77 ± 6.68 | 0.09 ± 0 | 1.11 ± 0.12 |
| **0.6** | 36.44 ± 6.4 | 0.09 ± 0.01 | 1.12 ± 0.14 |

**FIGURE 8. CROISSANCE DE SOUCHES DE *B. BRUXELLENSIS* SENSIBLE (B002-14 T14 7), TOLERANTE (VP1545) ET RESISTANTE (VP1503) AUX SULFITES.** TRAVAUX D'AVRAMOVA ET AL., 2018 [18].

La plupart de ces résultats sont le fruit du travail de nombreux étudiants, que j'ai encadrés ou contribué à encadrer, parmi lesquels les travaux de thèse de Laura Chasseriaud (*T. delbrueckii*), Marta Avramova (*B. bruxellensis*), Ana Hranilovic (*L. thermotolerans*), mais

aussi les projets de stage d'étudiants en Master, comme Maura Magani et Emilien Peltier (*B. bruxellensis*), Sara Windholtz et Guillaume Comte (*T. delbrueckii*), ou Elodie Juquin (*Hanseniaspora* sp., *Pichia* sp., *Metschnikowia* sp.). Ces travaux sont encore en cours pour la plupart des espèces, à des degrés divers. En particulier, les marqueurs microsatellites développés pour les espèces *M. pulcherrima*, *P. kluyveri* ou *Schizosaccharomyces pombe* restent à publier.

## POLYPLOIDIE, HYBRIDATION ET INTROGRESSION : DES MECANISMES RECURRENTS CHEZ LES LEVURES

L'étude de la diversité génétique des espèces de levures fréquemment retrouvées en œnologie a mis en évidence l'adaptation de certaines populations à des environnements plus ou moins anthropisés, posant ainsi la question des mécanismes évolutifs sous-jacents. En particulier, les évènements de polyploidisation, d'hybridation et d'introgression, tous fortement imbriqués, semblent assez fréquents dans l'évolution des levures. Ainsi, l'étude d'un petit nombre de souches de *S. cerevisiae* utilisées en boulangerie a révélé l'existence de nombreux autotétraploïdes, présentant de surcroit un comportement tétrasomique à la méiose (appariement aléatoire des 4 chromosomes homologues avec formation de tétravalents) [95]. Ces résultats nous ont conduit à réévaluer l'occurrence de la polyploïdie chez les champignons en général et les levures en particulier, dans une revue bien citée par nos pairs [208]. Une approche de génomique comparative, appliquée à *S. uvarum* (Fig. 8), montre l'existence de multiples introgressions originaires de *S. eubayanus*, *S. kudriavzevii* ou encore *S. cerevisiae* [89]. Ces introgressions sont significativement plus fréquentes chez les isolats d'environnements anthropisés, et certaines semblent spécifiques d'un procédé en particulier (cidre ou vin) [106]. L'origine de ces introgressions n'est pas clairement établie, mais l'une des possibilités est qu'elles proviennent d'évènements d'hybridation inter-spécifique suivis de rétrocroisements.

Chez *B. bruxellensis*, les différentes sous-populations détectées à l'aide des marqueurs microsatellites se distinguent, en plus de leur association à des procédés spécifiques, par leur composition génomique plus ou moins complexe [17]: deux groupes de souches diploïdes ont été décrits, l'un associé au vin et l'autre au kombucha. Les quatre autres groupes comprendraient des souches allotriploïdes, possédant toutes un '*core genome*' diploïde ainsi qu'un génome haploïde supplémentaire provenant d'espèces distinctes et inconnues [234, 235]. Les mécanismes de formation de ces allotriploïdes ne sont pas connus, une possibilité est qu'ils dériveraient de l'hybridation inter-spécifique de gamètes non-réduits (diploïdes) et réduits (haploïdes). Cette hypothèse reste très spéculative, sachant que l'existence de reproduction sexuée pour *B. bruxellensis* n'est pas

démontrée, que les donneurs des génomes haploïdes surnuméraires restent à identifier et qu'au moins quatre évènements d'allotriploidisation indépendants auraient eu lieu.

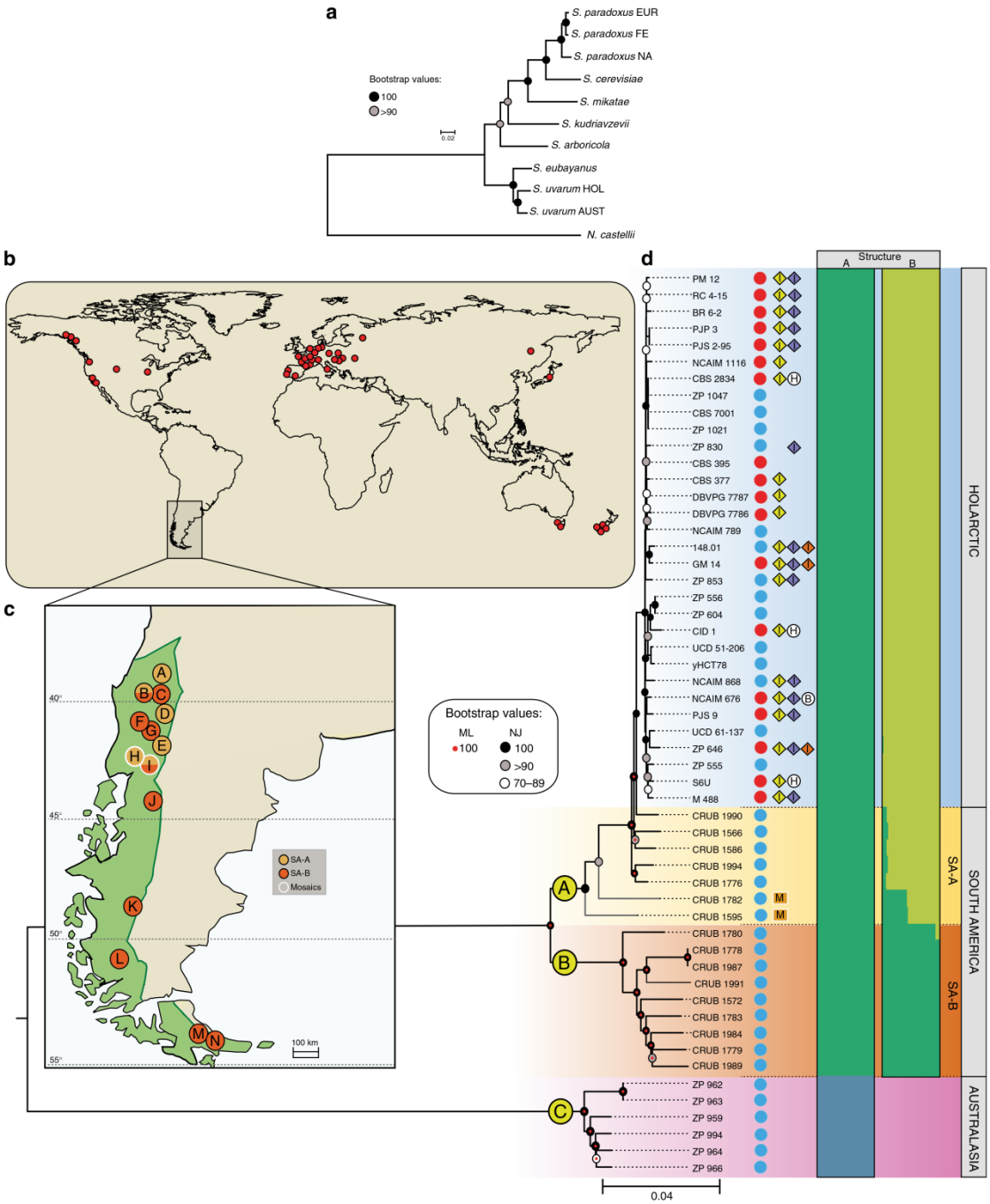



**FIGURE 9. DISTRIBUTION GEOGRAPHIQUE, PHYLOGENIE ET STRUCTURE DES POPULATIONS CHEZ *S. UVARUM*.** (A) PHYLOGENIE DU GENRE *SACCHAROMYCES*. (B) ET (C) ORIGINE GEOGRAPHIQUE DES 54 ISOLATS. (D) PHYLOGENIE DE 54 ISOLATS DE *S. UVARUM*.

La récurrence de ces phénomènes dans l'évolution des eucaryotes en général, et des levures en particulier, pose la question de l'origine de l'avantage apporté par l'hybridation. Le phénomène de supériorité des hybrides par rapport à ses parents porte le nom

d'hétérosis, ou vigueur hybride. Ce phénomène, très largement exploité pour l'amélioration des espèces d'intérêt agronomique, n'avait jamais été étudié dans un contexte œnologique. L'objectif du projet *HeterosYeast*, mené en collaboration avec l'UMR de Génétique Végétale du Moulon (Gif-sur-Yvette), l'unité de Mathématiques et Informatique Appliquées (INRA, Jouy-en-Josas) et l'industriel Biolaffort, était d'identifier des prédicteurs de vigueur hybride chez la levure. Pour se faire, des hybrides synthétiques ont été produits à partir de souches parentales homozygotes pures. Sept souches de *S. cerevisiae* et 4 souches de *S. uvarum* ont été utilisées pour la production des 55 hybrides intra- et interspécifiques possibles (dispositif diallèle). Les hybrides et leurs souches parentales ont été phénotypés en conditions œnologiques. Le suivi d'un grand nombre de fermentations (~400) a nécessité le développement d'outils bio-informatiques (scripts R) permettant leur analyse instantanée, utilisés depuis quotidiennement par les autres membres de l'équipe. Nos résultats indiquent que les hybrides interspécifiques présentent des caractéristiques intéressantes sur le plan biotechnologique : réduction du rendement en éthanol, augmentation de la production en esters éthyliques (impliqués dans la note fruitée des vins) [57]. Par ailleurs, les hybrides intra- et inter-spécifiques sont moins sensibles que leurs parents à des variations environnementales et présentent des caractéristiques phénotypiques plus constantes, un phénomène appelé homéostasie ou robustesse et que nous décrivons pour la première fois chez des microorganismes [57].

Au niveau protéomique, plus de 1300 protéines ont été quantifiées. Les hybrides interspécifiques se démarquent des hybrides intra-spécifiques et des souches parentales par l'existence d'un groupe d'environ 100 protéines présentant de l'hétérosis (*i.e.* protéines qui sont plus abondantes chez les hybrides interspécifiques que chez le meilleur de leurs parents) [56]. Une analyse *in silico* montre que l'hétérosis au niveau protéique est significativement corrélé au nombre de facteurs de transcription régulant l'expression des gènes correspondants, indiquant qu'un mécanisme sous-jacent de l'hétérosis serait lié à la complexité de la régulation transcriptionnelle [56].

Ce projet a généré un très gros jeu de données à différents niveaux d'organisation cellulaire (protéomique, caractères de fermentation et traits d'histoire de vie). L'analyse fine de ce jeu de données, surdimensionné (plus de variables – les protéines – que d'observations – les fermentations), a été poursuivie à l'aide d'approches mathématiques et statistiques par nos collaborateurs (UMR de Génétique Végétale du Moulon & équipe Mathématique et Informatique Appliquées (Jouy-en-Josas), et est toujours en cours de finalisation [236, 237].

Depuis mon arrivée au sein de l'UR Œnologie, mes travaux antérieurs ont donc été centrés sur l'analyse de la diversité génétique et phénotypique des levures du raisin et du

vin. Sur l'échelle allant de recherche fondamentale à recherche appliquée, la plupart des projets évoqués ci-dessus s'inscrivent plutôt dans la catégorie 'recherche fondamentale'. Cependant, la spécificité de l'Institut des Sciences de la Vigne et du Vin en général, et de l'UR Œnologie en particulier, reste ses liens très forts avec la filière vitivinicole et les praticiens. Ainsi, dès qu'une possibilité d'application émerge, elle est immédiatement transférée sur le terrain, soit par nos collaborateurs industriels, soit par le biais des cellules de transfert de technologie adossées à l'unité (notamment Microflora pour les innovations microbiologiques). Dans le paragraphe suivant, je m'attache à décrire quelques exemples concrets d'applications finalisées de mes travaux de recherche.

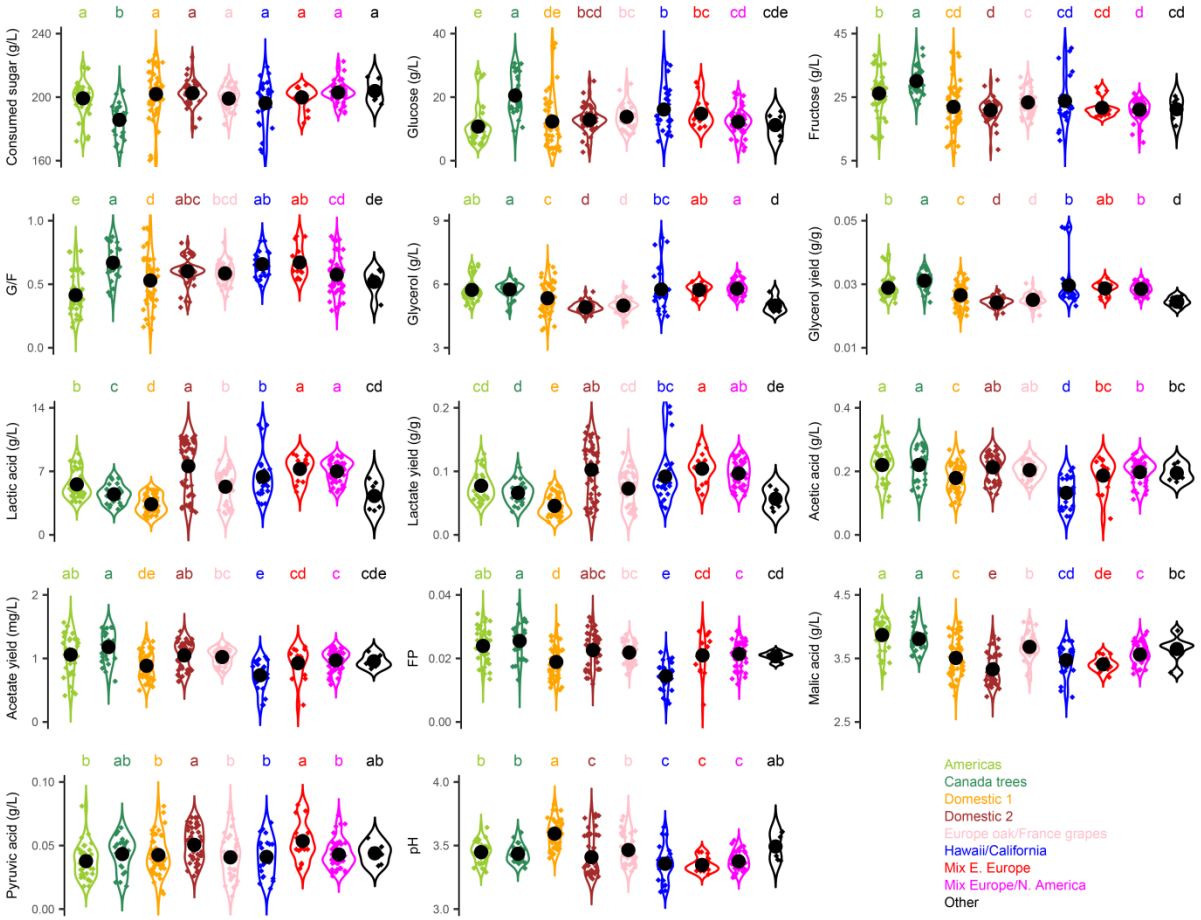



**FIGURE 10. PHENOTYPAGE DE 94 SOUCHES DE *LACHANCEA THERMOTOLERANS* APPARTENANT A 9 GROUPES GENETIQUES DIFFERENTS.** D'APRES HRANILOVIC ET AL., 2018 [8].

# II-C- ETUDIER LA DIVERSITE, QUELLES APPLICATIONS ?

## SELECTION DE SOUCHES POUR L'OENOLOGIE

Les travaux portant sur la diversité des levures peuvent conduire à de nombreux débouchés, l'exemple le plus évident est la sélection (et la commercialisation) de souches pour l'œnologie, à l'instar de *Lachancea thermotolerans*. L'étude de cette espèce a commencé par le génotypage d'une collection d'environ 170 isolats, puis par la caractérisation phénotypique de 94 souches en conditions œnologiques [8, 9]. Ce travail, initié en collaboration avec Vladimir Jiranek (Université d'Adélaïde), a permis l'accueil d'une étudiante en thèse pour 6 mois en 2016, Ana Hranilovic, que j'ai encadrée lors de son séjour en France. Ce travail se poursuit aujourd'hui avec le projet SoLt (Selection of *Lachancea thermotolerans*), financé par l'industriel Biolaffort, dont l'objectif est la sélection de souches de *L. thermotolerans* pour leur commercialisation. L'espèce est en effet décrite comme possédant plusieurs propriétés d'intérêt œnologique : capacité d'acidification des vins, impact organoleptique, mais aussi diminution du rendement en éthanol. En effet, l'une des conséquences associées au changement climatique concerne une teneur accrue en sucre des raisins, et donc une plus forte teneur en éthanol des vins. Or, les consommateurs (et les autorités de santé publique) plébiscitent plutôt des vins avec un degré alcoolique moindre. Pour atteindre cet objectif, une des stratégies consiste à utiliser des levures ayant un rendement de conversion sucre/alcool faible, propriété décrite chez *L. thermotolerans*. Ana Hranilovic, aujourd'hui post-doctorante (Université de Bordeaux et Adélaïde), poursuit le travail engagé en thèse. Ses travaux de phénotypage ont montré que les différentes sous-populations de *L. thermotolerans* présentaient une variabilité phénotypique parfois importante pour certains des caractères d'intérêt (e.g. production d'acide lactique allant de 1.8 à 12 g/L, Fig. 9). Etonnamment, certaines souches de *L. thermotolerans* commercialisées par des industriels concurrents de notre partenaire Biolaffort semblent relativement peu performantes pour les propriétés d'intérêt. Différents essais de vinifications ont été réalisés par Ana, incluant des moûts différents (rouge, blanc, provenant de France ou d'Australie). Deux souches particulièrement intéressantes ont été retenues et sont maintenant en cours de validation en conditions réelles, dans des chais partenaires.

Les autres objectifs de SoLt sont d'identifier les facteurs biotiques et abiotiques permettant de maitriser les propriétés œnologiques de l'espèce : identification de souches de *S. cerevisiae* compatibles avec *L. thermotolerans* pour la FA (*cf* III-B Interactions entre microorganismes), conditions d'inoculations (co-inoculation, inoculation séquentielle, ratio entre *S. cerevisiae* et *L. thermotolerans*) ; mais aussi de décrire les conséquences de son

l'utilisation : impact sur la conduite des fermentations malolactiques et les caractéristiques sensorielles des vins, etc.

Au-delà de la sélection *per se* de souches, l'étude de la diversité des espèces permet d'identifier de nouveaux critères d'intérêt pour les sélectionneurs. J'ai choisi de décrire brièvement deux exemples, portant sur l'hérédité mitochondriale et la phase de latence :

- Parmi les levures non-conventionnelles d'intérêt pour l'oenologie figurent les hybrides interspécifiques de *Saccharomyces*. Au cours de nos travaux sur les hybrides interspécifiques entre *S. cerevisiae* x *S. uvarum*, nous avons étudié l'impact de l'hérédité mitochondriale. Nous avons montré que les hybrides possédant le génome mitochondrial de *S. cerevisiae* ont une croissance plus rapide et une capacité respiratoire plus importante que les hybrides isogéniques correspondants (possédant le génome mitochondrial de *S. uvarum*) [54]. Des travaux menés en collaboration avec l'université de Valence (Espagne) montrent de plus que les hybrides possédant l'ADNm-*S.cerevisiae* sont plus tolérant au stress oxydatif et à la déshydratation [94]. A contrario, l'hérédité mitochondriale semble n'avoir aucun effet sur les paramètres de fermentation alcoolique dans les conditions testées [54]. Ce résultat est important dans l'optique d'une commercialisation des hybrides interspécifiques pour le marché œnologique : en effet, la production des levains sous forme de levures sèches actives se fait en condition respiratoire. A caractéristiques fermentaires équivalentes, il est donc plus pertinent de sélectionner des hybrides interspécifiques ADNm-*S.cerevisiae*, possédant une capacité de production accrue. Ces travaux nous ont donc permis d'identifier l'hérédité mitochondriale comme un paramètre clé pour la sélection de levures œnologiques d'origine hybride.
- La phase de latence correspond au temps nécessaire pour qu'un microorganisme s'adapte physiologiquement à son milieu et débute sa croissance. En œnologie, pendant les phases préfermentaires, une communauté de levures complexe est présente, comprenant des levures *Saccharomyces* sp. et non-*Saccharomyces*. Dans ce contexte, la durée de la phase de latence apparait comme un paramètre clé de la compétition entre espèces de levure, avec le raisonnement qu'une souche ayant une phase de latence courte devrait s'implanter plus rapidement dans le milieu au détriment des autres. Une approche de détection de QTL a été menée par Philippe Marullo pour identifier les bases génétiques de la phase de latence chez *S. cerevisiae*. Plusieurs translocations ont été identifiées comme impliquées dans la durée de la phase de latence, notamment en présence de sulfite [238]. La construction de souches de *S. cerevisiae* isogéniques, présentant une phase de latence longue ou courte, a permis de vérifier que, lors de la reconstruction de communauté de levures complexe, l'utilisation de *S. cerevisiae* avec une phase de latence longue autorisait le développement des levures non-*Saccharomyces* [113]. De façon inattendue, nous avons mis en évidence que l'allongement

de la phase de latence était, à elle seule, associée à une plus grande complexité des vins produits (sans intervention d'autres espèces). Les mécanismes sous-jacents restent inconnus, mais une phase de latence longue pourrait être un nouveau critère de sélection des souches de *S. cerevisiae*.

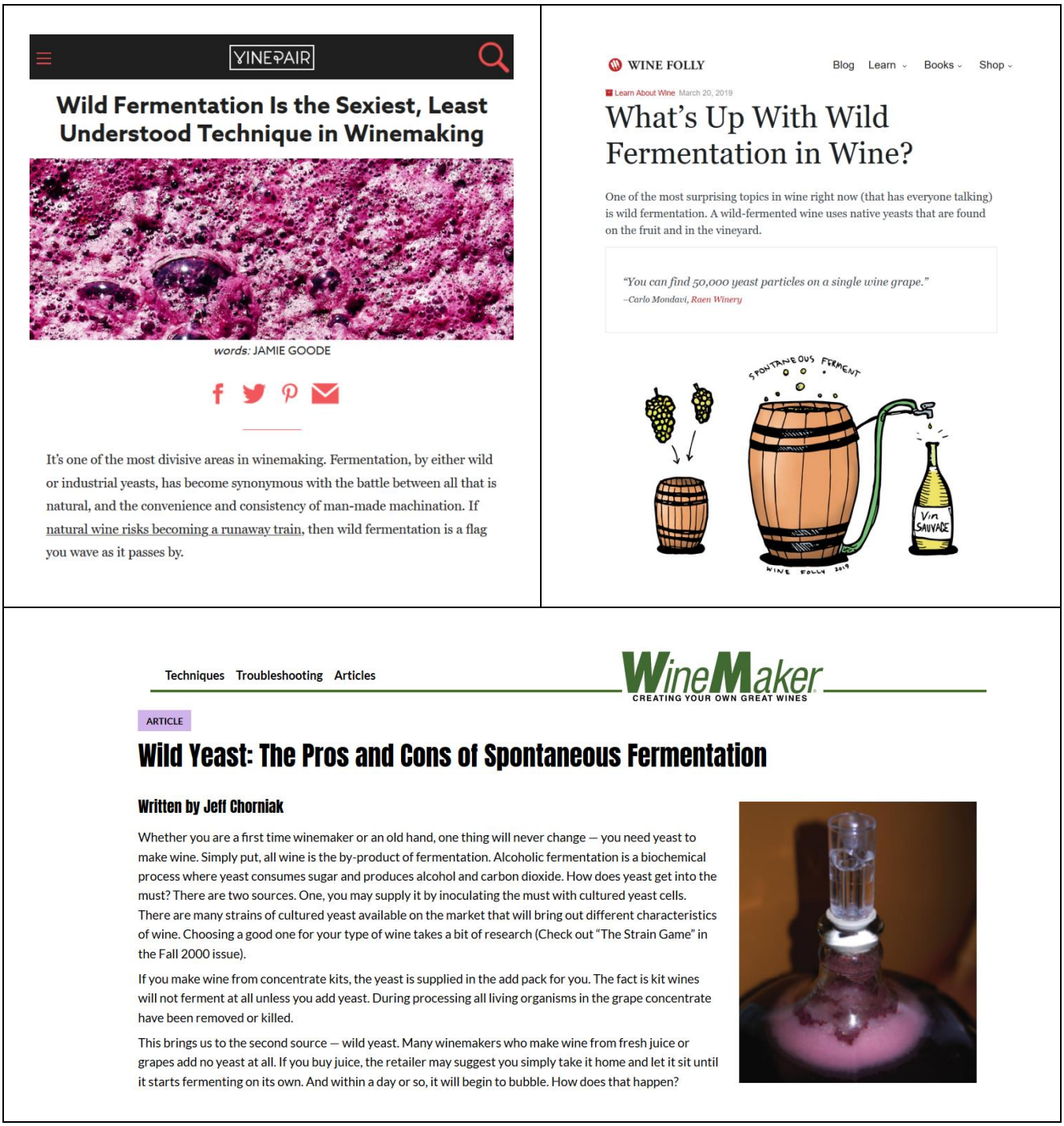



**FIGURE 11. REVUE DE PRESSE EN LIGNE SUR LA THEMATIQUE 'WILD FERMENTATION'.** HTTPS://VINEPAIR.COM (02/2018) ; HTTPS://WINEFOLLY.COM (03/2019) ; HTTPS://WINEMAKERMAG.COM (09/2005).

## EVALUER (ET FAIRE EVOLUER ?) LES PRATIQUES OENOLOGIQUES

La production de vin de qualité nécessite de nombreuses interventions humaines, regroupées sous le terme de 'pratiques œnologiques'. Ainsi, le code international des pratiques œnologiques de 2019, publié par l'OIV (Organisation Internationale de la Vigne et du Vin), recense plusieurs centaines d'interventions, procédures ou traitements tels que l'éraflage des grappes de raisin, le levurage, l'addition de sulfites, le collage des vins, etc. L'incidence de ces pratiques sur les différentes populations de levures n'est pas toujours bien décrite, en particulier en ce qui concerne les espèces indigènes, naturellement présentes dans les moûts. L'objectif du projet *Preferment* – piloté par Isabelle Masneuf-Pomarède et en collaboration avec les industriels Biolaffort et Pernod-Ricard – était donc d'évaluer l'impact de certaines pratiques lors des phases préfermentaires. Des microvinifications ont été réalisées en moût de Chardonnay, afin de suivre la dynamique des différentes populations de levure par PCR quantitative. L'incidence de facteurs externes, liés aux pratiques œnologiques (levurage, sulfitage, degré de clarification, température de pré-fermentation) a été testée. Nos résultats indiquent que les espèces naturellement présentent dans les moûts réagissent différemment vis-à-vis des facteurs abiotiques : par exemple, le développement de *T. delbrueckii* varie suivant le degré de clarification et la température, mais n'est pas modifié par le sulfitage [112]. A l'inverse, le développement d'*Hanseniaspora sp.* est plus important en l'absence de sulfitage, et associé à une plus grande production d'acide acétique.

L'utilisation et la valorisation des levures indigènes est une tendance en pleine progression ces dernières années chez certains vinificateurs (Fig. 11). Les bénéfices supposés restent discutés, les détracteurs des 'wild yeast' y voient plus un argument marketing dans l'air du temps qu'une pratique ayant un réel impact sur la qualité et la typicité des vins ou sur la durabilité des pratiques. Un des rôles de l'ISVV est d'enrichir le débat avec des données expérimentales, qui doivent être communiquées aussi bien à la communauté scientifique qu'à la profession vitivinicole. Ainsi, un certain nombre de nos résultats sont valorisés, en plus des habituelles publication scientifiques, par des communications techniques, lors de congrès/conférences à destination des praticiens [118, 136]. Nos collaborateurs industriels s'appuient d'ailleurs en partie sur ces travaux pour leur communication [239].

Parmi les pratiques œnologiques fréquentes, mais dont l'emploi est débattu, on retrouve le sulfitage, sujet d'inquiétude pour les consommateurs. La réglementation européenne N° 1169/2011 a fixé quatorze allergènes à déclaration obligatoire, dont les

sulfites (en concentrations supérieures à 10 mg/litre). En œnologie, les sulfites sont utilisés pour leurs propriétés antioxydantes, anti-oxydasiques (i.e. contre l'oxydation enzymatique) et antiseptiques. Du point de vue du microbiologiste, c'est cette dernière propriété qui est recherchée, et qui permet, en théorie, de lutter contre la flore d'altération des vins (comme *B. bruxellensis* ou les bactéries acétiques par exemple). Lors de nos travaux sur l'espèce *B. bruxellensis*, nous avons mis en évidence des comportements contrastés des différentes sous populations vis-à-vis de la présence de sulfite : les souches diploïdes sont sensibles aux sulfites, tandis que deux des groupes triploïdes, essentiellement associés au vin (groupes 'rouge' et 'turquoise') sont tolérants ou résistants aux sulfites aux doses habituellement recommandées dans la pratique [18]. Des auteurs avaient déjà relevé l'existence de souches de *B. bruxellensis* résistantes/tolérantes [240, 241], sans toutefois préciser leur fréquence. Nous montrons que 35% des isolats de *B. bruxellensis* sont résistants ou tolérants au sulfite (45% si l'on considère seulement les isolats d'œnologie). Par ailleurs, les groupes résistants/tolérants aux sulfites sont présents dans quasiment toutes les régions viticoles, en proportions variables (Fig. 12). Ces travaux ont une portée importante pour la profession vitivinicole, et ont fait l'objet de plusieurs communications techniques à destination des praticiens [29, 33, 53, 242, 243]. Un test moléculaire, nommé TYP\Brett, permettant de déterminer le groupe génétique et donc de prédire la sensibilité aux sulfites, a été développé. Un titre de protection industriel a été déposé au niveau national en 2015 suivi d'une extension internationale en 2017 [80]. Les étapes de maturation du test ont été conduites avec Aquitaine Science Transfert (la SATT – Société d'Accélération du Transfert de Technologies – de la région Nouvelle Aquitaine). Ce test a, dans un premier temps, été commercialisé par Microflora, la cellule de transfert de l'unité, dans le courant de l'année 2018. Depuis décembre 2018, c'est le laboratoire d'analyse œnologique Excell/Sarco qui exploite commercialement la licence du TYP\Brett et qui poursuit son développement technique. Ce test permet au praticien d'adapter ses pratiques de lutte contre Brett, de ne sulfiter que si le traitement peut être efficace, et de se tourner vers des moyens de lutte alternatifs (filtration, autres traitements, etc.).

Parmi les traitements alternatifs disponibles, l'OIV a récemment autorisé l'utilisation dans le vin de chitosane fongique (OIV-OENO 338A/2009) pour ses propriétés antimicrobiennes (entre autres) mais dont l'efficacité est débattue. Le projet Chitowine, porté par Marguerite Dols-Lafargue et démarré en 2018, vise à préciser les modes d'actions du chitosane, son efficacité dans différentes matrices, et ce, pour un grand nombre de souches et d'espèces de levures (dont Brett) et de bactéries.

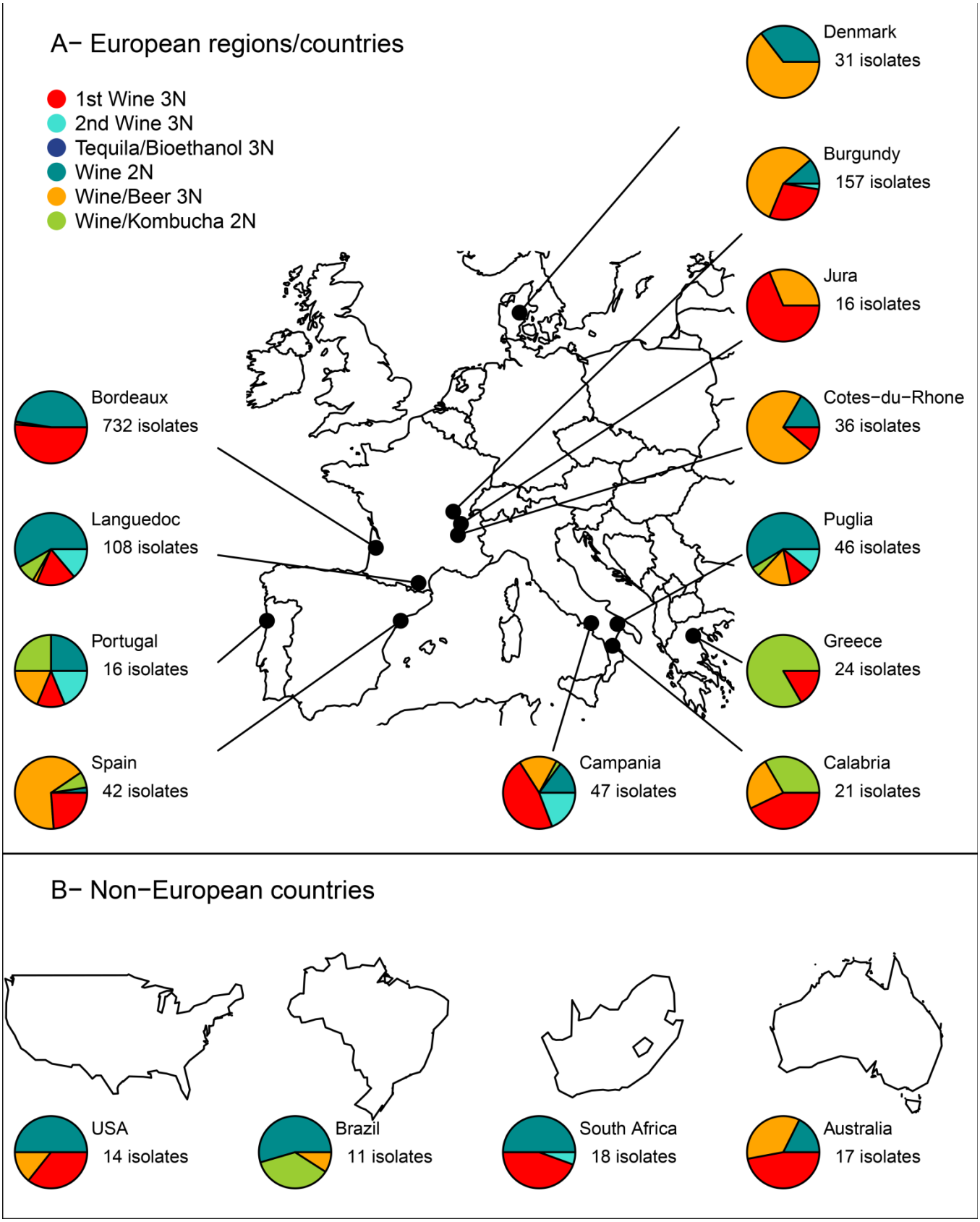



**FIGURE 12. DISTRIBUTION GEOGRAPHIQUE DE 1411 ISOLATS DU VIN DE B. BRUXELLENSIS DANS 6 GROUPES GENETIQUES.** D'APRES CIBRARIO ET AL., SOUMIS [3].

Etudier la diversité des levures du vin et du raisin trouve donc plusieurs applications très finalisées pour la filière vitivinicole. Certaines applications sont commercialisées ou commercialisables (i.e. la sélection de souches ou le développement de marqueurs moléculaires), tandis que d'autres sont immatérielles et se classent dans la catégorie générale de l'amélioration des connaissances. Ces connaissances nourrissent certains débats, l'œnologie – comme toutes les disciplines agricoles – étant riche en courants de pensée et sensible aux effets de mode. Nos travaux nous conduisent parfois à (tenter de) lutter contre certaines idées reçues ou conclusions hâtives. Ainsi, en 2018, nous avons accepté l'invitation de l'éditeur du journal *Applied Microbiology and Biotechnology* qui nous proposait de publier une mini-revue intitulée « *The complexity of wine: clarifying the role of microorganisms* ». La complexité d'un vin, que Denis Dubourdieu (fondateur et directeur de l'ISVV de 2009 à 2016) définissait comme « l'antidote de l'ennui » [244], est un attribut recherché par les amateurs du vin comme par les professionnels. L'objectif de cette mini-revue était de rappeler que, en dépit des raccourcis fréquents dans la littérature (y compris scientifique), la complexité d'un vin n'est ni liée à sa complexité chimique, ni à sa complexité microbiologique. Cette revue, écrite en commun avec nos collègues chimistes (Sophie Tempère, Axel Marchal, Jean-Christophe Barbe), a reçu un bon accueil de la communauté scientifique.

Mes travaux de recherche ont donc permis d'améliorer nos connaissances de la diversité génétique et phénotypique des levures du raisin et du vin, que ces espèces soient considérées comme flores positives ou d'altération. Au gré des projets, de nouveaux outils ou approches ont été développés, dont certains sont aujourd'hui implantés en routine au laboratoire. Ces connaissances ont été transférées vers la communauté scientifique (≈ 40 articles avec *peer review*, ≈ 90 communications orales ou affichés dans des congrès) ou vers la profession (≈ 15 publications et communications techniques). Certains de ces travaux se sont traduits par des applications concrètes pour la filière vitivinicole (test moléculaire TYP\brett). Dans la partie suivante, je vais détailler certaines pistes de recherche que je souhaite continuer (ou commencer) à explorer.

# III- PROJETS DE RECHERCHE

*On n'exécute pas tout ce qui se propose,*
*Et le chemin est long du projet à la chose.*

*Tartuffe, Molière.*

Mes travaux antérieurs ont permis de répondre à certaines questions concernant la diversité des levures, tout en soulevant de nombreuses autres. Sur le plan scientifique, les perspectives sont généralement multiples, mais les conditions bassement matérielles (*i.e.* la recherche de financement) permettent généralement de faire sans difficulté le tri dans les poursuites de projet... J'ai choisi de découper ce chapitre 'projets' en trois grands thèmes. Comme pour la présentation de mes travaux antérieurs, ce découpage est un peu artificiel tant la plupart des projets que nous menons sont imbriqués les uns dans les autres, avec des questions de recherche, des modèles biologiques, des approches et des outils qui se chevauchent. Le degré de réflexion de ces différents thèmes est plus ou moins abouti, il en va de même pour leurs financements.

Au fil des projets passés, l'importance de certains facteurs a été étudiée de façon récurrente : le facteur souche (= diversité intra-spécifique), le facteur espèce (= diversité inter-spécifique) ou encore le facteur environnement. Ces facteurs ont généralement été pris en compte individuellement. La suite logique est donc de s'intéresser aux interactions possibles entre ces différents facteurs, parmi lesquelles les interactions « Levure x Matrice » et les interactions entre microorganismes du vin. Les interactions « Levures x Matrice » ont déjà été un peu explorées lors du projet *Adaptalevure* chez *S. cerevisiae*, mais avec un petit nombre de souches. Mon souhait est d'aller plus loin, et d'explorer ces interactions chez l'espèce *B. bruxellensis*, naturellement associée à différentes matrices alimentaires (vin et bière notamment). De la même façon, si certains projets antérieurs ont permis d'initier l'étude des interactions entre microorganismes du vin, de très nombreuses perspectives sont possibles pour explorer ces phénomènes plus en profondeur, j'en décrirai quelques-unes. Enfin, certains projets sont nourris par des frustrations, la frustration de rester à un niveau d'étude très descriptif et pas assez fonctionnel, faute de temps, faute de moyens financiers, parfois faute d'approches adaptées. C'est l'un des inconvénients de la recherche dite appliquée, qui se traduit parfois par des études un peu superficielles. Le troisième thème de recherche que je vais donc évoquer portera sur la poursuite de l'étude de phénotype d'intérêt pour l'oenologie, mais à un niveau d'analyse plus moléculaire.

# III-A- INTERACTIONS LEVURE X MATRICE

Alors que l'espèce *B. bruxellensis* représente l'un des contaminants majeurs en œnologie, elle est paradoxalement utilisée comme auxiliaire technologique dans certains autres procédés fermentaires (production de bière, ou de kombucha). Toutefois, la maitrise de ces procédés reste encore aléatoire, et l'apparition de certains défauts (production d'acide acétique, etc.) est fréquente avec Brett.

Cette dualité (contaminant ou levain) est très atypique et pose la question des mécanismes et facteurs à l'origine de l'adaptation de l'espèce à ces différents procédés. Le projet BRETTADAPT, financé par l'ANR (AAPG2018, démarré au 1er janvier 2019) se propose donc d'identifier les facteurs génétiques, environnementaux et abiotiques sous-tendant l'adaptation de *B. bruxellensis* aux procédés fermentaires. Pour ce faire, une approche multi-échelle est envisagée, incluant génomique comparative et phénotypage à large échelle, en collaboration avec l'équipe GMGM (UMR 7156 Génétique Moléculaire, Génomique et Microbiologie, Université de Strasbourg).

Au niveau génomique, environ 200 souches représentatives de la diversité de l'espèce seront séquencées *de novo*, en combinant les techniques Illumina et MinION (génération de short-reads et long-reads respectivement). Une approche de génomique comparative sera menée, afin d'élucider les points clés de l'histoire évolutive de l'espèce. Ce travail sera piloté par l'équipe GMGM dirigée par Joseph Schacherer, qui a récemment publié le séquençage complet de 1011 génomes de *S. cerevisiae* [226]. Notre unité aura la charge de la caractérisation phénotypique de ces mêmes souches dans différents milieux : moût de raisin, moût de brasserie, vin et bière. L'utilisation de milieux correspondants à des produits déjà fermentés (bière, vin) et aux moûts correspondants permettra d'étudier le comportement de *B. bruxellensis* en tant que 1er et 2nd 'fermenteur'. Des bioréacteurs miniaturisés (12ml) seront utilisés, associés à des dosages robotisés de métabolites d'intérêt. Des caractères clés pour l'adaptation seront mesurés (viabilité, croissance des populations, etc.), mais aussi des caractères d'intérêt technologiques (capacité de bioadhésion, d'invasion, production de composés comme les phénols volatils ou l'acide acétique, etc.). Des approches de protéomique et de métabolomique (ciblée sur les composés volatils, impliqués dans l'arôme des aliments) seront également mises en œuvre. Une thèse, dont je suis la directrice, a démarré en ce sens en octobre 2019 (doctorant : Jules Harrouard). Ces travaux devraient permettre d'identification de signatures génomique et phénotypique de l'adaptation à ces différents milieux. Ces signatures seront validées ou invalidées sur le plan fonctionnel par l'analyse de mutants et/ou des expériences de compétition entre souches. Enfin, le projet BrettAdapt comprend un volet plus exploratoire,

qui vise à décrire l'écosystème microbien associé au développement de Brett, que ce soit en œnologie ou en brasserie. Ce travail devrait permettre de préciser les facteurs biotiques impliqués dans l'émergence de *B. bruxellensis*. Les retombées attendues de ce projet sont nombreuses : production de larges jeux de données pour la communauté scientifique, identification des facteurs génétiques, environnementaux et biotiques sous-tendant l'adaptation de *B. bruxellensis* aux procédés fermentaires. Plus généralement, sur le plan fondamental, ce travail nous permettra une meilleure connaissance d'une espèce de microorganisme anthropisée. Enfin, sur le plan appliqué, l'objectif final est de mieux maitriser les contaminations par *B. bruxellensis* en œnologie, et d'optimiser les nouveaux procédés fermentaires (nouveaux 'types' de bière, kombucha) utilisant cette espèce.

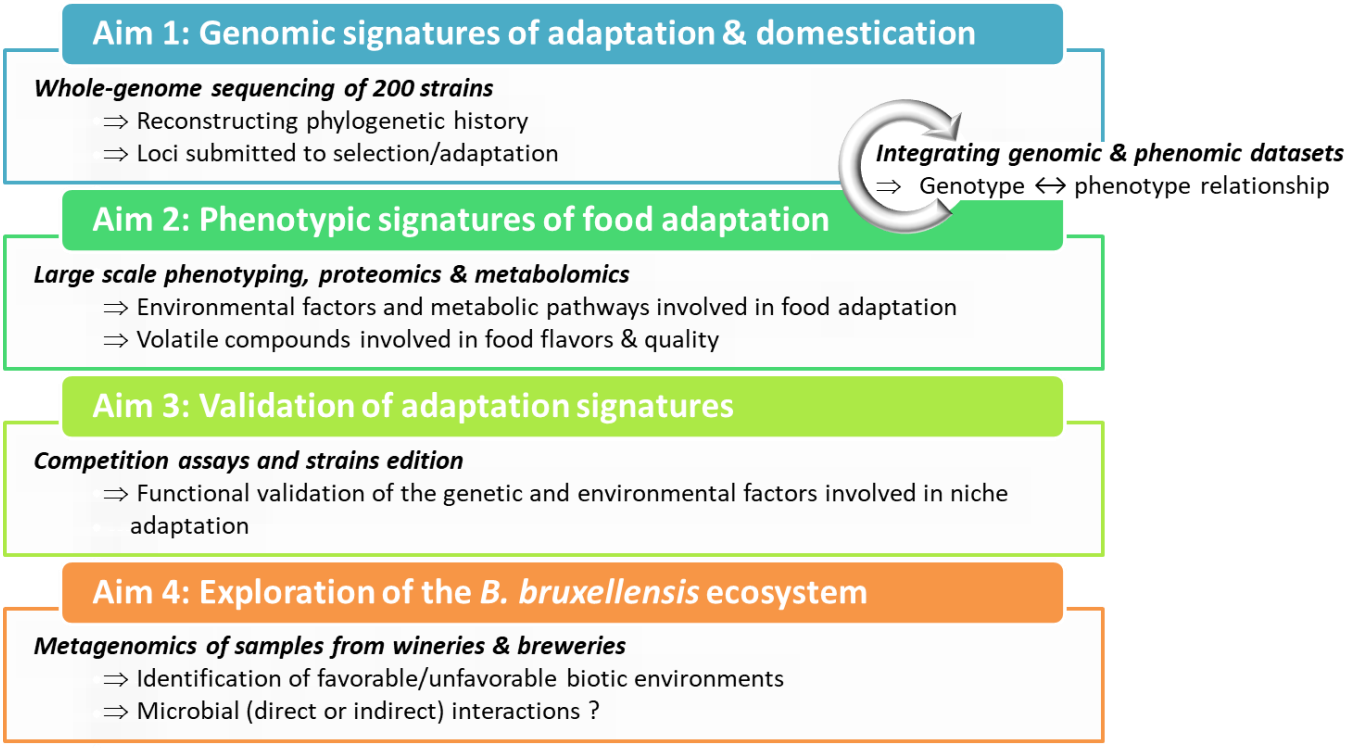



**FIGURE 13. RESUME DU PROJET ANR BRETTADAPT (2019-2022).** DEUX PARTENAIRES ACADEMIQUES SONT IMPLIQUES, L'UR ŒNOLOGIE ET L'EQUIPE GMGM DE UNIVERSITE DE STRABOURG.

# III-B- INTERACTIONS ENTRE MICROORGANISMES

Dans la plupart des procédés biotechnologiques, plusieurs espèces de microorganisme cohabitent dans le même environnement, et peuvent interagir entre elles. C'est le cas en œnologie, où de nombreuses espèces de microorganismes coexistent et se succèdent. Le vinificateur, pour maîtriser la qualité des vins, doit donc contrôler ces différents microorganismes. Or, les interactions entre les espèces microbiennes du vin sont mal décrites, mais leur impact potentiel est conséquent : la combinaison de certains microorganismes peut avoir des effets synergiques sur les populations, tandis que d'autres combinaisons entrainent la mort précoce de certaines espèces [157, 245, 246]. Ces effets synergiques peuvent être technologiquement intéressants si les espèces considérés sont œnologiquement 'positives' (par exemple dans le cas d'inoculations mixtes *Saccharomyces*/NS), mais catastrophiques s'ils participent à l'implantation ou au développement d'une espèce d'altération des vins. Quant aux interactions dites 'négatives' sur le plan des populations (*eg* impactant négativement le développement d'une des parties), elles constituent un formidable levier de lutte contre ces mêmes organismes d'altération, et peuvent permettre la mise en place de stratégie de biocontrôle. Dans les paragraphes suivants, je décrirai quelques projets visant à mieux décrire les phénomènes d'interaction entre microorganismes et à identifier les mécanismes moléculaires sous-jacents.

## INTERACTIONS ENTRE MICROORGANISMES DE TYPE *CELL-TO-CELL CONTACT*

Parmi les mécanismes d'interactions entre microorganismes décrits en œnologie, le phénomène de *cell-to-cell contact* (impliquant des contacts physiques entre cellules) pourrait jouer un rôle clé [157, 247]. Ce phénomène reste toutefois très difficile à caractériser à l'aide du matériel habituellement disponible en laboratoire, où les populations de microorganismes sont mélangées et donc en contact. Un outil unique, un fermenteur à double compartiment, permettant la croissance de deux populations de microorganisme en mélange ou séparées (c.a.d avec ou sans contact cellulaire) a été développé au laboratoire, sous la direction de Marina Bely. Les deux compartiments sont séparés par une membrane et le milieu est homogénéisé par une pompe péristaltique pilotée par ordinateur. Ce système a été appliqué avec succès à deux espèces de levure, *S. cerevisiae* et *T. delbrueckii* [157, 248], et a fait l'objet de la thèse de Laura Chasseriaud,

que j'ai participé à encadrer. Une approche protéomique a été appliquée afin d'identifier les mécanismes moléculaires sous-jacents. L'analyse a révélé que l'abondance de certaines protéines varie, avec ou sans contact cellulaire, et ce pour les deux espèces [114]. Cette approche a permis de dégager plusieurs voies de signalisation cellulaire qui pourraient être impliquées dans les interactions de type *'cell-to-cell' contact*, comme par exemple la production de ROS (Reactive Oxygene Species) ou l'implication dans l'équilibre redox. Des analyses complémentaires, réalisées lors du stage de M2 de Lydia Jimenez Gadea (2017) ont permis de valider l'existence de compétition entre espèces pour l'oxygène dissout dans le milieu fermentaire, et de confirmer des contenus en ROS différents entre espèce en présence/absence de contact cellulaire. L'ensemble de ces résultats fait l'objet d'une publication en cours de rédaction [114].

Ce phénomène de *cell-to-cell contact* a été décrit en œnologie pour plusieurs combinaisons de souches impliquant des espèces différentes, comme *S. cerevisiae* x *L. thermotolerans* [245, 247], *S. cerevisiae* x *Starm. bacillaris* [249], *S. cerevisiae* x *S. kudriavzevii* [250]. L'utilisation de plus en plus fréquente d'espèces non conventionnelles en co-inoculation avec *S. cerevisiae* nécessite d'identifier des combinaisons de souches compatibles, et la compréhension des mécanismes moléculaires sous-jacents représente un enjeu important pour la commercialisation des levures d'œnologie. Un travail d'envergure (non financé pour l'heure) pourrait être entrepris, impliquant la caractérisation du phénomène pour de nombreuses espèces, de nombreuses souches par espèces, mais aussi pour des matrices différentes (moûts blancs, rouges, liquoreux par exemple). Le préalable à ce travail est le développement de réacteurs miniaturisés, le fermenteur à double compartiment que nous avons développé n'existe qu'en deux exemplaires et nécessite de grande quantité de milieu (2.4L). Le suivi précis de la consommation en oxygène et du stress oxydant des différentes populations pourraient permettre de préciser l'implication de ces phénomènes. Une approche métabolique non ciblée pourrait également permettre d'identifier les composés impliqués dans le phénomène de *cell-to-cell contact*.

### BRETT&CIE : INTERACTIONS ET IMPACT DES PRATIQUES ŒNOLOGIQUES

Malgré l'accumulation des connaissances sur *B. bruxellensis*, plusieurs phénomènes observés empiriquement restent mal compris et peu étudiés. De nombreux auteurs et praticiens constatent que les 'Brett' se développeraient plus facilement au cours des FA et fermentations malolactiques (FML) difficiles [251], ainsi qu'entre ces deux étapes, tirant ainsi parti du 'vide microbiologique' [252]. Plusieurs travaux, aux résultats parfois

contradictoires, suggèrent l'existence d'interactions entre *Brettanomyces*/*Saccharomyces* [253, 254], mais aussi entre *Brettanomyces*/non-*Saccharomyces* [254, 255]. La cooccurrence de *Bretts* et d'autres microorganismes (levures mais aussi bactéries) pourrait engendrer différents types d'interactions, allant de la compétition pour l'utilisation de substrats carbonés et azotés (en faibles teneurs en fin de fermentation alcoolique), à des interactions de type *cell-to-cell contact* précédemment décrites. Il est donc nécessaire de mieux caractériser les interactions entre *B. bruxellensis* et les autres microorganismes, afin de mieux comprendre les facteurs environnementaux les impactant et d'identifier les mécanismes moléculaires impliqués. Ces aspects font l'objet du projet intitulé « Brett&cie », financé par l'industriel Lallemand depuis septembre 2018.

Le dispositif expérimental mis en place consiste à comparer la croissance de *B. bruxellensis* en présence de différentes souches de *S. cerevisiae*, ou de mélanges *S. cerevisiae*/NS, en prenant compte la variabilité génétique de l'espèce *B. bruxellensis* (test des principaux groupes génétiques isolés en œnologie). Les souches de *S. cerevisiae* et de non-*Saccharomyces* testées sont celles commercialisées par l'industriel Lallemand. La croissance des différentes espèces est suivie par des approches cultures dépendantes et par qPCR dans du vin sur plusieurs mois. Si nous mettons en évidence l'existence de souches de vinification favorisant/défavorisant la croissance de 'Brett', nous pourrons mieux rationaliser la lutte contre *B. bruxellensis*, entreprendre la caractérisation d'un plus grand nombre de souches et d'espèces, et rechercher les phénomènes d'interaction sous-jacents. La poursuite de ce projet reste encore un peu floue dans l'attente des premiers résultats, il en va de même pour son financement.

## L'ECOLOGIE SYNTHETIQUE POUR MIEUX COMPRENDRE LES INTERACTIONS ENTRE MICROORGANISMES

Les interactions entre microorganismes sont un enjeu clé pour l'œnologie mais aussi pour d'autres procédés alimentaires impliquant des communautés microbiennes complexes. Des travaux préliminaires, réalisés par l'équipe Ecologie Microbienne des Aliments, de l'institut Micalis (UMR1319/INRA-AgroParisTech), ont récemment démontré la pertinence des approches d'écologie synthétique (reconstruction de communautés microbiennes simplifiées et contrôlées) couplée à des analyses –*omics* (transcriptomique, métabolomique) pour l'étude des interactions microbiennes dans une matrice alimentaire type 'viande'. La mise en commun de données et de connaissances obtenues pour différents types de matrices alimentaires pourrait ouvrir de nouvelles pistes de travail. Fort de ce constat, Stéphane Chaillou et Marie Champomier-Verges ont réuni un consortium de 7 partenaires autour du projet METASIMFOOD (demande de financement ANR, AAPG2020). Quatre matrices alimentaires ont été retenues, le vin, le kéfir de fruit, la choucroute et le

fromage végétal (aussi appelé 'fauxmage' ou 'vromage'). Chacun de ces procédés fermentaires fait face à de nombreux changements : le réchauffement climatique impacte (et va continuer d'impacter) les propriétés physico-chimiques des matrices alimentaires, et les évolutions sociétales engendrent également des modifications de process (comme la réduction des intrants). L'objectif de ce projet est donc de préciser l'impact de ces différents changements sur les communautés microbiennes impliquées dans ces procédés fermentaires à l'aide d'approches et d'outils communs (et comparables), et de proposer des pistes de sélection pour les consortiums microbiens du 'futur'. Au sein de l'unité de recherche Œnologie, ce projet implique aussi bien des microbiologistes pour la reconstruction et le suivi des consortiums microbiens (levures-bactéries-phages) que des chimistes pour les analyses métabolomiques (RMN, GC). Les données multi-omiques obtenues seront utilisées pour la construction de réseaux d'interactions métaboliques [256] et de modèles prédictifs.

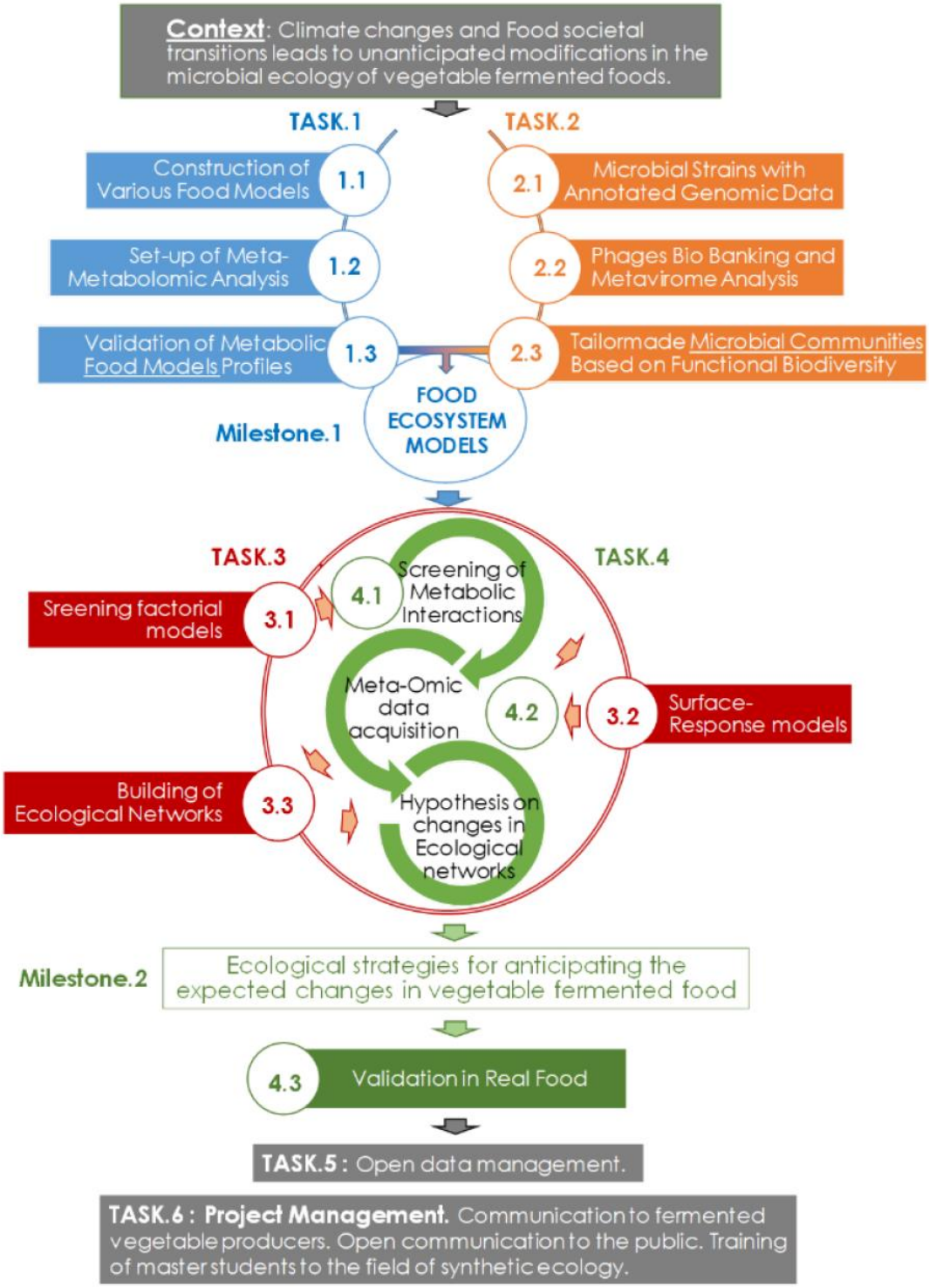



**FIGURE 14. ORGANISATION DU PROJET METASIMFOOD (ANR AAPG 2020).**

# III-C- PHENOTYPES D'INTERET OENOLOGIQUE

Parmi les phénotypes d'intérêt sur lesquels nous avons travaillé, il en est deux qui m'intriguent particulièrement et pour lesquels identifier les mécanismes sous-jacents me parait nécessaire : la capacité de bioadhésion de *B. bruxellensis* (qui fait l'objet du projet BRETTADHERE porté par Isabelle Masneuf-Pomarède, 2020-2022), et la capacité à produire du lactate de *L. thermotolerans* (projet non financé pour l'heure).

## RECHERCHES SUR LES PROPRIETES DE BIOADHESION DE *B. BRUXELLENSIS*

*B. bruxellensis* est capable d'adhérer à différentes surfaces, pour ensuite former des biofilms [257]. Des résultats portants sur un nombre très restreint d'isolats [77] montre que cette bioadhésion varie en fonction de la souche testée. Cette variabilité intra-espèce est également présente pour les propriétés physico-chimiques de surface des cellules, fortement impliquées dans les phénomènes d'adhésion. En effet, la formation de biofilms passe par une première étape d'adhésion qui met en jeu différentes interactions physico-chimiques (électrostatiques, van der Waals, Lewis acide-base, hydrophobes en particulier) dépendantes des propriétés de surface des cellules. Ces propriétés sont liées à leur composition de surface incluant ainsi les protéines, les polysaccharides, les lipides, etc.

Des travaux préliminaires ont récemment permis de confirmer, pour un petit nombre de souches, que la formation de biofilms était dépendante de la nature de la souche considérée [1]. Etant donnée la très grande variabilité génétique de *B. bruxellensis*, il est aujourd'hui nécessaire d'étendre ces travaux à un plus grand nombre de souches représentatifs des groupes génétiques de l'espèce pour avancer dans la compréhension des phénomènes de bioadhesion.

Sur le plan fondamental, ce projet vise à évaluer les caractéristiques de surface (charge globale, caractère hydrophobe ou hydrophile, propriétés Lewis acide-base, présence d'exopolysaccharides, composition en lipides…) et les capacités de bioadhésion et de formation de biofilm de Brett. La variabilité de ces propriétés au sein de l'espèce sera également évaluée en prenant en compte la diversité génétique connue. L'hypothèse de l'existence d'une relation entre groupe génétique, caractéristiques physico-chimiques de surface, propriétés d'adhésion et de colonisations (formation de biofilms) sera testée. La disponibilité des séquences génomiques de nombreuses souches (cf projet BrettAdapt) permettra de rechercher des gènes candidats en lien avec ces caractéristiques, et de les tester formellement (étude de mutants). L'impact des facteurs environnementaux (type de matériaux acier, époxy, bois, éthanol, température, pH, qualité de l'eau) sera également testé.

D'un point de vue appliqué, l'objectif est de compléter l'offre actuelle en matière d'outil de détection de *B. bruxellensis* en cave et d'améliorer le conseil vis-à-vis des procédures d'hygiène. Nous verrons s'il est possible de mettre au point un test de détection des souches présentant des aptitudes spécifiques de bioadhésion. En particulier, si le lien entre groupe génétique et propriétés de bioadhésion est confirmé, le marqueur moléculaire TYP\Brett pourrait permettre de prédire la capacité d'adhésion des souches de *B. bruxellensis* et ainsi adapter les préconisations de protocoles de désinfection à mettre en œuvre.

Outre l'unité de recherche Œnologie, ce projet implique plusieurs partenaires, l'un académique (équipe de Marie-Noelle Bellon-Fontaine, UMR Génie et Microbiologie des Procédés Alimentaires, GMPA, AgroParisTech, INRA, Université Paris-Saclay), l'autre industriel (le laboratoire d'œnologie Excell/Sarco, exploitant du brevet TYP\Brett). Une thèse CIFFRE sur ce sujet démarre début 2020 (encadrée par Isabelle Masneuf-Pomarède).

## *LACHANCEA THERMOTOLERANS* ET LA PRODUCTION D'ACIDE LACTIQUE

Nos travaux antérieurs ont confirmé que l'espèce *Lachancea thermotolerans* présentait des caractéristiques d'intérêt pour l'œnologie, en particulier sa capacité à produire de l'acide lactique. Cependant, nous montrons également qu'une grande variabilité existe à l'intérieur de l'espèce, avec une différence d'ordre 7 entre les souches fortement et faiblement productrices de lactate. Cette variabilité est liée au facteur 'souche', mais aussi au facteur 'groupe génétique' [8]. La production de lactate, si elle est d'ores et déjà exploitée par la profession œnologique, est pourtant mal comprise : son rôle physiologique reste hypothétique chez *L. thermotolerans*, de même que les facteurs (biotiques, voire abiotiques) la contrôlant. Il s'agit pourtant d'une spécificité intrigante : la plupart des levures (dont *S. cerevisiae*) ne produisent pas naturellement de lactate, qui est plutôt l'apanage des bactéries lactiques. Chez ces dernières, le lactate est impliqué dans le maintien de l'équilibre redox des cellules. Ce rôle est traditionnellement joué par le glycérol chez les levures, et est associé à la production d'acétate afin de maintenir la balance NADH/NAD+. Or, nos travaux suggèrent l'existence de corrélations entre lactate, glycérol, acétate et éthanol variables d'un groupe génétique à un autre, suggérant l'existence de différentes stratégies métaboliques. Cette spécificité souligne le manque de données concernant les flux de carbones au niveau du métabolisme central chez *L. thermotolerans* et la régulation de sa balance redox. Or, l'on peut supposer que l'adoption de différentes stratégies métaboliques pourrait sous-tendre l'adaptation à différents environnements, comme démontré chez *S. cerevisiae* [258, 259]. Il serait donc pertinent de développer un projet visant à mieux comprendre les différentes stratégies métaboliques chez *L. thermotolerans*, et explorer le lien avec l'anthropisation de certaines sous-populations.

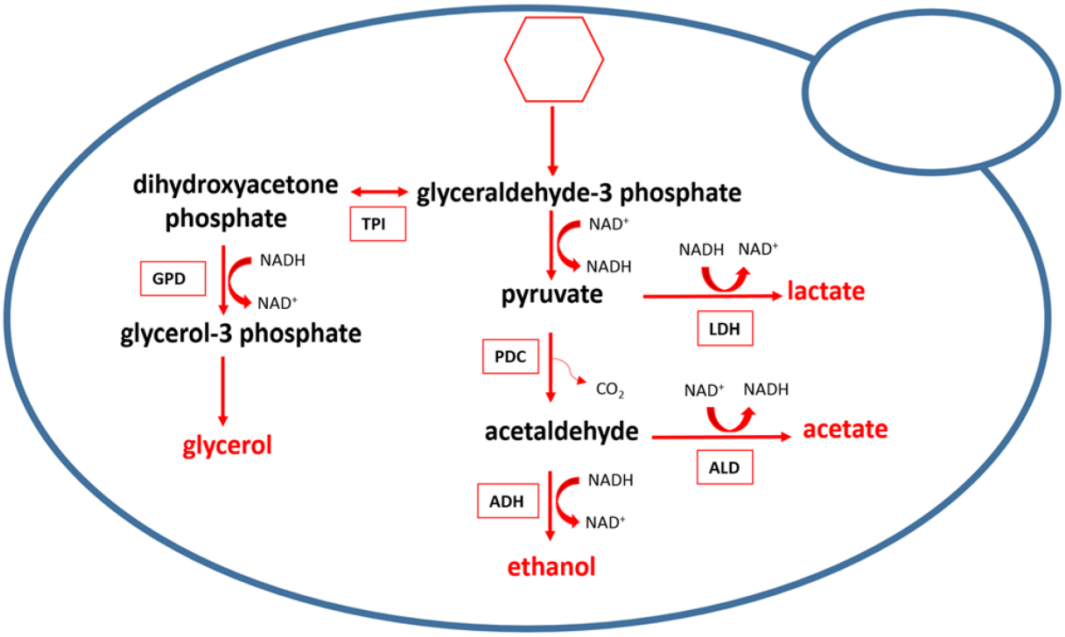



**FIGURE 15. PRODUCTION DES PRINCIPAUX METABOLITES SORTANTS PENDANT LA FERMENTATION ALCOOLIQUE CHEZ LACHANCEA THERMOTOLERANS.** PDC : PYRUVATE DECARBOXYLASE; ADH: ALCOHOL DEHYDROGENASE; ALD: ALDEHYDE DEHYDROGENASE; LDH: LACTATE DEHYDROGENASE; GPD: GLYCEROL-3-PHOSPHATE DEHYDROGENASE; TPI: TRIOSEPHOSPHATE ISOMERASE.

Un tel projet nécessiterait l'utilisation de souches avec des comportements contrastés et appartenant à des groupes génétiques différents, déjà disponibles au laboratoire. Des approches omics-, associant transcriptomique, métabolomique et fluxomique, permettraient de tester différentes hypothèses métaboliques. Des travaux similaires ont été conduits par l'équipe SPO (INRA, Montpellier) sur l'espèce *S. cerevisiae*, ce projet (encore très hypothétique et peu abouti) pourrait prendre la forme d'une collaboration entre nos deux équipes.

# IV- CONCLUSION ET SELECTION DE PUBLICATIONS

Depuis 2006 et mon arrivée au sein de l'UR Œnologie, j'ai centré mes travaux sur l'étude de la diversité génétique et phénotypique des levures du raisin et du vin. Certaines approches historiques disponibles au laboratoire (suivi des cinétiques fermentaires, dosage de composés d'intérêt) ont évolué grâce à la mise en place d'outils haut-débit de plus en plus miniaturisés. Cette mutation sur le plan technique a été possible grâce au déploiement parallèle d'outils bio-informatique et bio-statistique, indispensables pour le traitement de larges jeux de données, et dont certains sont aujourd'hui utilisés en routine au laboratoire. Des approches -omics, à différents niveaux d'intégration cellulaire ont été importées ou développées (ou sont en cours de développement), sans pour autant mettre de côté les approches plus classiques de microbiologie ou de génétique des populations. Ces travaux ont été financés par différents acteurs, publiques (ANR, région, etc.) ou privés, et ont nécessité l'engagement de nombreux collègues, collaborateurs et étudiants. Nos travaux ont permis d'améliorer nos connaissances des levures du raisin et du vin sur plan fondamental, et quelques applications finalisées ont vu le jour.

Si, pour chaque question initiale posée, quelques éléments de réponse ont été apportés, dix nouvelles questions ont émergées, car c'est la définition même de la recherche. Ces nouvelles questions ont nourri les projets en cours, de BrettAdapt à MetaSimFood, financés (ou finançables) à court ou moyen terme. C'est sans doute l'un de mes plus grand regrets, de ne pas avoir les moyens financiers et humains pour développer des projets à plus long terme comme par exemple pour collecter du matériel et des échantillons pour les générations futures. Nous vivons une période charnière pour l'œnologie : des contraintes environnementales (changement climatique) et sociétales (évolution vers des modes de consommation et de production plus durables et responsables) sont en train de profondément modifier les pratiques vitivinicoles, et auront sans doute un impact marqué sur les consortiums microbiens. Une vision à long terme de la recherche (et les moyens qui vont avec) permettrait, par exemple, d'organiser des campagnes de prélèvement et de mise en collection des consortiums levuriens sur une échelle de temps large, au cours des décennies à venir, pour pouvoir, dans un futur un peu lointain (plusieurs dizaines d'années) les mettre en lien avec l'évolution des pratiques. Ce travail de collecte et de conservation, assez ingrat, pourrait permettre aux futures générations de chercheurs d'avoir accès à des données et à du matériel biologique unique. Il n'existe pas, à ma connaissance, de soutien financier pour des projets à long-terme, dont le seul objectif serait de collecter du matériel ou des données non-immédiatement exploitables pour ceux qui les collectent, mais pour les générations futures… Et c'est bien dommage !

Pour finir, j'ai sélectionné 5 publications représentant la diversité de nos travaux : trois publications scientifiques décrivant des approches et des résultats originaux, mais aussi un article de revue scientifique et une publication technique destinée à la profession.

**Albertin, W., P. Marullo, M. Bely, M. Aigle, A. Bourgais, O. Langella, T. Balliau, D. Chevret, B. Valot, T. da Silva, C. Dillmann, D. de Vienne, and D. Sicard. 2013. 'Linking post-translational modifications and variation of phenotypic traits', *Mol Cell Proteomics*, 12: 720-35.**

Dans cet article, nous avons exploré le lien existant entre l'abondance des protéines impliquées dans une voie métabolique ciblée (la glycolyse et la fermentation alcoolique) et les caractères phénotypiques qui en découlent : flux glycolytique, production d'éthanol, etc. Nous avons également mesuré les traits d'histoire de vie (taille de la population, taille des cellules, etc.). Il s'agit d'une publication dont je suis très fière car elle a demandé un travail conséquent à plusieurs niveaux. Sur le plan des analyses, nous avons utilisé pour la première fois au laboratoire des outils statistiques permettant de co-analyser des données obtenues à différents niveaux d'intégration cellulaire. Et sur le plan expérimental, nous avons employé une approche qui peut paraitre un peu obsolète aujourd'hui (quantification de l'abondance des protéines par l'électrophorèse bidimensionnelle), mais qui nous a donné accès aux différentes formes post-traductionnelles de chaque protéine d'intérêt. Malgré les avancées technologiques dans le domaine de la spectrométrie de masse, l'étude des modifications post-traductionnelles (MPT) reste encore limitée à quelques protéines d'intérêt (et/ou seulement pour quelques types spécifiques de MPT, phosphorylation et glycosylation en tête). Il y a là un monde gigantesque à découvrir, les analyses in silico prédisant une myriade de PTM possibles pour chaque protéine – dont certaines nous paraissent aujourd'hui exotiques (myristoylation, glypiation, etc.) – et dont l'impact au niveau phénotypique reste à découvrir... Il nous faudra attendre le développement d'approches haut-débit efficaces pour explorer plus largement cet univers [260], mais j'ai bon espoir de disposer un jour d'une technologie adaptée à ce genre d'analyses !

**Blein-Nicolas, M., W. Albertin, T. da Silva, B. Valot, T. Balliau, I. Masneuf-Pomarede, M. Bely, P. Marullo, D. Sicard, C. Dillmann, D. de Vienne, and M. Zivy. 2015. 'A Systems Approach to Elucidate Heterosis of Protein Abundances in Yeast', Mol Cell Proteomics, 14: 2056-71.**

Des compétences assez variées ont été nécessaires pour cette étude originale de l'hétérosis : nous avons construit un dispositif demi-diallèle chez la levure (soit tous les croisements possibles entre *n* individus parentaux, mais sans croisement réciproque). Deux espèces parentales de levure ont été utilisées, *S. cerevisiae* et *S. uvarum*, toutes deux présentes en œnologie. Plusieurs mois de persévérance ont été nécessaires pour produire 55 hybrides

intra- et inter-spécifiques à partir de 11 souches parentales, à l'aide d'un micromanipulateur, l'outil le plus emblématique du levuriste (mais aussi celui qui demande le plus de savoir-faire et de patience). Nous avons également développé des outils de caractérisation génétique de ces hybrides (marqueurs nucléaires et mitochondriaux), puis nous avons phénotypé hybrides et souches parentales en condition œnologique avant de quantifier leur protéome. Là encore, des outils d'analyses bio-informatiques spécifiques ont été nécessaires pour exploiter l'immense jeu de données généré, dont certaines facettes sont toujours en cours d'analyse…

**Avramova, M., A. Cibrario, E. Peltier, M. Coton, E. Coton, J. Schacherer, G. Spano, V. Capozzi, G. Blaiotta, F. Salin, M. Dols-Lafargue, P. Grbin, C. Curtin, W. Albertin, and I. Masneuf-Pomarede. 2018. '*Brettanomyces bruxellensis* population survey reveals a diploid-triploid complex structured according to substrate of isolation and geographical distribution', Scientific reports, 8.**

Cette publication est un exemple assez classique des travaux que nous avons menés récemment sur plusieurs espèces de levure d'intérêt en œnologie, ici la levure d'altération des vins *B. bruxellensis*. Nous avons assemblé une large collection d'isolats (>1400), provenant du monde entier (29 pays répartis sur les 5 continents) et de substrats variés (vin, mais aussi bière, tequila, bioéthanol, kombucha, etc.). Cette collection est avant tout un effort collectif, et le résultat des relations de confiance que nous entretenons avec nos collaborateurs nationaux et internationaux (à tous, merci !). En parallèle, nous avons développé des outils de génotypage spécifique de l'espèce (marqueurs microsatellite). Les différentes sous-populations ont ensuite été caractérisées pour des phénotypes d'intérêt, comme par exemple la résistance/tolérance aux sulfites pour *B. bruxellensis*.

**Albertin, W., M. Avramova, A. Cibrario, P. Ballestra, M. Dols-Lafargue, C. Curtin, and I. Masneuf-Pomarede. 2017. "*Brettanomyces bruxellensis* : diversité génétique et sensibilité aux sulfites." In Revue des Oenologues, 31-33.**

Cet article est une publication technique (sans comité de lecture scientifique), destinée à la profession vitivinicole, et sollicitée par l'un des éditeurs de la *Revue des Œnologues*. J'ai choisi cette publication car elle reflète l'une des missions de l'UR Œnologie et de l'ISVV : le transfert et la vulgarisation des connaissances vers la filière. Ici, il s'agit de décrire nos recherches sur la diversité génétique et phénotypique de l'espèce *B. bruxellensis*, et d'expliquer en quoi ces travaux peuvent être utiles aux professionnels. En l'occurrence, nous avons développé un marqueur moléculaire permettant de prédire la résistance/tolérance aux sulfites des '*Bretts*', outil aujourd'hui commercialisé par le laboratoire d'analyses œnologiques bordelais Excell/Sarco.

**Tempère, Sophie, Axel Marchal, Jean-Christophe Barbe, Marina Bely, Isabelle Masneuf-Pomarede, Philippe Marullo, and Warren Albertin. 2018. 'The complexity of wine: clarifying the role of microorganisms', Applied Microbiology and Biotechnology.**

Fin 2017, nous avons été sollicités par l'éditeur du journal *Applied Microbiology and Biotechnology* pour l'écriture d'une revue invitée sur la complexité du vin. Les articles de revue sont des outils intéressants, pour ceux qui les lisent mais aussi pour ceux qui les écrivent : le travail de bibliographie, qui devrait être la base de nos travaux, est bien trop souvent malmené par la pression du 'Publish or perish'. L'écriture de revue nous offre la possibilité de revenir au fondement de notre travail de chercheur tout en étant 'productif' sur le plan bibliométrique. Cette revue a été co-écrite avec nos collègues spécialistes de la chimie des arômes et de l'analyse sensorielle, une opportunité de mettre en commun compétences et connaissances, et de débattre des courants de pensées dans un exercice collectif très instructif. L'objectif était de (tenter de) rectifier certaines idées reçues fréquemment rencontrée dans la littérature scientifique et technique, et entre autres, de rappeler que la complexité d'un vin n'est ni liée à sa complexité chimique, ni à sa complexité microbiologique. Un exercice d'humilité intéressant pour des microbiologistes qui idéalisent parfois le rôle joué par leur modèle de prédilection… Les levures sont indispensables – mais pas suffisantes – pour la vinification !

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# Linking Post-Translational Modifications and Variation of Phenotypic Traits*[S]

**Warren Albertin‡§¶‖‖, Philippe Marullo§¶‖, Marina Bely§¶, Michel Aigle**, Aurélie Bourgais‡, Olivier Langella‡‡, Thierry Balliau‡‡, Didier Chevret§§, Benoît Valot‡‡, Telma da Silva§¶¶¶, Christine Dillmann¶¶, Dominique de Vienne¶¶, and Delphine Sicard§¶**

**Enzymes can be post-translationally modified, leading to isoforms with different properties. The phenotypic consequences of the quantitative variability of isoforms have never been studied. We used quantitative proteomics to dissect the relationships between the abundances of the enzymes and isoforms of alcoholic fermentation, metabolic traits, and growth-related traits in *Saccharomyces cerevisiae*. Although the enzymatic pool allocated to the fermentation proteome was constant over the culture media and the strains considered, there was variation in abundance of individual enzymes and sometimes much more of their isoforms, which suggests the existence of selective constraints on total protein abundance and trade-offs between isoforms. Variations in abundance of some isoforms were significantly associated to metabolic traits and growth-related traits. In particular, cell size and maximum population size were highly correlated to the degree of N-terminal acetylation of the alcohol dehydrogenase. The fermentation proteome was found to be shaped by human selection, through the differential targeting of a few isoforms for each food-processing origin of strains. These results highlight the importance of post-translational modifications in the diversity of metabolic and life-history traits.** ***Molecular & Cellular Proteomics 12: 10.1074/mcp.M112.024349, 720–735, 2013.***

The key problem in understanding genotype-phenotype relationships is the complexity arising from multiple levels of cellular functioning. Among them, metabolic networks involve series of interconnected chemical reactions catalyzed by enzymes, allowing the transformation of input substrates into intermediate or final metabolites. These networks play an essential role in an organism's growth, reproduction, and ability to maintain cell integrity and to respond to environmental changes (1, 2). The metabolic fluxes, as well as the metabolite concentrations, are governed by the activity of the enzymes, which depends on three types of factors: kinetic parameters, enzyme abundance, and activation state of the enzyme. The kinetic parameters are determined by the sequence and the three-dimensional structure of the protein (3). The abundance of the enzymes is the result of numerous molecular processes taking place from the transcriptional to the translational level, including epigenetic modifications of the DNA and chromatin (4), transcriptional regulation by transcription factors (5), mRNA capping and splicing and small RNA regulation (6), protein turnover (7), etc. The enzyme activation state is primarily because of post-translational modifications of the native protein, themselves highly regulated (8, 9). Other mechanisms involved in enzyme activity are protein-protein interactions and allosteric regulation, such mechanisms being sometimes mediated through post-translational modifications (10, 11). The resulting isoforms can display differences in activity, affinity for partners (protein or effectors), and stability (12). The most studied modification is the reversible activation and inactivation of enzymes by phosphorylation (11, 13, 14), but other modifications are documented, such as acetylation that alters enzymatic activity and stability (15, 16) or fatty-acid modifications affecting cellular localization (17).

Thus, there are multiple levels to modulate metabolic phenotypes, and the identification of the most effective ones has been the subject of much interest (18–26). Recent data suggest that upstream levels of regulation have moderate control over metabolic changes. For example, genes involved in redox regulation in *Arabidopsis thaliana* have quite stable expression whereas the corresponding fluxes and metabolite contents display marked genetic variations (27). Similarly, various studies on bacteria have shown that transcription is not sufficient to explain the variation of metabolic fluxes or phenotypes in *Escherichia coli* (28), *Bacillus subtilis* (29), *Co-*

From the ‡CNRS, UMR 0320/UMR 8120 Génétique Végétale, F-91190 Gif-sur-Yvette, France; §Univ. de Bordeaux, ISVV, EA 4577, Unité de recherche Œnologie, F-33140 Villenave d'Ornon, France; ¶INRA, ISVV, USC 1219 OENOLOGIE, F-33140 Villenave d'Ornon, France; ‖SARCO/LAFFORT Group, 7 rue Franc Sanson, F-33370 Floirac, France; **Université Lyon 1, UMR 5240 Microbiologie Adaptation et Pathogénie, F-69622 Villeurbanne, France; ‡‡PAPPSO, UMR 0320/UMR 8120 Génétique Végétale, F-91190 Gif-sur-Yvette, France; §§PAPPSO, UMR MICALIS, Domaine de Vilvert, F-78352 Jouy-en-Josas, France; ¶¶Univ Paris-Sud, UMR 0320/UMR 8120 Génétique Végétale, F-91190 Gif-sur-Yvette, France

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*rynebacterium glutamicum* (30), *Synechocystis* sp (31), or *Mycoplasma pneumonia* (19). Systems biology studies, including transcriptomic and proteomic approaches, have suggested that the transcriptome alone does not provide a reliable indication of flux distribution in metabolic networks in yeast (32–34). By contrast, manipulating post-translational processes may have marked consequences on metabolic flux. For example in plants, abolition of the post-translational regulation of just one enzyme (a nitrate reductase) is sufficient to increase the corresponding flux of nitric oxide (35). In *Bacillus subtilis*, the phosphorylation state of one protein (Crh, a phosphocarrier protein) controls the flux through the methylglyoxal pathway, which is an alternative route of glycolysis in bacteria (36). In *Salmonella enterica,* lysine acetylation was shown to coordinate central metabolic pathways such as carbon use (37) or glycolysis and the TCA cycle in human (15). Thus, phosphorylation, acetylation, and other post-translational modifications emerge as major regulators of central metabolic pathways, yet are largely underestimated because of the lack of reliable approaches for systematic analyses (38). Their genetic and plastic variability together with their effects on the phenotype remain to be studied.

The present work focuses on the genetic and plastic variability of enzyme and isoform abundances in yeast, and on the possible consequences of this variability on metabolic and "life-history" traits, *i.e.* traits characterizing the lifespan of the organism such as growth or survival. Quantitative proteomics based on two-dimensional electrophoresis (2-DE)[1] is well adapted for this purpose, because the different isoforms of a protein often have different electrophoretic mobility, resulting in distinguishable spots. We applied quantitative proteomics to *Saccharomyces cerevisiae* alcoholic fermentation (AF), a central metabolic pathway exploited for millennia in three important human food-processes: beer and wine production (39–41), and bread leavening (42). The yeast AF enzymes are well-known and most of them have been identified on 2-DE maps (43–47). In a previous work, we showed that life-history traits (carrying capacity and cell size) and metabolic phenotypes (maximum $CO_2$ flux, ethanol, acetate, and glycerol content) displayed large variation, with medium effects usually higher than the strain effects (48). On the other hand, trade-offs were found between metabolic and life-history traits (49). A recent work showed that the expression variation of a few genes involved in the upper part of glycolysis could drive changes in life-history strategies (50), indicating that life-history traits might be under the control of some metabolic enzymes.

All these observations prompted us to investigate the possible control of metabolic and life-history traits by a large panel of AF enzymes tested under various conditions. Our experimental design included nine food-processing strains grown in triplicate in three different synthetic media mimicking the dough/wort/grape must found in bakery, brewery, and enology, to: (1) quantify thoroughly the abundances of 18 AF enzymes and their isoforms in a sample of 27 medium x strain combinations; (2) compare the genetic and plastic variability of the enzymes and their isoforms; (3) search which enzymes or isoforms, if any, are related to $CO_2$ flux, AF metabolite concentrations, and life-history traits, and thus may exert control over metabolism and life-history strategy. Our results highlight the preponderant role of post-translational modifications in the variation of metabolic phenotypes and life-history traits.

## EXPERIMENTAL PROCEDURES

A detailed Materials and Methods section is available as Supporting Information.

*Biological Material and Synthetic Fermentative Media*—Nine *S. cerevisiae* strains were used (supplemental Table S1), from enology (E1 to E4), brewery (B1 and B2), and distillery origins (D1 to D3). All strains were grown in triplicates in three synthetic fermentative media differing by their amount of sugar, nitrogen, pH, osmotic pressure, and anaerobic growth factors to reflect main changes of fermentation medium between brewery (BREM), bakery (BAM), and winery (WIM) contexts (supplemental Table S2).

*Metabolic and Life-history Traits*—For each of the 81 fermentations (nine strains × three media × three repetitions), we measured the following metabolic and life-history traits: $CO_2$ specific flux (the $CO_2$ production rate *per* cell, g/h/cell), ethanol production (% vol/cell), acetic acid concentration (g/cell), glycerol concentration (g/cell), biomass (gcell), carrying capacity (*K* or maximum population size in cells/ml), and cell size ($\mu$m [diameter]).

*Quantitative Proteomics*—One sample *per* fermentation (81 fermentations) was harvested at comparable physiological stage (maximal $CO_2$ production rate before nutriment starvation). One 2-DE gel per sample was run and stained with colloidal-blue, which offers a linear relationship between spot quantification and protein abundance (47) and thus allows accurate comparison of spot abundance between and within 2-DE gels. Spots of interest were quantified using Progenesis software (Nonlinear Dynamics, Newcastle, UK) and identified using mass spectrometry (MS). Almost all enzymes involved in glycolysis and ethanol pathways were identified, or at least the major and most abundant isozymes in case of paralogous genes.

*Statistical Analyses*—The variation of each isoform or enzyme abundance (in the latter case the isoforms of the enzyme were summed) was investigated through a mixed ANOVA model:

$$Z = \mu + medium_i + strain_j + block_k + position_l + batch_m + medium * strain_{ij} + \varepsilon_{ijklm}$$

where $Z$ is the variable, *medium* is the medium effect ($i = 1, 2, 3$), *strain* is the strain effect ($j = 1, \ldots, 9$), *block* is the random block effect (effect of each weekly experimental repetition, $k = 1, \ldots, 11$), *position* is the random position effect (bioreactor position, $l = 1, \ldots, 15$), *batch* is the random 2-DE batch effect ($m = 1, \ldots, 6$), *medium* * *strain* is the interaction effect between medium and strain factors, and $\varepsilon$ is the residual error. For further analyses (hierarchical clustering, PCA, LDA, regression analysis, etc.), we used the mean abundances predicted by the ANOVA model, that is, corrected for the random effects (block, position, and batch effects). The final data set is available as supplementary Data set S4. Hierarchical clustering was made using R (Ward's clustering method and Euclidean distances). Proteomic-trait

[1] The abbreviations used are: 2-DE, two-dimensional electrophoresis; BREM, brewery medium; BAM, bakery medium; WIM, winery medium; LDA, linear discriminant analysis; AF, alcoholic fermentation; MS, mass spectrometry; MCA, metabolic control analysis.

relationships were explored using multiple linear regression to find enzymes and isoforms whose abundance was significantly related to metabolic and life-history traits. The impact of human domestication was investigated using linear discriminant analysis (LDA) to discriminate beer, distillery, and wine strains using R. Discriminant isoforms were identified through stepwise variable selection and through the calculation of the « ability to separate » (AS) criterion.

## RESULTS

To explore the extent of phenotypic diversity of enzymes abundance in alcoholic fermentation pathway, we chose nine food-processing strains of *S. cerevisiae* (supplemental Table S1) from different food origins, and we performed anaerobic alcoholic fermentations in triplicate using three different synthetic media (supplemental Table S2) that mimicked the dough/wort/grape must found in bakery, brewery, and enology (48). For each of the 81 fermentations (9 strains × 3 media × 3 repetitions), cell samples for proteomics assays were harvested during the fermentations when the $CO_2$ production rate *per* cell (the flux) was close to its maximum, so that the cells displayed comparable physiological stage. Using quantitative proteomics, we identified and quantified the relative abundance of 15 enzymes of glycolysis and ethanol pathways, one enzyme of acetate pathway and two enzymes of glycerol pathway (Fig. 1). Those 18 enzymes were representative of the alcoholic fermentation metabolic process and will be thereafter called the fermentation proteome. For most enzymes, several spots, corresponding to different post-translational forms (isoforms) were identified (Fig. 1), allowing subsequent analyses both at the enzyme level (sum of all isoforms for each enzyme) and at the post-translational modification level (individual isoforms). The few suspected allelic variants identified by 2-DE (shifting trains of spots, Fig. 1*B*) were confirmed by gene sequence (supplementary Information Data set S1). In these last cases, we compared isoforms having the same position within the train of spots (acidic, basic and intermediary isoforms) rather than co-located spots (see Materials and Methods in Supplementary Information). The mean coefficient of variation between biological triplicates for isoforms was 18.4%, which is low enough to accurately detect small abundance variations. Proteomic data were released in the PROTICdb database, a web-based application designed for large-scale proteomic programs to store and query data related to protein separation by 2-DE and protein identification by MS (http://moulon.inra.fr/protic/adaptalevure. See Supplementary Information for details).

*The Fermentation Proteome is Constrained*—We first analyzed the different sources of variation for protein abundance at different levels: At the whole fermentation proteome level (sum of enzymes of glycolysis, ethanol, acetate, and glycerol pathways), at the enzymes level (sum of isoforms for each enzyme), and at the post-translational level using individual isoforms (Table I). Considered globally, the sum of the abundance of the enzymes involved in the fermentation proteome (42 isoforms) represents on average 32.87 ± 1.89% of the total analyzed proteome (2265 ± 209 spots depending on the 2-DE gel). Variance analysis (ANOVA) revealed that such fermentation pool displayed no medium, no strain, and no medium × strain interaction effects, indicating that the enzymatic pool allocated to glycolysis, ethanol, acetate, and glycerol pathways is invariant whatever the medium and strain considered. Within the fermentation proteome, the abundance from one enzyme to another (sum of all isoforms for each enzyme) varied greatly (supplemental Fig. S1), with highly abundant proteins (Tdh2p, Tdh3p, Eno2p, Fba1p) and poorly represented enzymes (PfK1p, Ald6p, Pgi1p, Hor2p, Rhr2p). However, although abundance had important variation within enzymes, among strains, the proportion allocated to each enzyme appeared to be globally conserved (Fig. 2). Indeed, the mean coefficients of variation of the 18 enzymes (CV = 0.26) and 42 isoforms (CV = 0.35) were significantly lower than the mean coefficient of variation of the 688 other common spots (non-AF proteins) on the 2-DE gels (CV = 1.24, Kolmogorov-Smirnov test, $p$ value = $2.48 \times 10^{-10}$ and $2.89 \times 10^{-15}$, respectively). However, although the abundance of AF enzymes appeared more constrained than the whole proteome, significant variations were found, in particular for the enzymes of the last part of glycolysis (except Tdh3p and Gpm1p), as well as for the enzymes of ethanol, acetate, and glycerol pathways (Table I). A significant strain effect was found for most enzymes (13/18), which accounted for 21% to 68% of total variation (Table I). The medium effect was significant for only 6/18 enzymes and accounted for much less of the total variation (between 4 and 28%, Table I). The medium × strain interaction effect was significant for 2/18 enzymes, and accounted for 15% to 16% of total variation of the enzyme. Finally only 5/18 enzymes exhibited no strain or medium effect, and the average abundance of Pgi1p, Fba1p, and Tpi1p, corresponding to the first part of glycolysis, was similar in all the 27 medium × strain combinations. Therefore, we found a significant variation for enzyme abundance, which was better explained by genetic differences between strains than by plastic changes in response to variations of the culture medium.

*The Isoforms of a Given Enzyme Display Different Patterns of Variation*—The different isoforms of the fermentation enzymes were also analyzed individually (Table I). The isoforms of a given enzyme generally displayed different abundance patterns that were hidden when the analysis was performed at the enzyme level (sum of the isoforms). For example, three spots were identified for Gpm1p. Summing all isoforms, Gpm1 displayed neither medium nor strain effects, whereas spot 3313 displayed strain effect (higher relative abundance for E2 and D1 strains). For most enzymes, isoforms vary in different ways with respect to genetic and environmental factors (Table I). In addition, for some enzymes, the abundance variation of the different isoforms compensate, in part, for each other. For example, the global variance of Pdc1p abundance was twice lower than the sum of the variances of the

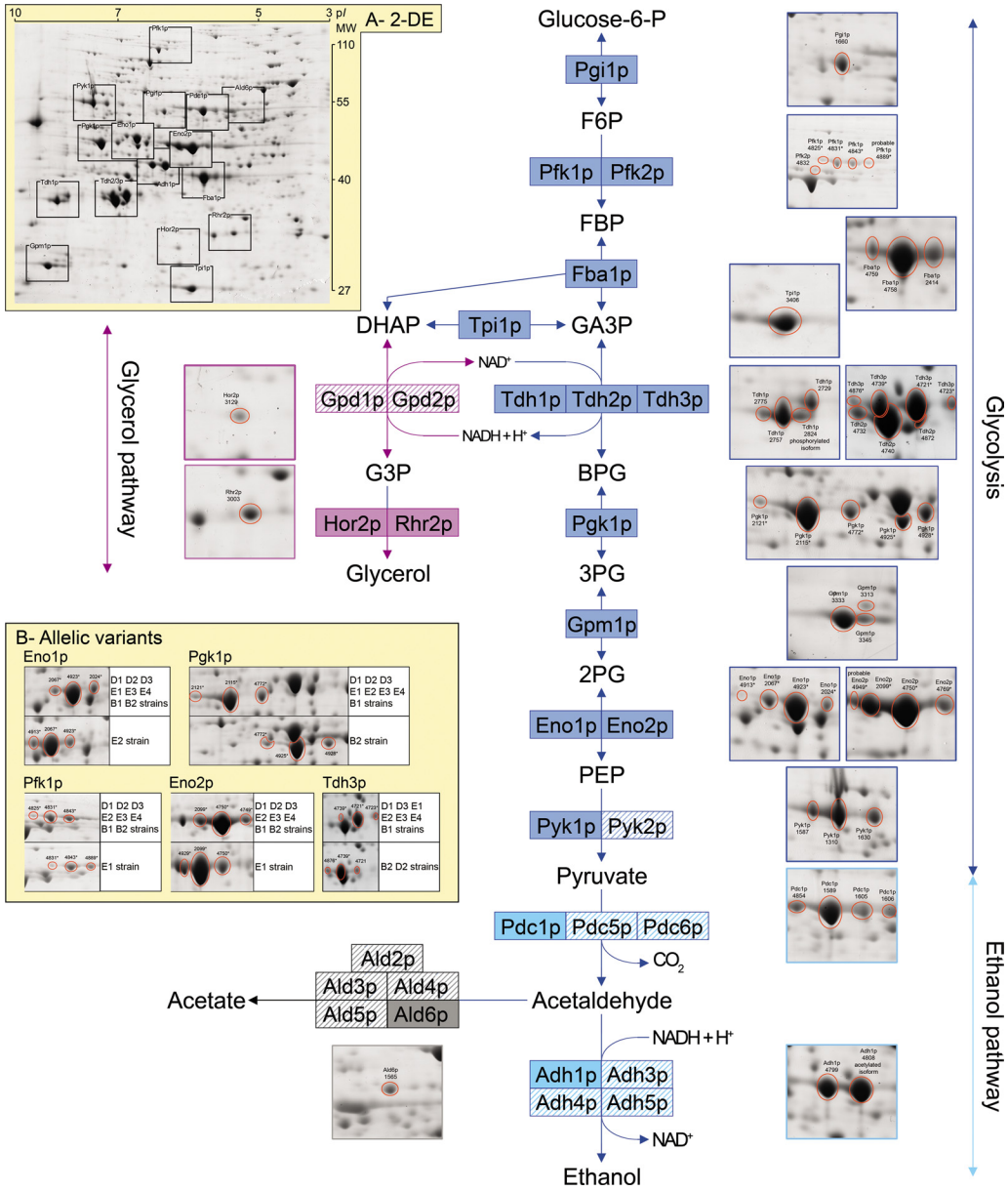


FIG. 1. **Linking glycolysis, ethanol, glycerol, and acetate pathways to 2-DE proteomics.** Plain boxes: identified enzymes. Striped boxes: unidentified enzymes corresponding to minor or low-abundant isozymes. Stars indicate specific spots corresponding to allelic variants (see *B*). *A*, 2-DE: Localization of the different enzymes within the master gel (co-electrophoresis of all samples). MW and p*I* stand for Molecular Weight (kDa) and isoelectric point, respectively. *B*, Allelic variants: five allelic variants were identified for Pfk1p, Pgk1p, Tdh3p, Eno1p, and Eno2p on the basis of the electrophoretic mobility of the corresponding spots, and were confirmed by protein sequence (supplementary Information Dataset S1). The detail of enzymes and metabolites abbreviations is Pgi, *P*hospho*g*luco*i*somerase; Pfk, *P*hospho*f*ructo*k*inase; Fba, *F*ructose-*b*iphosphatase *a*ldolase; Tpi, *T*riose-*p*hosphate *i*somerase; Tdh, *T*riose-phosphate *d*e*h*ydrogenase; Pgk, 3-*P*hospho*g*lycerate *k*inase; Gpm, *G*lycerate *p*hospho*m*utase; Eno, *E*nolase; Pyk, *P*yruvate *k*inase; Pdc, *P*yruvate *d*ecarboxylase; Adh, *A*lcohol *d*e*h*ydrogenase; Gpd, *G*lycerol-3-*p*hosphate *d*ehydrogenase; Hor, *H*yperosmolarity-*r*esponsive (DL-glycerol-3-phosphatase); Rhr, *R*elated to *HOR2* (DL-glycerol-3-phosphatase); Ald, *Al*dehyde *d*ehydrogenase; Glucose-6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, Fructose-1,6-biphosphate; DHAP, dihydroxyacetone phosphate; G3P, Glycerol-3-phosphate; GA3P, glyceraldehyde-3-phosphate; BPG, glycerate-1,3-biphosphate; 3PG, glycerate-3-phosphate; 2PG, glycerate-2-phosphate; PEP, phosphoenol-pyruvate.

four individual Pdc1p isoforms ($1.08 \times 10^{-3}$ *versus* $2.26 \times 10^{-3}$). This showed that the different Pdc1p isoforms had negative covariance. Indeed, the abundance of three Pdc1p isoforms (1605, 1606, and 1589) was significantly lower for strain D3 whereas the abundance of the remaining Pdc1p isoform (spot 4854) was significantly higher, compensating in part for the variation of the others. Isoform compensation was found for four enzymes (Fba1p, Tdh2p, Eno2p, Pdc1p) that displayed less variation when considering the variance of the sum of the isoforms rather than the sum of the variance of the

TABLE I

*Results of the ANOVAs: sums of squares for abundance of enzymes and their isoforms involved in glycolysis, ethanol, acetate, and glycerol pathways. For some enzymes/isoforms. data transformation was necessary to obtain normally-distributed residues: log transformation for the fermentation proteome, Pgi1p-1660, Pfk2p-4832, Fba1p-4759, Tdh1p-2775, Tdh2p-4872, Tdh2p-4740, Tdh3p-all isoforms, Tdh3p-acidic, Tdh3p-intermediary, Tdh3p-basic, Pgk1p-basic, Gpm1p-all isoforms, Gpm1p-3333, Gpm1p-3345, Gpm1p-3313, Eno1p-all isoforms, Eno2p-all isoforms, Eno2p-intermediary, Eno2p-acidic, Pyk1p-1587, Pdc1p-1606, Adh1p-all isoforms, Adh1p-4799, Ald6p-1565; inverse transformation for Fba1p-all isoforms, Fba1p-4758, Eno1p-acidic, Pyk1p-all isoforms, Pyk1p-1630, Pyk1p-1310, Pdc1p-4854, Adh1p-4808; and finally square root transformation for Tdh1p-2824 and Eno1p-intermediary. Variance was calculated across the 27 strain x medium combinations*

| Metabolic pathway | Enzymes | Isoforms reference | % of total sum of squares (df)[a] | | | | Variance |
|---|---|---|---|---|---|---|---|
| | | | medium (2) | strain (8) | medium × strain (16) | residual (52) | |
| Fermentation proteome | all AF enzymes | all isoforms | 3.55 | 10.84 | 28.86 | 56.74 | 6.21E-02 |
| Glycolysis | Pgi1p | 1660 | 0.33 | 19.9 | 17.9 | 61.87 | 1.86E-05 |
| | Pfk1p | all isoforms | 2.2 | 34.9*** | 27.62 | 35.29 | 1.37E-05 |
| | | intermediary | 2.57 | 43.99*** | 24.85 | 28.59 | 3.85E-06 |
| | | basic | 1.96 | 31.2** | 19.09 | 47.75 | 1.81E-07 |
| | | acidic | 5.12 | 27.45** | 28.87 | 38.57 | 2.02E-06 |
| | Pfk2p | 4832 | 0.82 | 35.26** | 27.79 | 36.13 | 1.94E-06 |
| | Fba1p | all isoforms | 2.18 | 9.57 | 24 | 64.25 | 5.69E-04 |
| | | 4759 | 2.14 | 21.07 | 26.3 | 50.5 | 5.23E-05 |
| | | 4758 | 0 | 8.3 | 31.08 | 60.62 | 4.87E-04 |
| | | 2414 | 9.22 | 17.38 | 16.95 | 56.45 | 9.35E-05 |
| | Tpi1p | 3406 | 1.71 | 11.57 | 19.81 | 66.9 | 9.08E-05 |
| | Tdh1p | all isoforms | 18.73** | 21.9** | 20.82 | 38.55 | 3.31E-03 |
| | | 2775 | 13.79** | 19.81* | 27.79 | 38.61 | 2.58E-04 |
| | | 2757 | 14.46* | 20.09* | 19.27 | 46.18 | 9.84E-04 |
| | | 2729 | 12* | 21.56** | 28.25 | 38.19 | 5.41E-05 |
| | | 2824 | 10.55 | 29.07** | 10.8 | 49.58 | 1.02E-04 |
| | Tdh2p | all isoforms | 1.77 | 37.89*** | 24.82 | 35.52 | 1.71E-03 |
| | | 4732 | 2.56 | 50.81*** | 11.24 | 35.4 | 7.68E-05 |
| | | 4872 | 4.21* | 77.63*** | 5.2 | 12.96 | 9.01E-05 |
| | | 4740 | 0.49 | 56.38*** | 17.4 | 25.73 | 1.73E-03 |
| | Tdh3p | all isoforms | 1.3 | 14.91 | 33.52 | 50.27 | 1.63E-03 |
| | | basic | 1.51 | 17.71 | 22.79 | 57.99 | 5.16E-05 |
| | | intermediary | 1.22 | 12.24 | 34.52 | 52.02 | 1.13E-03 |
| | | acidic | 1.28 | 79.49*** | 5.79 | 13.44 | 3.11E-04 |
| | Pgk1p | all isoforms | 8.49* | 48.63*** | 9.48 | 33.39 | 2.68E-03 |
| | | basic | 20.61*** | 34.57*** | 13.09 | 31.74 | 2.70E-05 |
| | | intermediary | 5.58 | 42.82*** | 11.97 | 39.63 | 1.61E-03 |
| | | acidic | 7.67* | 53.39*** | 5.53 | 33.41 | 9.13E-05 |
| | Gpm1p | all isoforms | 9.22 | 6.03 | 23.08 | 61.67 | 2.98E-04 |
| | | 3333 | 9.71 | 2.83 | 23.73 | 63.72 | 1.98E-04 |
| | | 3345 | 5.47 | 6.35 | 17.42 | 70.75 | 1.39E-05 |
| | | 3313 | 2.89 | 24.26** | 32.97 | 39.88 | 3.74E-05 |
| | Eno1p | all isoforms | 27.55*** | 26.87*** | 18.24 | 27.34 | 2.31E-03 |
| | | basic | 27.34*** | 27.65*** | 15.64 | 29.37 | 6.54E-05 |
| | | intermediary | 21.82*** | 26.52** | 15.69 | 35.96 | 1.30E-03 |
| | | acidic | 16.07** | 31.99*** | 15.23 | 36.71 | 1.02E-04 |
| | Eno2p | all isoforms | 3.39 | 27.88** | 20.81 | 47.92 | 1.88E-03 |
| | | basic | 4.18 | 32.77*** | 21.16 | 41.9 | 5.08E-04 |
| | | intermediary | 1.52 | 22.85* | 32.17 | 43.46 | 1.38E-03 |
| | | acidic | 1.9 | 15.67 | 14.51 | 67.92 | 6.40E-05 |
| | Pyk1p | all isoforms | 1.7 | 41.85*** | 19.56 | 36.89 | 2.99E-03 |
| | | 1587 | 0.79 | 21.51 | 22.41 | 55.29 | 8.72E-05 |
| | | 1310 | 1.1 | 36.36*** | 22.03 | 40.51 | 1.46E-03 |
| | | 1630 | 2.74 | 42.2*** | 14.38 | 40.68 | 1.55E-04 |
| Ethanol | Pdc1p | all isoforms | 7.69 | 20.88* | 24.64 | 46.79 | 1.08E-03 |
| | | 4854 | 0.65 | 55.53*** | 11.52 | 32.3 | 9.72E-04 |
| | | 1589 | 2.28 | 66.54*** | 11.99 | 19.19 | 1.16E-03 |
| | | 1605 | 6.71 | 39.02*** | 9.49 | 44.78 | 1.15E-04 |
| | | 1606 | 3.64 | 29.71* | 10.56 | 56.1 | 1.65E-05 |
| | Adh1p | all isoforms | 0.94 | 33.43*** | 23.38 | 42.24 | 7.22E-04 |
| | | 4799 | 1.78 | 49.82*** | 17.3 | 31.1 | 1.90E-04 |
| | | 4808 | 0.92 | 27.33** | 28.75 | 43 | 2.45E-04 |
| Glycerol | Hor2p | 3129 | 6.76** | 63.72*** | 14.62* | 14.9 | 3.75E-05 |
| | Rhr2p | 3003 | 4.08** | 68.49*** | 15.92** | 11.52 | 1.95E-04 |
| Acetate | Ald6p | 1565 | 23.67 | 18.32 | 19.39 | 38.61 | 8.96E-06 |

[a] df: degree of freedom. Significance is indicated as follow: * significant at 5%; ** significant at 1%; *** significant at 0.1% (Benjamini-Hochberg correction for multiple testing).

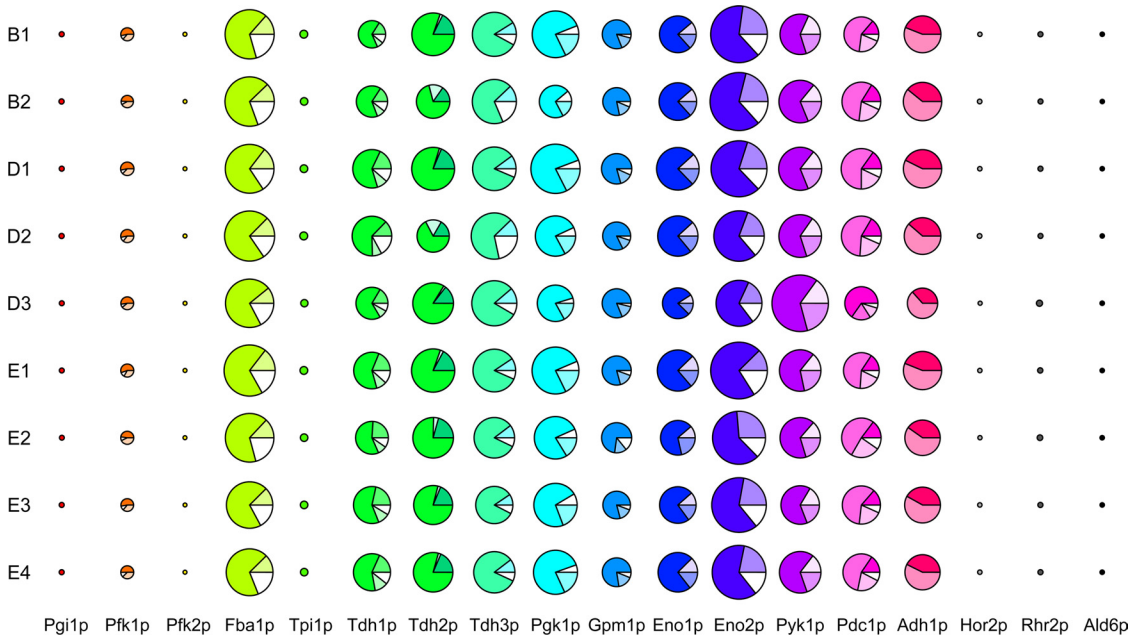


FIG. 2. **Distribution of the fermentation proteome within nine food-processing strains.** B1 and B2, brewery strains; D1 to D3, distillery strains; E1 to E4, enology strains. The 18 enzymes involved in glycolysis, ethanol, glycerol and acetate pathways are illustrated by pies whose size is proportional to the mean enzyme abundance over the three media. Within each pie the different isoforms (if any) are represented by pie's slice of different colors.

isoforms (Table I). Isoforms compensation was visible on Fig. 2: the total enzyme abundances (illustrated by pie sizes) varied weakly between strains whereas the distribution of the different isoforms of the enzymes (illustrated by pie's slices) was more variable for Tdh2p, Pdc1p, Fba1p, and Eno2p. Overall, analyses of variance showed that the different isoforms of a given enzyme can respond differently to environmental and genetic changes, and in some cases can compensate each other.

*Global Patterns of Isoform Abundance Variation Reflect the Strain Genetic Diversity*—To represent global patterns of protein abundance variation, we performed a hierarchical clustering of all 27 medium × strain combinations on the basis of individual mean isoform abundance over replicates (Fig. 3*A*). The resulting dendrogram showed a clustering according to the strains rather than the culture media and was close to the one obtained from genetic data (Fig. 3*B*). The hierarchical clustering was thus congruent with the analyses of variance of the isoforms and suggested that the variations in isoform abundance of the fermentation proteome are mainly genetically determined and displays limited plastic variation.

Because yeast strains used in different food processes may have experienced independent human domestication (51–53), we searched for enzymes and/or isoforms that could be involved in differentiating the strains according to their food origin (beer, distillery, and wine). We ran a LDA on the basis of isoform relative abundance with the food origin of the strains as grouping factor (Fig. 4*A*). The *a posteriori* probability to infer correctly the food origin of a strain was 0.96, indicating that it was possible to find a linear combination of isoforms that almost perfectly separated the samples according to the food origin of the strains, whatever the culture medium. A stepwise variable selection was then performed to determine which isoforms allowed such food origin discrimination and the ability to separate (AS) criterion was calculated (Fig. 4*B*). The isoform with the highest AS was Pdc1p-4854 that accounted for 32.56% of food origin discrimination, indicating that human domestication differentially targeted this isoform, directly or indirectly. Indeed, Pdc1p-4854 was significantly more abundant in distillery strains than in beer and wine strains (Fig. 4*C*). The acidic isoform of Tdh3p also accounted for 15.39% of food origin discrimination and separated wine strains from both distillery and beer strains. The unique isoform of Ald6–1565, one isoform of Tdh1p (2729), and one isoform of Pgk1p (intermediary) were associated with 7.51%, 7.13%, and 6.54% of food-origin discrimination, respectively. The other isoforms had lower ability to separate food origins (<5%). Notice that only few isoforms of the same enzyme appeared among the most discriminant factors in the LDA: one isoform out of the four of Pdc1p, one isoform out the three of Tdh3p, Tdh1p, and Pgk1p. This result indicates that the fermentation proteome was significantly shaped by human domestication, through the differential targeting of some isoforms of a few enzymes involved in fermentation.

*The $CO_2$ Specific Flux is Related to Variation in Abundance of Specific Isoforms of Different Enzymes*—The net outcome of glycolysis and alcoholic fermentation is the production of ethanol and ATP from glucose, along with $CO_2$ release. Step-

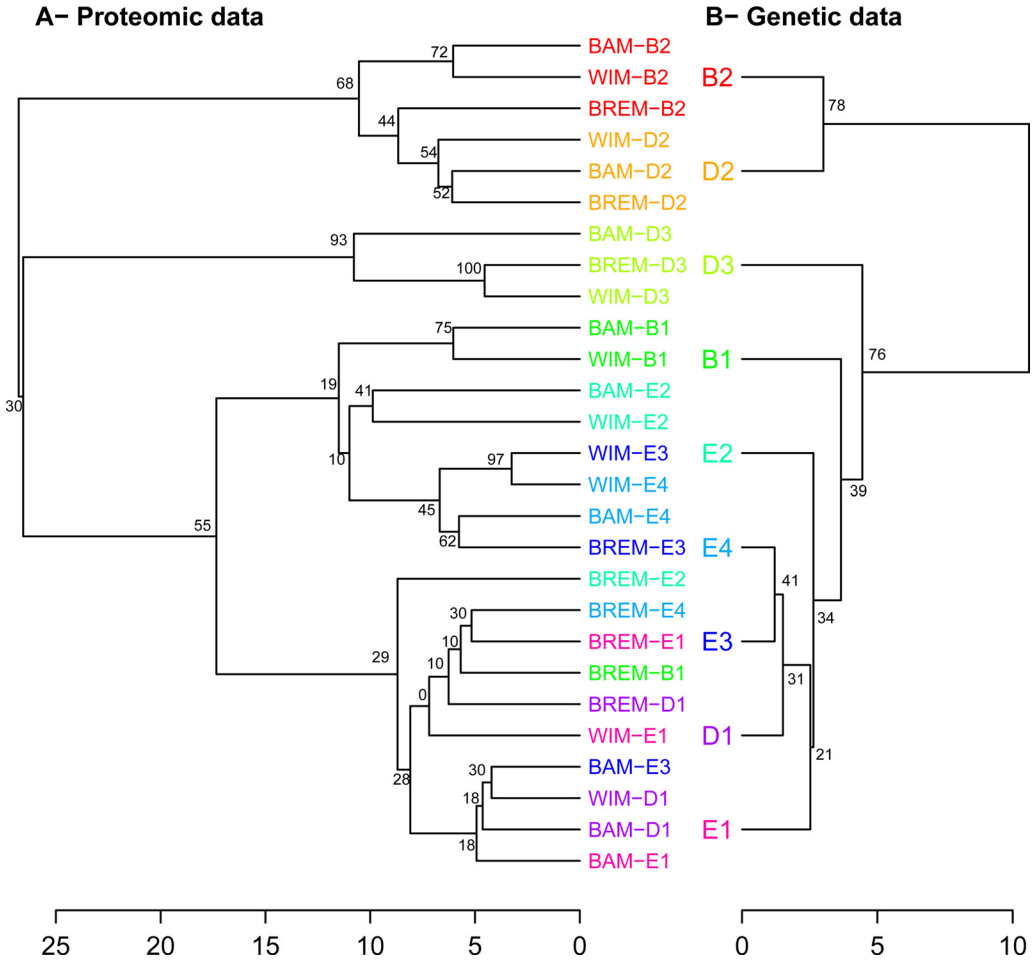


FIG. 3. **Hierarchical clustering using proteomic and genetic data.** *A*, Proteomic relationships between nine food-processing strains in three fermentative media using the fermentation proteome data. *B*, Genetic relationships between nine food-processing strains using eight microsatellites. The robustness of the nodes was assessed through multiscale bootstrap resampling.

wise multiple regression analyses were performed to determine which enzymes or isoforms of the fermentation proteome (if any) were related to $CO_2$ specific flux (*i.e.,* the $CO_2$ production rate *per* cell). For enzyme-flux regression analysis, the best model (*i.e.,* lowering AIK criterion) accounted for 44.49% of the variation of the $CO_2$ specific flux. For the isoform-flux regression analysis, the best model accounted for 79.50% of the variation of the $CO_2$ specific flux (Table II), suggesting that the efficiency of the fermentation process is more related to the abundance of specific forms of different enzymes than to global enzyme abundances. Therefore we considered individual isoforms for further functional analysis. Eighteen isoforms were found to be significantly associated with the variation of the $CO_2$ specific flux (Fig. 5*A*). Among them, the Pdc1p-1589 isoform accounted for 12.47% of the $CO_2$ flux variation, which was the largest part of variation explained by a single isoform. However, its abundance was negatively correlated to the flux, which suggests that it may correspond to an inactive or poorly active form of Pdc1p. In addition, two isoforms of Tdh1p (spots 2757 and 2729) accounted for 10.07% and 8.50% of the flux variation, respectively, and both were positively correlated to the flux. An isoform of the alcohol dehydrogenase, Adh1p (spot 4799) was also found negatively correlated to the flux and accounted for 7.57% of its variation. Finally, an enzyme involved in the glycerol pathway, Hor2p (spot 3129), was found negatively related to the $CO_2$ flux and accounted for 5.02% of the flux variation, whereas the isoforms of the other enzymes accounted for less than 5% of the flux variation. Thus, $CO_2$ flux variation was associated with the variation in abundance of specific isoforms rather than by the variation in abundance of all isoforms belonging to a peculiar enzyme. Moreover, each isoform accounted for a limited proportion of the flux variation, suggesting that the control of $CO_2$ flux is distributed among the different isoforms.

*The $CO_2$ Specific Flux is Related to Phosphorylation and N-Terminal Acetylation of Some Enzymes*—Additional experiments were run with a high-resolution mass spectrometer (QExactive, Thermo Scientific) to identify the underlying post-translational modification(s) differentiating the isoforms of the three enzymes (Pdc1p, Tdh1p, and Adh1p) that accounted for

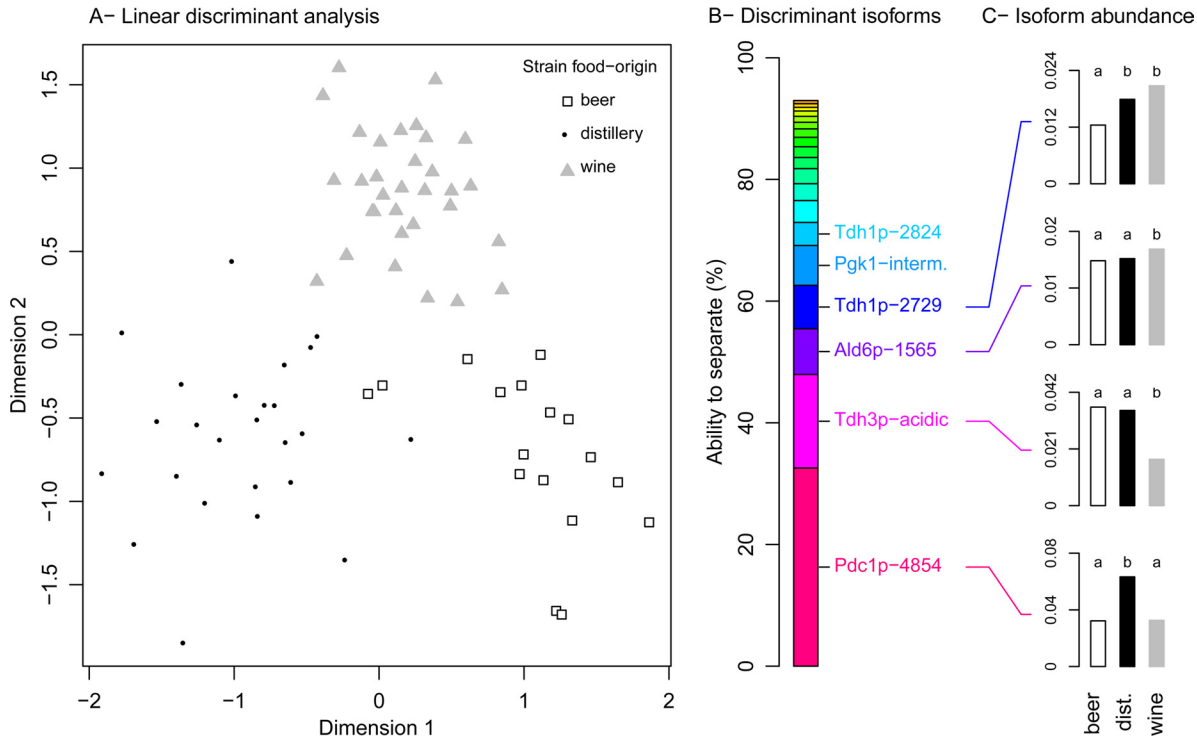


FIG. 4. **Discrimination of the food origin of the strains on the basis of the fermentation proteome.** *A*, Linear discriminant analysis of the food origins of the strains (beer, distillery, and wine strains) on the basis of the isoform relative abundances. *B*, Values of the "Ability to Separate" (AS) criterion for the most significant isoforms. The other isoforms have an AS below 4%. *C*, Isoform mean relative abundance for each food origin. Means with different letters differ significantly (Duncan's multiple comparison, $p < 0.05$).

TABLE II

*Percentage of variation accounted for by the multiple regression model for whole enzymes and for individual isoforms for seven metabolic or life-history traits*

| Metabolic of life-history traits | Whole enzymes | Individual isoforms |
|---|---|---|
| Flux | 44.49% | 79.50% |
| Ethanol per cell | 46.14% | 60.23% |
| Glycerol per cell | 49.96% | 69.05% |
| Acetate per cell | 46.53% | 68.00% |
| Biomass per cell | 34.22% | 56.58% |
| K (carrying capacity) | 67.28% | 87.60% |
| Cell size | 60.92% | 88.39% |

the highest parts of variation of $CO_2$ specific flux. The mass spectrometry (MS) data were used to search specifically for phosphorylation and N-terminal acetylation, which are very common post-translational modifications in yeast and were previously described for Pdc1p, Tdh1p, and Adh1p (http://www.ibgc.u-bordeaux2.fr/YPM/, http://www.phosphogrid.org).

For the four isoforms of Pdc1p, we were unable to identify the causal post-translational modifications, due either to the absence of phosphorylation or N-terminal acetylation for this enzyme or to accessibility problems of the modified peptides. For Tdh1p-2824, we identified a phosphorylated serine (position 201) that discriminated Tdh1p-2824 and Tdhp-2757 (which may correspond to the native protein). Protein phosphorylation induces an acidic shift, which is congruent with isoform position on gel (Fig. 1). The post-translational modifications associated with the two other isoforms of Tdh1p (2729 and 2775) were not identified, but multiple combinations of post-translational modifications are possible, rendering hazardous their identification by mass spectrometry.

For Adh1p, we identified an N-terminal acetylation (after methionine excision) harbored by Adh1p-4808, whereas Adh1p-4799, the other isoform, probably corresponded to the native protein. N-terminal acetylation also induces an acidic shift, in accordance with Adh1p isoform location on 2-DE gels. Hence, from a functional viewpoint, our mass spectrometry analyses indicated that $CO_2$ flux variation was mainly negatively associated with the variation in abundance of an unknown post-translational modified form of Pdc1p, the phosphorylation status of Tdh1p and the N-terminal acetylation status of Adh1p.

*Final Metabolite Concentrations are Related To Some Specific Isoforms of Different Enzymes*—During alcoholic fermentation, most of the consumed glucose (89.08%) is used to produce ethanol. The remaining glucose is used for biomass production (5.96%), glycerol (3.86%) and acetate (0.61%) synthesis (48). Other minor by-products, such as carbohydrate storage, represent less than 5‰ of initial glucose content. However, the AF product concentration at the end

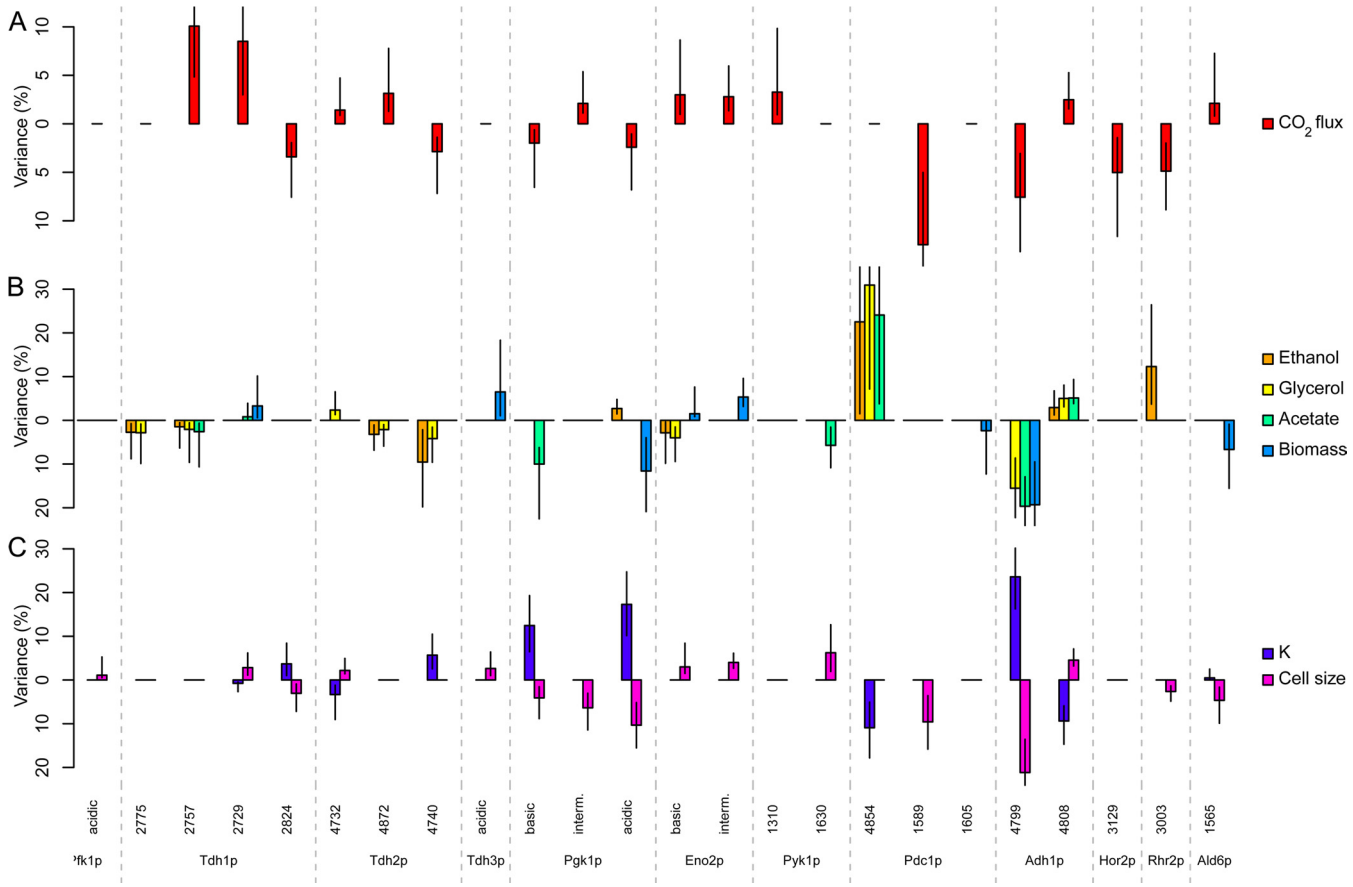


FIG. 5. **Relationship between seven metabolic and life-history traits and individual isoforms of the enzymes involved in alcoholic fermentation.** The best model accounting for trait variation was established using stepwise multiple regression (see equations in supplemental Information - text file). All the isoforms included in the model are presented. *A*, Percentage of variance of the $CO_2$ specific flux per cell accounted for by each isoform. *B*, Percentage of variance accounted for by each isoform for the concentration of ethanol, glycerol, and acetate per cell, and for biomass per cell. *C*, Percentage of variance accounted for by each isoform for two life-history traits: *K* (carrying capacity) and cell size.

of the fermentation process varies greatly depending on the medium and the strain for a given fermentation volume (48). To determine whether some enzymes or isoforms could be related to the final concentration of AF products and production of biomass, stepwise multiple regression and bootstrap resampling were applied for ethanol, acetate, glycerol, and biomass production *per* cell. With no exception, individual isoforms accounted for a larger part of variation of each of the four AF products than did the whole enzymes (Table II).

Nine isoforms were found significantly related to ethanol production *per* cell (Fig. 5*B*), the first one being Pdc1p-4854 that accounted for 22.52% of its variation with a positive correlation between the abundance of the isoform and ethanol production. Two other isoforms, Rhr2p-3003 and Tdh2p-4740, accounted for a substantial part of the variation (12.29% and 9.55%, positive and negative correlation respectively). For acetate production (Fig. 5*B*), the fittest model included seven isoforms, two of them accounting for most of the variation: Pdc1p-4854 (24.08%, positive correlation) and Adh1p-4799 (19.66%, negative correlation). Variation in glycerol production was also mostly associated with the variation in abundance of these two isoforms (30.93% variation, positive correlation for Pdc1p-4854 and 15.54% variation, negative correlation for Adh1p-4799), whereas it was not associated with the variation of Hor2p and Rhr2p isozymes, that are directly involved in the glycerol pathway (Fig. 5*B*). Finally, regarding biomass production *per* cell, the fittest regression model contained eight isoforms of which the first ones, Adh1p-4799, Pgk1p-acidic, and Ald6p-1565, were negatively correlated to biomass and accounted for 19.31%, 11.59%, and 6.67% of variation respectively. Thus, the fate of glucose during AF seems to be mostly related to some specific isoforms of a few enzymes: increased level of Pdc1p-4854 was associated with an increase of ethanol, glycerol, and acetate production, whereas increased level of the nonacetylated form of Adh1p (Adh1p-4799) was associated with a decrease of biomass formation and to a lesser extent of acetate and glycerol concentrations. For Pdc1p, the isoform associated with ethanol, glycerol, and acetate production (Pdc1p-4854) differs from the one associated with $CO_2$

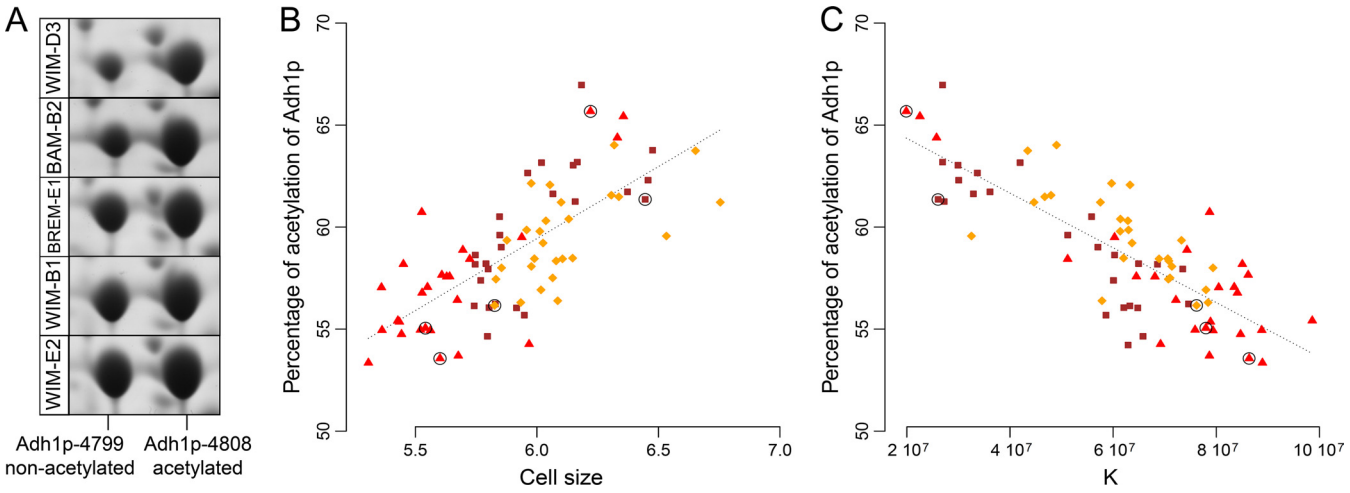


FIG. 6. **Relationships between Adh1p isoform ratio and two life-history traits, cell size, and carrying capacity.** *A*, 2-DE gel portions of the two isoforms of Adh1p ordered in decreasing Adh1p isoform ratio (spot 4799/spot 4808). B1 and B2, brewery strains; D3, distillery strains; E1 to E4, enology strains. BAM, BREM, and WIM: bakery, brewery, and winery media. *B*, Relationship between cell size (diameter, $\mu$m) and Adh1p isoforms ratio (spot 4799/spot 4808). A significant negative correlation was found ($\rho = -0.74$, $p < 10^{-15}$). *C*, Relationship between *K* (cells *per* ml) and Adh1p isoform ratio (spot 4799/spot 4808). A significant positive correlation was found ($\rho = 0.77$, $p < 10^{-15}$). The points corresponding to the 2-DE gel portions (*A*) are black-circled.

specific flux variation (Pdc1p-1589) but belong to the same enzymes.

*"Ant" and "Grasshopper" Strategies are Mainly Determined by the Degree of Acetylation of Adh1p*—In previous works, two life-history traits, cell size and maximum population size (*K*, the carrying capacity) were found to be related to the glycolytic flux and to define a range of life-history strategies distributed between two extreme behaviors, metaphorically designated as ants and grasshoppers (49, 50, 54). Similarly to what was done for metabolic traits, stepwise multiple regression and bootstrap resampling on isoform abundances were applied to cell size and *K*, the carrying capacity (Fig. 5*C*). The two isoforms of alcohol dehydrogenase, Adh1p, accounted for a large part of variation of these two life-history traits. Variation in abundance of the non-N-terminal acetylated isoform (Adh1p-4799) was found to be positively associated with *K* and negatively associated to cell size (23.59% and 21.16% of variation respectively), whereas it was the opposite for the acetylated isoform of Adh1p (spot 4808) (9.38% and 4.55% for *K* and cell size, negative and positive correlation respectively) . Some isoforms of Pgk1p and Pdc1p were also significantly related to *K* and cell size: Pgk1p-acidic, Pgk1pbasic, and Pdc1p-4854 accounted for 17.29%, 12.46%, and 10.92% of *K* variation, whereas Pgk1p-acidic and Pdc1p-1589 accounted for 10.33% and 9.58% of cell size variation. Interestingly, this analysis allowed us to explore the metabolic bases for the correlation between *K* and cell size. From the isoforms that were retained by the multiple regression, eight (Tdh1p-2729, Tdh1p-2824, Tdh2p-4732, Pgk1p-basic, Pgk1p-acidic, Adh1p-4799, Adh1p-4808, and Ald6p-1565) were positively correlated with one trait, and negatively correlated with the other, which is consistent with the negative correlation previously observed between the two life-history traits. The other isoforms were specific for either *K* or cell size. In addition, we found a spectacular specialization of the isoforms of Adh1p, with isoform-4799 correlated with high K and low cell size whereas isoform-4808 was correlated to low K and high cell size (Fig. 5*C*). Although these two isoforms accounted for around 26–33% of the variation for cell size and *K* by linear regression, it raised up to 52–65% when considering the degree of acetylation of Adh1p (Adh1p-4808/sum of both Adh1p isoform). As shown Fig. 6, the percentage of acetylated Adh1p is highly positively correlated to *K* ($\rho = 0.77$, $p < 10^{-15}$) and highly negatively correlated to cell size ($\rho = -0.75$, $p < 10^{-15}$). This shows that ant and grasshopper strategies are mainly associated with the degree of N-terminal acetylation of the alcohol dehydrogenase.

*Using Natural Variation for Metabolic Engineering*—Although multiple regression analyses gave a good indication of the correlation existing between metabolic or life-history traits and isoform abundances, they cannot predict whether the change of abundance of a single isoform will have a large impact on the traits. Indeed, an isoform may be significantly correlated to a trait but the slope may be low (a variation in abundance has little effect on the flux) and/or the range of variation in abundance of the isoform may be restricted. To determine the extent to which the different isoforms could affect maximum $CO_2$ flux during alcoholic fermentation, we used the equation of the multiple regression model previously established. For each strain, we used the mean abundance of their isoforms observed in all three media (supplementary Information Data set S2), except for one isoform the concentration of which varied over its natural range of variation in the nine strains. This allowed us to predict how the $CO_2$ specific

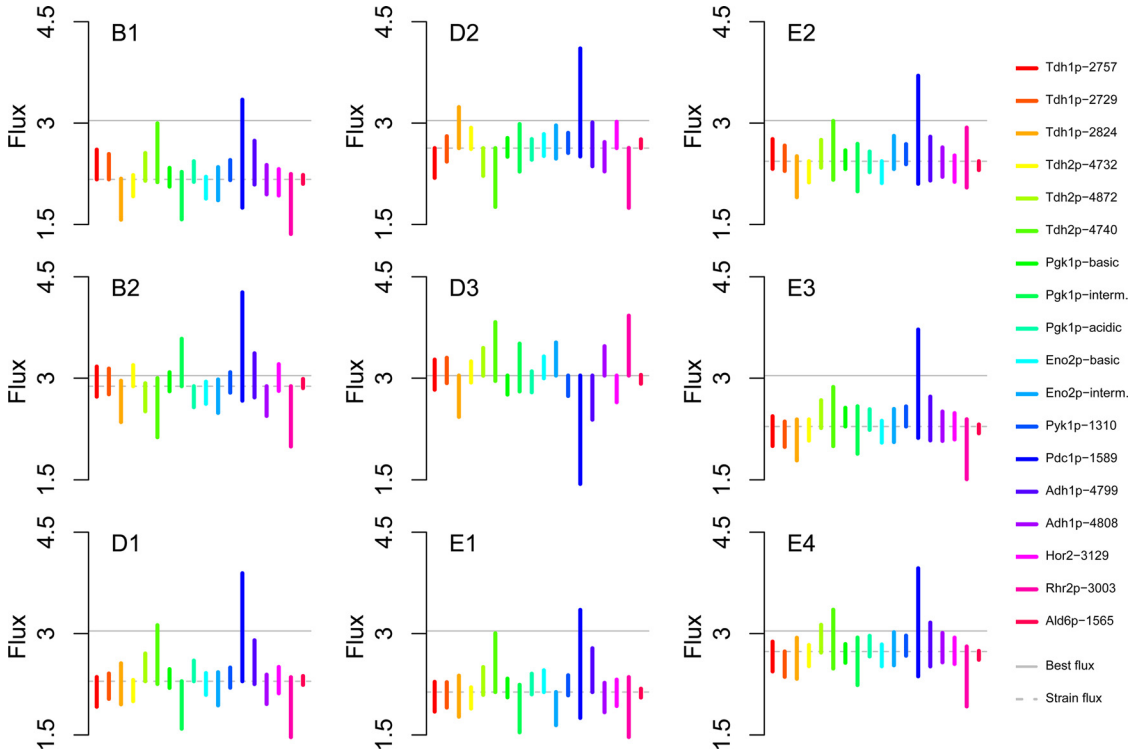


FIG. 7. **Predicted response of $CO_2$ flux to individual isoform variation.** To predict the response of $CO_2$ flux to individual isoform variation, we used the equation of the multiple regression accounting for $CO_2$ flux from isoform abundance. For each strain, the abundance of all isoforms but one was fixed equal to their mean over the three media, and the last isoform varied over the range of natural variation among the nine strains. D1 to D3, distillery strains; E1 to E4, enology strains. BAM, BREM, and WIM: bakery, brewery, and winery media. The $CO_2$ specific flux is expressed in $10^{-11}$ g $h^{-1}$ $cell^{-1}$. The "best flux" is indicated by the gray horizontal lines and the "strain flux" by the dotted line.

flux changes when the abundance of one isoform changes (bar lengths and positions, Fig. 7). For instance, the natural range of variation of Ald6p-1565 was associated with a very low $CO_2$ flux variation, whereas the response of the flux upon Pdc1p-1589 variation was large. The magnitude of these predicted variations was not strain-dependent (the same equation of multiple regression was used), but there were clear differences between strains regarding the max-min flux profiles over the isoforms (bar positions on the *y* axis in Fig. 7). In D3 strain, which had the highest observed flux ($3.04 \times 10^{-11}$ g/h/cell), but the lowest abundance of Pdc1p-1589 (remember that the correlation between the flux and Pdc1p-1589 is negative), changing Pdc1p-1589 abundance was associated with a flux decrease. But for the eight remaining strains, Pdc1p-1589 variation was mostly associated with a strong increase of the flux, even exceeding D3's (best) flux. For example, strain B1 had a mean flux of $2.17 \times 10^{-11}$ g/h/cell. The abundance decrease of Pdc1p-1589 isoform could be associated to a virtual flux increase of 54% ($3.35 \times 10^{-11}$ g/h/cell) compared with the measured B1's flux. For B2 the virtual flux was even more increased: B2 strain had a mean flux of $2.88 \times 10^{-11}$ g/h/cell that could be increased up to $4.27 \times 10^{-11}$ g/h/cell along with the virtual abundance decrease of Pdc1p-1589. This result elects Pdc1p-1589 as a relevant molecular target for further flux improvement for most strains. In a lesser extent Tdh2p-4740 could also be targeted to increase flux in all strains but B1 and D2, and Rhr2p-3003 in D3 and E2.

## DISCUSSION

*Global Constraints on the Fermentation Proteome*—We reported here a comprehensive study of the sources of variation for the yeast fermentation proteome during alcoholic fermentation, and its relationship with metabolic and life-history trait variation. We compared strains from different food origins grown in different fermentation media, and chose the peak $CO_2$ production rate as the reference physiological stage. At this stage the enzymatic pool allocated to the fermentation proteome, which represents one third of the total proteome, appeared to be constant over the media and strains considered. Previous work has suggested that enzyme concentrations cannot increase indefinitely and are probably bounded because of cellular constraints in space and energy (55), avoiding macromolecular crowding (56) and lowering the energetic cost associated with enzyme transcription, translation, and maintenance under limited resources (57, 58). Although our data demonstrated the existence of such a constraint at the level of the whole fermentation proteome, we have also shown that the AF enzymes have reduced variance compared with non-AF proteins, highlighting the existence of evolution-

ary constraints. Such constraints may be related to the existence of macromolecular complexes associating enzymes belonging to the same metabolic pathway. These so-called metabolons (59) were described for glycolytic enzymes in several organisms ranging from plants (60, 61), to human (62, 63) and yeast (64). Metabolon allows passing (channeling) the intermediary metabolites from one enzyme to the consecutive one within a given metabolic pathway, forming a metabolite tunnel. Substrate channeling is assumed to increase the efficiency and velocity of metabolic pathways, relative to the performances of a set of independent enzymes, but also to prevent the release of unstable intermediates (65). In addition, protein associations may protect the metabolic pathways in stressful environments (66). Indeed, we can hypothesize that the abundance of metabolon enzymes is closely regulated, which is consistent with the strong evolutionary constraint observed here for AF enzymes.

*Variation and Trade-offs Between Isoform Abundances*—For most of the AF enzymes studied here, several spots were identified in the 2-DE gels. Those isoforms could be unambiguously attributed to post-translational modifications: (1) all the isoforms were detected in all nine strains. Within a given strain, different isoforms corresponding to a given enzyme encoded by a given gene necessarily arose through post-translational modifications; (2) in case of proteins encoded by paralogous genes (Tdh1/Tdh2/Tdh3, Eno1/Eno2, etc.) mass spectrometry specific peptides allowing the clear discrimination of the paralogs were identified (supplementary Information Data set S3); (3) in the only five cases where we observed a shift of electrophoretic mobility of one enzyme for a particular strain (Fig. 1*B*), *all* the spots corresponding to the enzyme shifted, indicating a change in an amino acid, confirmed from gene sequences; (4) we were able to identify the post-translational modifications for some isoforms. Adh1p-4808 was identified as the acetylated form of Adh1p, whereas Adh1p corresponded to the native protein. Tdh1p-2824 displayed a phosphorylated residue (serine 201), whereas Tdh1p-2757 corresponded to the nonphosphorylated isoform. The post-translational modifications differentiating the other isoforms could not be identified.

Our data allowed us to measure the magnitude of the abundance variation of the isoforms of each enzyme, and to compare them to the variation observed at the whole enzyme level represented by the sum of isoform abundances. Many enzymes had isoforms that displayed different ranges of genetic and environmental variation, indicating isoform specialization. For some enzymes, most of the variation for protein abundances was observed at the isoform level, and the abundances of the different isoforms of a given enzyme were negatively correlated between strains, resulting in a lower genetic variation at the enzyme level. Moreover, variation of isoform abundances seemed to have clear functional consequences. Some specific isoforms—rather than all the isoforms of some enzymes—were associated to the variation of $CO_2$ flux, AF products, cell size, or carrying capacity (*K*). A few isoforms were also associated to differences between the food origins of the strains. The relationships between isoforms and phenotypic traits can be interpreted in three ways: (1) the isoforms of the enzymes control the phenotype, (2) the phenotype regulates back isoforms abundance, (3) trade-offs related to different allocations of the same resources lead to correlations between isoforms and traits. As post-translational modifications of enzymes are largely involved in the modulation of catalytic activity, ranging from inactivation to full activation, in protein-protein interactions or the regulation of enzymatic turnover (12), we interpret our results as the consequence of isoform control on phenotypic traits. To our knowledge, this is the first time that post-translational modifications are shown to be associated with traits related to fermentation metabolism, suggesting that "fine-tuning" of yeast AF is sustained at the post-translational level.

*Sharing-out the Control of Flux*—In metabolic control analysis (MCA), the flux control coefficient is a dimensionless measure of how much a flux varies in response to an infinitesimal change in the rate of a particular reaction (67, 68). Provided the flux-enzyme (or isoform) relationship is concave hyperbolic, flux control coefficients can also be estimated from changes in enzyme activities (69). By extension, the proportion of variance of $CO_2$ flux accounted for by the abundance variation of an individual isoform can be regarded as a proxy of the flux control exerted by the isoform. Although the relationship between flux control coefficient and the proportion of accounted for variance is complex (70), an enzyme that accounts for a significant part of the flux variance has necessarily a nonnull control coefficient. In our work, 18 isoforms were found to be related to the $CO_2$ flux, each isoform accounting for a limited proportion of flux variation (maximum 12.34%), in agreement with MCA that predicts flux control to be split over several enzymes rather than one. The $CO_2$ flux control was mainly distributed among an unknown post-translational modification of Pdc1p, the phosphorylation status of Tdh1p and the degree of N-terminal acetylation of Adh1p. Some isoforms of Pfk1p and Pfk2p exhibited strong genetic variation but no association with the $CO_2$ flux, which suggests that those enzymes have no control of the $CO_2$ flux. Conversely, our experimental set-up did not allow us to say anything about the control of the flux for isoforms that showed no genetic or environmental variations like Pgi1p and Tpi1p. Our results can be compared with previous works that specifically under- or overexpressed some enzymes of the fermentation proteome. From a MCA perspective, allosteric enzymes Pfkp and Pyk1p are unlikely to exert a high control on the glycolytic flux (71). This was confirmed experimentally for Pfkp (72–74) which is consistent with our own findings. However, Pyk1p was found to exert a significant level of control over both the rate and direction of carbon flux in yeast during growth on glucose (73), as well as in *Lactococcus lactis* (75). Accordingly, the Pyk1p-1310 isoform was found significantly

associated with $CO_2$ flux in our study. Our results also show some discrepancies with what had been previously observed. For instance Schaaff *et al.* (74) reported that the yeast glycolytic flux remained unaffected by the overexpression of hexokinase, Pfk1p, Pgk1p, Pyk1p, Pdc1p, Gpm1p, or Adh1p, whereas we found a strong association between the $CO_2$ flux and some isoforms of Pgk1p, Pyk1p, Pcd1p, and Adh1p. Similarly, over and underexpression of Pgi1p and Fba1p were shown to be associated with changes in glucose consumption rate, cell size, and the carrying capacity *K* (50), whereas we found no genetic and no environmental variation for isoform abundance of those enzymes. However, those results may not be contradictory, keeping in mind that the variations that we observed between strains are the result of evolutionary processes that occurred during yeast domestication, whereas Schaaf *et al.* (74) and Wang *et al.* (50) had analyzed expression mutants. Indeed, in the MCA perspective, the enzyme selection coefficients for changing the flux are proportional to the flux control coefficients (76). Enzymes having a strong control on the flux are expected to be the primary targets of selection and show less genetic variation within populations. In *Drosophila*, a survey of within and between species polymorphism of 17 enzymes pointed the glucose-6P branchpoint as a specific target of selection (77). It is often assumed that the first steps in a metabolic pathway are exerting strong control over flux (78). This prediction was verified for the pathway of anthocyanin biosynthesis by comparing the rate of evolution of enzyme genes along the pathway in three plant species: Rausher *et al.* (79) showed that upstream enzymes of the pathway were much less variable than downstream enzymes. In yeast, previous works suggested that specific selective pressures shaped the first part of the glycolysis: Pgi1p, Fba1p, and Tpi1p have been conserved as single copy even after independent whole-genome duplication, meaning that one duplicated copy has been lost (80–82). Our results show less genetic variation between strains for the abundance of enzymes of the upper part of the glycolytic pathway, and more variation for the abundance of downstream enzymes, together with strong associations with $CO_2$ flux as well with metabolic or life-history traits. We propose to interpret these features as evidence for unequal sharing-out of the flux control, with a higher control exerted by the upstream enzymes leading to higher evolutionary constraints. Because of those constraints, human selection for modulating the $CO_2$ flux or other traits related to food processing has been only possible through small changes in the abundance of less constrained enzymes, downstream in glycolysis or belonging to the glycerol, acetate, or ethanol pathways, resulting in a higher genetic diversity observed today for those enzymes.

*Human Domestication Shaped the Fermentation Proteome*—Using linear discriminant analysis and subsequent studies, we showed that the fermentation proteome was significantly shaped by human domestication, through the differential targeting of a few isoforms. The distillery strains were separated from beer and wine strains on the basis of Pdc1p-4854, an isoform associated to the main AF metabolites (ethanol, acetate, glycerol concentration per cell). Wine and beer strains have been specifically selected to lower acetate production that is responsible for a well-known off-flavor, the vinegar taste (83), whereas this feature is less important for distilling yeasts. This could explain why winemakers and brewers' selection significantly lowered this isoform. In addition, winemaking selection appeared to have specially targeted the acidic isoform of Tdh3p. In a recent work, Jimenez-Marti and coworkers suggested that Tdh3p might account for strain adaptation to enological conditions (84). This could explain why isoforms of this enzyme had been specially targeted for wine strains. Industrial yeast improvement strategies could build on this result of empirical human domestication and target the regulation of the post-translational forms of the others enzymes involved in metabolic control such as Pdc1p or Adh1p.

*Pdc1p and Adh1p: The Last, but not the Least, Steps of Alcoholic Fermentation*—Deciphering the relationships between the fermentation proteome and metabolism revealed the implication of several isoforms belonging to different enzymes. In particular, isoforms of two proteins, Pdc1p and Adh1p, were associated to alcoholic fermentation: Pdc1p-1589 isoform was found to be related to the $CO_2$ flux, whereas Pdc1p-4854 accounted for an important part (20–32%) of the final ethanol, glycerol, and acetate concentrations. Importantly, this last isoform's abundance was low, and considering the four identified Pdc1p's spots as a whole would have hidden Pdc1p-4854 variation and forbidden the identification of Pdc1p as an essential enzyme controlling AF metabolites production in addition to its role in $CO_2$ flux control. Previous work described the presence of N-terminally acetylated and nonacetylated isoforms of Pdc1p in yeast (85), and close comparison of our 2-DE gels with the yeast proteome map (http://www.ibgc.u-bordeaux2.fr/YPM/) suggests that Pdc1p-4854 may correspond to the nonacetylated isoform whereas Pdc1p-1589 and Pdc1p-1605 may be acetylated forms. Pdc1p N-terminal acetylation is achieved through the excision of the initial methionine and subsequent addition of an acetyl group. Our mass spectrometry analyses allowed us to identify the native (nonacetylated) peptide for Pdc1p-4854 (MSEITLGK), whereas this peptide was not identified for other Pdc1p isoforms. We hypothesized that our mass spectrometry analyses failed to detect the acetylated peptide ($^{*}$SEITLGK) because of its short length (peptides with length inferior to eight amino acids usually have low yet not-significant *p* values ($>0.05$) and are thus missed by the analysis).

Besides Pdc1p, Adh1p's isoforms were found to be significantly related to different metabolic and life-history traits: the nonacetylated isoform of Adh1p (4799) was associated with a significant part of the variation of glycerol, acetate, and biomass formation per cell (15–20%), and was also related to two fitness traits (21–24%), the carrying capacity (K) and cell size,

that define ant and grasshopper life-history strategies. In particular, the degree of acetylation of Adh1p was strongly related to these traits, highlighting the functional importance of N-terminal acetylation. In yeast, the N-terminal acetyltransferase responsible for Adh1p acetylation is NatA (85). NatA is composed of two main subunits (Nat1p and Ard1p), whose mutants are related to the disappearance of acetylated isoforms of several enzymes, including Adh1p and Pdc1p (85). N-terminal acetylation is one of the most common protein modifications in eukaryotes, as 85% of the proteins have an acetylated isoform (86), but it displays several specificities. Unlike other post-translational modifications, N-terminal acetylation is irreversible and occurs during protein synthesis after about 50 amino acid residues have emerged from the ribosome (87). Thus, N-terminal acetylation is sometimes designed as a cotranslational rather than post-translational modification. The biological significance of N-terminal acetylation is still unclear, because no global trend regarding the functional consequences of N-terminal acetylation emerges. For some proteins, N-terminal acetylation may act as a degradation signal (88), whereas for others N-terminal acetylation may protect from proteolytic degradation and subsequently increases their half-life (89). N-terminal acetylation was also shown to be involved in protein sorting and addressing to cellular organelles (90) or to membrane (91). Indeed, although Adh1p is frequently described as a cytoplasmic protein, it is also associated with the plasma membrane (92) like many other fermentation enzymes (Pgi1p, Tpi1p, Eno1p, Eno2p, Tdh1p, Tdh2p, Tdh3p, Pgk1p, Pyk1p). In addition, these enzymes display protein-protein interactions in yeast (93) and also in Human (94), suggesting they may form a large metabolon complex whose plasma membrane localization (92) may be useful for the rapid processing of the glucose entering the cell. This is particularly true for larger cells in which independent cytosolic proteins have less chance to be close together and form an enzyme-to-enzyme channeling of glycolytic intermediates. Moreover, in human erythrocytes, glycolytic enzymes are organized into complexes on the membrane (63) *via* N-terminal residues of some proteins (95) and phosphorylation (63), highlighting the importance of post-translational modifications in metabolon efficiency. We speculate that a few fermentation enzymes could act as a "plasma membrane anchor" of fermentation metabolon, such as acetylated Adh1p. This would be in agreement with the fact that larger cells show a higher degree of N-terminal acetylation of Adh1p. It could also explain why the nonacetylated isoform of Adh1p is negatively correlated to the flux, in contrast to the acetylated one.

In any case, although the real impact of N-terminal acetylation is unknown for Adh1p, we showed a clear correlation between the degree of acetylation of Adh1p and both cell size and carrying capacity, as well as between the nonacetylated isoform and $CO_2$ flux/glycerol/acetate/biomass production. Recently, some works have shown that lysine acetylation was involved in the control of central metabolic pathways in both prokaryotes and eukaryotes (15, 37), but to our knowledge, this is the first time that N-terminal acetylation of an enzyme is shown to be unambiguously associated with both metabolic and life-history traits.

In conclusion, using *Saccharomyces cerevisiae* alcoholic fermentation as a model, we have highlighted the importance of post-translational modifications such as phosphorylation and N-terminal acetylation in metabolic control. Isoforms were also shown to govern other key fitness traits related to cell growth, showing their importance from the functional and evolutionary viewpoints and underlining the usefulness of large-scale approaches at the post-translational level.

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S This article contains supplemental Information, Data sets S1 to S4, Tables S1 and S2 and Fig. S1.

|||| To whom correspondence should be addressed: CNRS, 210 Chemin de Leysotte, Villenave d'omon 33882, France. Tel.: +33 5 57 57 58 64; Fax: +33 5 57 57 58 13; E-mail: albertin@moulon.inra.fr.

**Author contributions:** P.M., M.B., M.A., C.D., D.V. and D.S. designed research; W.A., P.M. M.B. and A.B. performed research; T.B., D.C. and B.V conducted mass spectrometry analyses, O.L. and T.B. released proteomic data through PROTICdb, W.A., P.M., M.B., M.A., T.S., C.D., D.V. and D.S. analyzed data and wrote the paper.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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# A Systems Approach to Elucidate Heterosis of Protein Abundances in Yeast*[S]

**Mélisande Blein-Nicolas[A], Warren Albertin[B,C], Telma da Silva[A,D], Benoît Valot[E], Thierry Balliau[A], Isabelle Masneuf-Pomarède[C,F], Marina Bely[C], Philippe Marullo[C,G], Delphine Sicard[H,I], Christine Dillmann[H], Dominique de Vienne[H], and Michel Zivy[J,K]**

**Heterosis is a universal phenomenon that has major implications in evolution and is of tremendous agro-economic value. To study the molecular manifestations of heterosis and to find factors that maximize its strength, we implemented a large-scale proteomic experiment in yeast. We analyzed the inheritance of 1,396 proteins in 55 inter- and intraspecific hybrids obtained from *Saccharomyces cerevisiae* and *S. uvarum* that were grown in grape juice at two temperatures. We showed that the proportion of heterotic proteins was highly variable depending on the parental strain and on the temperature considered. For intraspecific hybrids, this proportion was higher at non-optimal temperature. Unexpectedly, heterosis for protein abundance was strongly biased toward positive values in interspecific hybrids but not in intraspecific hybrids. Computer modeling showed that this observation could be accounted for by assuming concave relationships between protein abundances and their controlling factors, in line with the metabolic model of heterosis. These results point to nonlinear processes that could play a central role in heterosis. *Molecular & Cellular Proteomics 14: 10.1074/mcp.M115.048058, 2056–2071, 2015.***

Nonadditive inheritance in hybrids, whereby the phenotype of offspring is not the average of the parental phenotypes, is commonly observed in all species. For monogenic traits, the departure from additivity is called dominance (1) or overdominance if the phenotypic value of the hybrid is outside the range defined by the parental values (2). For polygenic traits, it is called heterosis. Heterosis is commonly associated to macroscopic traits, but it also applies to less integrated traits such as metabolite abundances (3, 4), fluxes and enzyme activities (5–7), mRNA, and protein amounts (8).

The concept of heterosis is not universally shared and depends on the scientific communities. Strictly speaking, heterosis is defined as the superiority of the hybrid over the mean parental value (mid-parent heterosis, MPH[1]) or over its parent exhibiting the highest value (best-parent heterosis, BPH). This definition and the associated terminology are historical and come from the fact that heterosis was commonly associated to traits such as growth rate, biomass, size, yield, or fertility, for which higher values are beneficial. However, lower values can also occur (*e.g.* (9)). The definition of heterosis has therefore been broadened to include also the cases where the hybrid is below the mean parental value (negative MPH) or below its parent exhibiting the lowest value (worst-parent heterosis, WPH). In this paper, we will adopt this second definition, motivated by its lack of presumption about whether the changes observed in hybrids are beneficial or detrimental.

Heterosis has fascinated scientists and breeders for more than 100 years. It has major implications in evolution and domestication of crop plants (10, 11), and it has been exploited since the 1930s in plant breeding to produce hybrids of high agronomic value (12). In this context, heterosis has proven to efficiently accelerate the process of selection for various crops (reviewed in (13)). Heterosis is opposite to inbreeding depression, that is supposed to be predominantly caused by the homozygosity of deleterious recessive alleles (14). Heterosis can provide a heterozygote advantage by buffering against these alleles and confers genetic plasticity to adapt to environmental changes (11).

From the [A]INRA, PAPPSO, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France; [B]CNRS, UMR 0320/UMR 8120, Génétique Végétale, F-91190 Gif-sur-Yvette, France; [C]Université Bordeaux, Unité de Recherche Œnologie, EA 4577, ISVV, 210 chemin de Leysotte, 33140 Villenave d'Ornon, France; [D]Ariana Pharmaceuticals, 28 rue du Docteur Finlay, 75015 Paris, France; [E]CNRS, Université de Franche-Comté, UMR 6249 Chrono-Environnement, F-25000, Besançon, France; [F]Bordeaux Sciences Agro, Gradignan, France; [G]BIOLAFFORT, F-33034 Bordeaux, France; [H]Univ Paris-Sud, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France; [I]INRA, UMR1083, 2 Place Viala, F-34060 Montpellier, France; [J]CNRS, PAPPSO, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France

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[1] The abbreviations used are: ANOVA: analysis of variance; MPH, mid-parent heterosis; BPH, best-parent heterosis; CFU, colony-forming unit; LC-MS/MS, liquid chromatography-tandem mass spectrometry; RPM, round per minute; TF, transcription factor; WPH, worst-parent heterosis; YPD, yeast extract peptone dextrose; ZALS, zwitterionic acid labile surfactant.



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Given the importance of heterosis for agriculture and because it is an intriguing phenomenon, many studies have focused on the understanding of its genetic and molecular bases (11, 15–25). Three nonexclusive hypotheses based on genetic effects are classically put forward to explain heterosis. First, the dominance hypothesis attributes heterosis to complementation: In the hybrid, the recessive alleles are masked by dominant and generally favorable alleles (26, 27). Second, the overdominance hypothesis assumes that heterosis is inherent to the heterozygous state (2, 28). Third, the epistasis hypothesis proposes that heterosis is due to intergenic interactions created in the hybrid (29, 30). Scientists have long sought a unifying theory to account for heterosis, but it is now commonly admitted that this phenomenon likely arises from the combination of several genetic mechanisms, the relative effects of which vary according to the trait, the cross, or the species (23, 25).

These genetic effects are consistent with the factors known to maximize the occurrence of heterosis. When compiling the results obtained so far across numerous studies, it appears that heterosis is of greatest magnitude for highly integrated, and hence polygenic, traits such as crop yield (23, 31); it is larger in allogamous than in autogamous species (31); it requires genetic divergence between parents; and interspecific crosses commonly produce higher levels of heterosis (21, 24, 32). However, these general trends are not sufficient for a reliable prediction of heterosis, which is a major challenge for plant and animal breeding and for biotechnology. Future strategies for heterosis prediction will have to rely both on an accurate description of its manifestations and on the detailed knowledge of the factors that maximize its strength. To address these issues, we performed a large-scale study of heterosis by analyzing the inheritance of the abundance of a high number of proteins in an unprecedented number of yeast hybrids grown in two conditions. The proteomic level is particularly relevant to the large-scale study of heterosis because protein abundances are polygenic molecular traits (33) that can be measured by high-throughput quantitative proteomics (34, 35).

Yeast has only rarely been used to study heterosis (9, 36–41). Yet, it is amenable to large-scale laboratory experiments, and it is of major industrial interest for wine making. Hybrids with exceptional performances were reported in *Saccharomyces cerevisiae* (36, 38, 42, 43), and several observations indicate that interspecific hybridization could be used in breeding to produce improved strains for wine making. For instance, many interspecific hybrids between *S. cerevisiae* and *S. uvarum* Beijerinck or *S. kudriavzevii* isolated in wine and natural environments showed important biotechnological potential, such as a better robustness than their parents (44–47). In addition, several wine strains empirically selected for their biotechnological properties proved to be interspecific hybrids (48, 49). For all these reasons, we chose to study heterosis for protein abundance in yeast strains from *S. cerevisiae* and *S. uvarum*, which are the two main species associated with grape juice fermentation (50).

By using shotgun proteomics, we analyzed more than 1,300 proteins in an experimental design, including 11 parental strains of *S. cerevisiae* and *S. uvarum* and their 55 intra- and interspecific hybrids, which were grown at two temperatures to take into account adaptation differences between parental species (18 °C and 26 °C optimal for *S. uvarum* and *S. cerevisiae*, respectively (44, 51, 52)). We showed that heterosis for protein abundance was strongly biased toward positive values in interspecific hybrids but not in intraspecific hybrids, which, to our knowledge, has never been reported. We also showed that our experimental results were consistent with results obtained from modeling approaches assuming nonlinear relationships between protein abundances and their controlling factors.

## EXPERIMENTAL PROCEDURES

*Yeast Strains*—Four diploid *S. uvarum* strains, seven diploid *S. cerevisiae* strains, and their 55 hybrids produced from a half diallel design (53) were analyzed in this study. Parental strains were derived from strains isolated from different geographical locations and from either natural or food-processing origins (Table I): the *S. cerevisiae* strains were isolated from diverse media (distillery, enology, oak exudate) to maximize the genetic diversity within this species (54); the *S. uvarum* strains, originating from grape must or cider fermentation, were chosen to cover a wide part of the genetic diversity of the *S. uvarum* species (Masneuf-Pomarède, I., personal communication). For each original strain, one meiospore was isolated with a micromanipulator (Singer MSM Manual, Singer Instrument, Somerset, UK). All the original strains but Alcotec 24 were homothallic (*HO*/*HO*); therefore, fully homozygous diploid strains were spontaneously obtained by fusion of opposite mating type cells. For A24 (*ho*/*ho*), one isolated haploid meiospore was diploidized via transient expression of the HO endonuclease (55). All strains were grown at 30 °C in YPD medium containing 1% yeast extract (Difco Laboratories, Detroit, MI), 1% bactopeptone (Difco), and 2% glucose, supplemented or not with 2% agar. When necessary, antibiotics were added at the following concentrations: 100 $\mu$g/ml for G418 (Sigma, L'Isle d'Abeau, France), and nourseothricin (Werner bioagent, Jena, Germany) and 300 $\mu$g/ml for hygromycin B (Sigma).

*Construction of the Half Diallel Design*—Hybrid construction was performed as described in Albertin *et al.* (53). Briefly, the 11 diploid parental strains were transformed with a cassette containing the *HO* allele disrupted by a gene of resistance to either G418 (*ho::KanR*), hygromycin B (*ho::HygR*), or nourseothricin (*ho::NatR*). Strain transformation allowed conversion to heterothallism for the homothallic strains. Then the mating-type (*MATa* or *MATalpha*) of antibiotic-resistant monosporic clones was determined using testers of well-known mating type. For each cross, parental strains of opposite mating type were put in contact 2 to 6 h in YPD medium at room temperature and plated on YPD-agar containing the appropriate antibiotics. Ten independent hybrids per cross were recovered. After recurrent cultures on YPD-agar corresponding to ~ 80 generations, the nuclear chromosomal stability of the hybrids was controlled by pulsed field electrophoresis (CHEF-DRIII, Bio-Rad, Marnes-La-Coquette, France) as well as homoplasmy (only one parental mitochondrial genome). One hybrid per cross was finally retained for further experiments.

*Yeast Strain Characterization*—Two polymorphic microsatellites specific to *S. cerevisiae* (Sc-YFR038 and Sc-YML091 (56)) and two

TABLE I
*Origin of parental strains*

| Species | Parental strains | Monosporic derivate | Collection/Supplier[a] | Isolation origin | Area of origin | Reference |
|---|---|---|---|---|---|---|
| *S. uvarum* | PM12 | U1 | ISVV | Grape must fermentation | Jurançon, France | (98) |
| *S. uvarum* | PJP3 | U2 | ISVV | Grape must fermentation | Sancerre, France | (98) |
| *S. uvarum* | Br6.2 | U3 | ADRIA NORMANDIE | Cider fermentation | Normandie, France | (95) |
| *S. uvarum* | RC4–15 | U4 | ISVV | Grape must fermentation | Alsace, France | (57) |
| *S. cerevisiae* | CLIB-294 | D1 | CIRM-Levures | Distillery | Cognac, France | (98) |
| *S. cerevisiae* | Alcotec 24 | D2 | Hambleton Bard | Distillery | UK | (100) |
| *S. cerevisiae* | CLIB-328 | E1 | CIRM-Levures | Enology | UK | (100) |
| *S. cerevisiae* | BO213 | E2 | LAFFORT Oenologie | Enology | France | (101) |
| *S. cerevisiae* | F10 | E4 | LAFFORT Oenologie | Enology | Bordeaux, France | (101) |
| *S. cerevisiae* | VL3 | E5 | LAFFORT Oenologie | Enology | Bordeaux, France | (102) |
| *S. cerevisiae* | YPS128 | W1 | SGRP | Forest, oak exudate | Pennsylvania, USA | (54) |

[a] ISVV, http://www.oenologie.u-bordeaux2.fr/.
ADRIA NORMANDIE, http://www.adria-normandie.com.
SGRP, http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html.
CIRM-Levures, http://www.inra.fr/internet/Produits/cirmlevures.
Hambleton Bard, http://www.hambletonbard.com.
LAFFORT Œnologie, http://www.laffort.com.

specific to *S. uvarum* (locus 4 and 9 (57)) were used to discriminate rapidly the hybrids from the parental strains. These four markers were amplified in a multiplex PCR reaction (95 °C for 5 min for initial denaturation step; 95 °C for 30 s, 55 °C for 90 s, and 72 °C for 60 s repeated 35 times; a final elongation step of 30 min at 60 °C). The PCR products were analyzed on an ABI3730 apparatus (Applied Biosystem, Villebon-sur-Yvette, France), and microsatellite lengths were analyzed using the Peak Scanner tool (Applied Biosystem).

*Alcoholic Fermentation in Grape Must*—All the 66 strains (11 parents and 55 hybrids) were grown in the same batch of white grape must obtained from Sauvignon grapes harvested in vineyards in the Bordeaux area (2009 vintage). Tartaric acid precipitation was stabilized, and turbidity was adjusted to 100 NTU (nephelometric turbidity unit) before storage at −20 °C. The sugar concentration was 189 $g.l^{-1}$, the nitrogen content was 242 $mg.l^{-1}$ and the pH was 3.3. The indigenous yeast population, estimated by YPD-plate counting after must thawing, was less than 20 CFU (colony-forming unit) *per* ml. Precultures of each strain were run in half-diluted must filtered through a 0.45 $\mu$m nitrate-cellulose membrane (24 °C, 150 rpm (rounds per minute)) during 24 h, after what one million cells per ml were sampled and added to a final volume of 125 ml of Sauvignon must. Then, fermentations were run into 125 ml glass reactors at two different temperatures (18 °C and 26 °C, 300 rpm) and repeated three times independently. In total, 396 alcoholic fermentations were performed (66 strains × 2 temperatures × 3 replicates) following a randomized experimental design. Of them, 31 failed due to the poor fermenting abilities of some strains (Table S1). The amount of $CO_2$ released was regularly determined by measurement of glass-reactor weight loss.

*Protein Extraction and Digestion*—Samples were harvested at 40% of $CO_2$ release to perform proteomic analyses. At this time, all strains had reached their maximum population size and performed alcoholic fermentation without growing. Only strain × temperature combinations with at least two successful fermentations were kept for further mass-spectrometry analysis (Table S1). Five milliliters of fermentative media were sampled and centrifuged (5 min, 2,750 g). The pellets were rinsed two times with 5 ml of water, frozen in liquid nitrogen, and stored at −80 °C until protein extraction. Total protein extracts were isolated via acetone precipitation as described in Blein-Nicolas *et al.* (58). Dried protein pellets were solubilized in 300 $\mu$l of a solution containing 6 M of urea, 2 M of thiurea; 10 mM of dithiothreitol (DTT); 30 mM of TrisHCl, pH 8.8; and 0,1% of zwitterionic acid labile surfactant (ZALS, Proteabio, Morgantown, WV, USA) and centrifuged for 10 min at 14,000 rpm. Protein concentration was determined using PlusOne 2-D Quant Kit (GE Healthcare, Velizy-Villacoublay, France) and adjusted to 4 $\mu g.\mu l^{-1}$. After a 10-times dilution in 50 mM of ammonium bicarbonate, proteins were reduced 1 h in 100 mM DTT, alkylated 1 h in 40 mM iodoacetamide, and digested overnight at 37 °C with 1/50 (w/w) trypsin (Promega, Charbonnière, France). Digestion was stopped by adding 0.4% of trifluoroacetic acid (TFA). Peptides were purified on solid phase extraction using polymeric C18 column (Phenomenex, Le Pecq, France) with a washing solution containing 0.06% acetic acid and 3% acetonitrile (ACN). After elution with 0.06% acetic acid and 70% ACN, peptides were speedvac-dried and suspended in 2% ACN and 0.08% TFA.

*LC-MS/MS Analysis*—LC-MS/MS analyses were performed using a NanoLC-Ultra System (nano2DUltra, Eksigent, Les Ulis, France) connected to a Q-Exactive mass spectrometer (Thermo Electron, Waltham, MA, USA). A 700 ng of protein digest were loaded onto a PepMap C18 precolumn (0.3 × 5 mm, 100 Å, 5 $\mu$m; NanoSeparations, Nieuwkoop, Netherlands) at 7.5 $\mu l{\cdot}min^{-1}$ and desalted with 0.1% formic acid and 2% ACN. After 3 min, the precolumn was connected to a PepMap C18 nanocolumn (0.075 × 150 mm, 100 Å, 3 $\mu$m). Buffers were 0.1% formic acid in water (A) and 0.1% formic acid and 100% ACN (B). Peptides were separated using a linear gradient from 5 to 35% buffer B for 40 min at 300 $nl{\cdot}min^{-1}$. One run took 60 min, including the regeneration step at 100% buffer B and the equilibration step at 100% buffer A.

Ionization was performed with a 1.3-kV spray voltage applied to an uncoated capillary probe (10 $\mu$m tip inner diameter; New Objective, Woburn, MA, USA). Peptide ions were analyzed using Xcalibur 2.2 (Thermo Electron) with the following data-dependent acquisition steps: (1) MS scan (mass-to-charge ratio (*m/z*) 400 to 1,400, 70 000 resolution, profile mode), (2) MS/MS (17,500 resolution, collision energy = 30%, profile mode). Step 2 was repeated for the eight major ions detected in step 1. Dynamic exclusion was set to 40 s. Xcalibur raw data files were transformed to mzXML open source format using msconvert software in the ProteoWizard 3.0.3706 package (59). During conversion, MS and MS/MS data were centroided.

*MS Data Availability*—The raw MS output files were deposited online using PROTICdb database (60–62) at the following URL: http://moulon.inra.fr/protic/heterosyeast2.



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*Protein Identification*—Protein identification was performed using the custom database described in Blein-Nicolas *et al.* (58). This database, containing 10,851 entries, was constructed from the translations of all systematically named ORFs of *S. cerevisiae* and *S. uvarum* downloaded from the Saccharomyces Genome Database (SGD project, http://www.yeastgenome.org/, versions dated October 5, 2010 and December 15, 2003, respectively). The proteins of *S. cerevisiae* and of *S. uvarum* encoded by orthologous genes were attributed unique labels. A contaminant database containing the sequences of standard contaminants and the sequences of 16 proteins of *Vitis vinifera* previously identified in extracts of yeast grown in grape juice was also interrogated. The decoy database comprised the reverse protein sequences of the custom database. Database search was performed with X!Tandem (version 2011.12.01.1; http://www.thegpm.org/TANDEM/) with the following settings. Enzymatic cleavage was declared as a trypsin digestion with one possible misscleavage. Carboxyamidomethylation of cysteine residues and oxidation of methionine residues were set to static and possible modifications, respectively. Precursor mass precision was set to 10 ppm. Fragment mass tolerance was 0.02 Th. A refinement search was added with the same settings, except that protein N-ter acetylations were also searched. Only peptides with an E-value smaller than 0.05 were reported.

Identified proteins were filtered and sorted by using X!Tandem-Pipeline (version 3.3.0, http://pappso.inra.fr/bioinfo/xtandempipeline/). Criteria used for protein identification were (i) at least two different peptides identified with an E-value smaller than 0.05 and (ii) a protein E-value (product of unique peptide E-values) smaller than $10^{-4}$. These criteria led to a false discovery rate estimated by using the decoy database of 0.12% and 1.15% for peptide and protein identification, respectively.

*Peptide Quantification and Processing Intensity Data*—Peptides were quantified based on extracted ion chromatograms using MassChroQ software version 1.2.2 (63) with the parameters given in File S1. The detection threshold on min and max were set at 30,000 and 50,000, respectively. Due to progressive fouling of the quadrupole, sensitivity losses were observed over time, leading to a global decrease of measured intensities, particularly for hydrophobic peptides. To take these sensitivity losses into account, samples were classified according to their running order and divided into five blocks representing homogeneous global intensities. For each peptide, the block effect was retrieved and subtracted from intensity measures by using an analysis of variance (ANOVA). Then, normalization was performed to take into account possible global quantitative variations between LC-MS runs. For each LC-MS run, the ratio of all peptide values to their value in the chosen reference LC-MS run was computed. Normalization was performed by dividing peptide values by the median value of peptide ratios.

Raw data (containing intensity measures of 25,060 peptides) were then filtered to remove (i) dubious peptides for which standard deviation of retention time was superior to 60 s, (ii) peptide × strain × temperature combinations quantified in only one replicate, and (iii) peptides shared by several proteins, representing less than 5% of all the quantified peptides. To avoid bias on the estimation of total protein abundances in hybrids, we removed parent-specific peptides by using peptides presenting presence/absence variation among parental strains as a proxy. However, parent-specific peptides were confounded with species-specific peptides, which represented nearly 65% of the valid peptides. To exploit as far as possible the data available for intraspecies crosses, we thus split the dataset into three subsets: one contained *S. cerevisiae* triplets (hybrid and its parents), another contained *S. uvarum* triplets, and the last one contained interspecific triplets. Parent-specific peptides were removed separately in the three subsets. To finish, in order to estimate the peptide effect properly, peptides quantified in less than four strains × temperature combinations in a given subset of data were removed.

*Detection of Protein Abundance Changes*—

Protein abundances were estimated independently in the three subsets of data by using

$$\log(I_{istr}) = \theta_{kst} + D_i + B_r + C_{tr} + \epsilon_{istr}$$

where $I_{istr}$ is the normalized intensity value for peptide $i$ in strain $s$, temperature $t$, and replicate $r$,

$\theta_{kst}$ is the natural logarithm of the abundance of protein $k$ in strain $s$ and temperature $t$,

$B_r \sim N(0,\sigma_B^2)$ is an error due to the biological variation of replicate,

$C_{str} \sim N(0,\sigma_C^2)$ is an error due to the technical variation of sample $str$,

$D_i \sim N(0,\sigma_D^2)$ is an error due to the LC-MS response of peptide $i$, and

$\epsilon_{istr} \sim N(0,\sigma_\epsilon^2)$ is the residual error.

Estimation of the parameters of the model was performed as described in Blein-Nicolas *et al.* (64). Protein abundance changes were detected by multiple test procedure across four different contrasts: (i) hybrid-mean of parents, (ii) hybrid–parent$_1$, (iii) hybrid–parent$_2$, (iv) parent$_1$–parent$_2$. Since several couples of strains × temperature combinations and several proteins were tested, p-values were adjusted for multiple testing by a Benjamini–Hochberg procedure (65). Of note, the statistical power was reduced in the subset of data containing interspecific hybrids compared with the two other subsets since intensity data were more drastically filtered (on average, there were 6.2 peptides per protein in the subset containing interspecific hybrids against 8.9 and 8.2 in the subsets containing *S. cerevisiae* hybrids and *S. uvarum* hybrids, respectively).

*Data Analysis*—Protein abundances estimated in different subsets of data were not directly comparable. To overcome this drawback, the subset of data containing interspecific hybrids (further named *B* for between) was taken as a reference, and the following linear regression was performed for each protein in the subsets of data containing intraspecific hybrids (referred to as *W* for within):

$$\theta_{pt}^W = \mathrm{a} + \mathrm{b}\theta_{pt}^B + \epsilon_{pt}$$

where $\theta_{pt}^W$ and $\theta_{pt}^B$ are the abundances estimated in parental strain $p$ at temperature $t$ in the subsets of data $W$ and $B$, respectively,

a and $b$ are the parameters of intercept and slope, respectively, and

$\epsilon_{pt}$ is the residual error.

The median of the coefficient of determination $R^2$ was 0.83, indicating that the protein abundances estimated separately in different subsets of data were globally well correlated. For proteins with $b$ significantly different from 0 (adjusted $p < .05$), estimators of $a$ and $b$ were used to correct the abundances estimated for intraspecific hybrids:

$$\omega_{ht}^W = (\theta_{ht}^W - \hat{\mathrm{a}})/\hat{\mathrm{b}}$$

where $\theta_{ht}^W$ is the abundance estimated in hybrid $h$ at temperature $t$ in the subset $W$. Then, protein abundances in the subset $B$ were gathered with the $\omega_{ht}^W$ computed in the subset $W$.

A total of 615 proteins quantified in more than 122 strains × temperature combinations were kept for data representation as heat map and principal component analysis. Missing data were imputed from a uniform distribution with minimum = 0 and maximum = $10^6$ under the hypothesis that they corresponded to low abundance values.

All data analyses and graphical representations were performed using R version 3.0.2 (66). Appropriate statistical tests were used for each kind of data: $\chi^2$ tests were used to compare distributions; Student and Mann–Whitney tests were used to compare means in the



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case of normally distributed and nonparametric data, respectively; Pearson and Kendall correlation tests were used to analyze associations between normally distributed data and counting data, respectively; and analyses of variance were performed by using a linear model for normally distributed data or generalized linear model with Poisson distribution for counting data. Residuals were examined for normality and independence.

*In Silico Simulation of Heterosis*—We wrote an R program to simulate heterosis in the framework of a nonlinear genotype–phenotype relationship (File S2). We assumed that the protein abundances were controlled by 10 factors ($i = 1, \ldots, 10$), the inheritance of which was either additive of nonadditive. We varied the number of polymorphic factors from 1 to 10. Each factor was defined by its value $E_i$ (concentration or activity of factor $i$) and its contribution to the abundance of the controlled protein, $a_i$. The $E_i$ values were drawn in a gamma distribution (mean = 6, coefficient of variation = 0.3; $\sim \Gamma(11.11, 0.54)$) and the $a_i$ in a uniform distribution $\sim U(1,10)$. These parameters were chosen to get distributions of protein abundances similar to the distributions observed for the most abundant proteins, which are right skewed and have coefficients of variation around 0.2–0.3. For a given protein, the $a_i$'s were the same for the parents and their hybrid. Homozygous parents were created by randomly attributing an $E_i$ value (allelic value) to each factor. The Euclidean distance between two parents $j$ and $j'$ was computed as follows:

$$D_{jj'} = \sqrt{\sum_{i=1}^{10}(E_{ij} - E_{ij'})^2}$$

Protein abundances were computed assuming a concave relationship between the factors and the abundances. To this end, we used a simple hyperbolic function derived from that of the metabolic control theory (67):

$$A_j = \frac{X}{\sum_{i=1}^{10}\left(\frac{1}{a_i E_{ij}}\right)},$$

where $A_j$ is the abundance of the protein in parent $j$ and $X$ is a constant.

To compute protein abundance in the hybrids, we took into account an index of inheritance $x_{ijj'}$ drawn in a normal distribution N (0.5, 0.15). If $x_{ijj'} = 0.5$, the factor was additively inherited in the hybrid between parents $j$ and $j'$, otherwise there was positive or negative deviation from additivity. If $x_{ijj'} = 0$ (respectively $x_{ijj'} = 1$), there was strict dominance of parent $j$ (respectively $j'$) over parent $j'$ (respectively $j$). Therefore, the abundance of a protein in a hybrid is written:

$$A_{jj'} = \frac{X}{\sum_{i=1}^{10}\left(\frac{1}{(x_{ijj'}[n_{ijj'} - 1] + 1)a_i E_{ij}}\right)},$$

where $A_{jj'}$ is the abundance of the protein in the hybrid between parents $j$ and $j'$, and $n_{ijj'} = E_{ij'}/E_{ij}$.

The simulations were performed with 20,000 proteins (Figs. 8*C* and 8*E* and Fig. S6) or 400,000 proteins (Figs. 8*B* and 8*D*)

## RESULTS

*Protein Quantification by LC-MS/MS*—A total of 396 alcoholic fermentations (66 strains × 2 temperatures × 3 replicates) were performed, of which 31 failed due to the poor fermenting abilities of some strains (Table S1). Yeast samples taken from the 365 successful fermentations were analyzed by shotgun label-free quantitative proteomics. Detailed information on all the peptides and proteins identified in all LC-MS/MS runs are shown in Table S2 and S3, respectively. Peptides were quantified by integrating precursor ion peak areas. The quantification measurements obtained for each peptide are shown in Table S4.

In total, 1,583 proteins were quantified in at least one strain × temperature combination (Table S5). Of them, 1,396 proteins were quantified both in a hybrid and its parents at the same temperature. These 1,396 proteins belonged to 16 functional categories following the MIPS Functional Catalogue Database (68) (Fig. S1, Table S6). Metabolism was the most represented category, with 534 proteins (31.1% coverage; Fig. S1).

Representation of protein abundances as a heat map showed that the strain × temperature combinations were separated in three main clusters corresponding globally to *S. uvarum* strains, interspecific hybrids, and *S. cerevisiae* strains (Fig. 1, clusters A, B, and C, respectively). Interspecific hybrids differed from all the other strains by a cluster of proteins that were globally more abundant than in the other strains (Fig. 1, cluster II). *S. uvarum* strains and *S. cerevisiae* strains differed by two clusters of proteins: one containing proteins that were more abundant in *S. cerevisiae* (Fig. 1, cluster I) and one containing proteins that were more abundant in *S. uvarum* (Fig. 1, cluster III). Except for a particular group containing the parental strain D2 and all its descendants including interspecific hybrids (Fig. 1, cluster D), the strains × temperature combinations within the clusters A, B, and C were grouped by temperature.

*Protein Inheritance Patterns*—To analyze the inheritance of protein abundances at a given temperature, we considered the triplets (formed by one hybrid and its parents) where at least two successful fermentations were obtained for each member. This was the case for 53 triplets at 18 °C and for 44 triplets at 26 °C (Table S1). For each protein × hybrid × temperature combination, we computed the deviation from additivity ($d$) as the difference between hybrid abundance and mid-parental abundance. A protein was considered as heterotic whenever $d$ was significantly different from zero (Wald test, adjusted $p < .05$, Table S7). A total of 97,360 protein × hybrid × temperature combinations were examined. For 65.2% (63,469) of them, no significant abundance variation was detected neither between a hybrid and its parent nor between parents (invariant proteins). The remaining 33,891 protein × hybrid × temperature combinations were classified depending on their inheritance pattern (Tables S7 and S8, Fig. 2): 66.8% (22,634) displayed additivity; 11.7% (3,965) displayed negative or positive MPH, meaning that the protein abundance in the hybrid was within the parental range; 11.0% (3,746) displayed BPH or WPH, meaning that the protein abundance in the hybrid fell outside the parental range; and 10.5% (3,546) corresponded to cases of unresolved heterosis

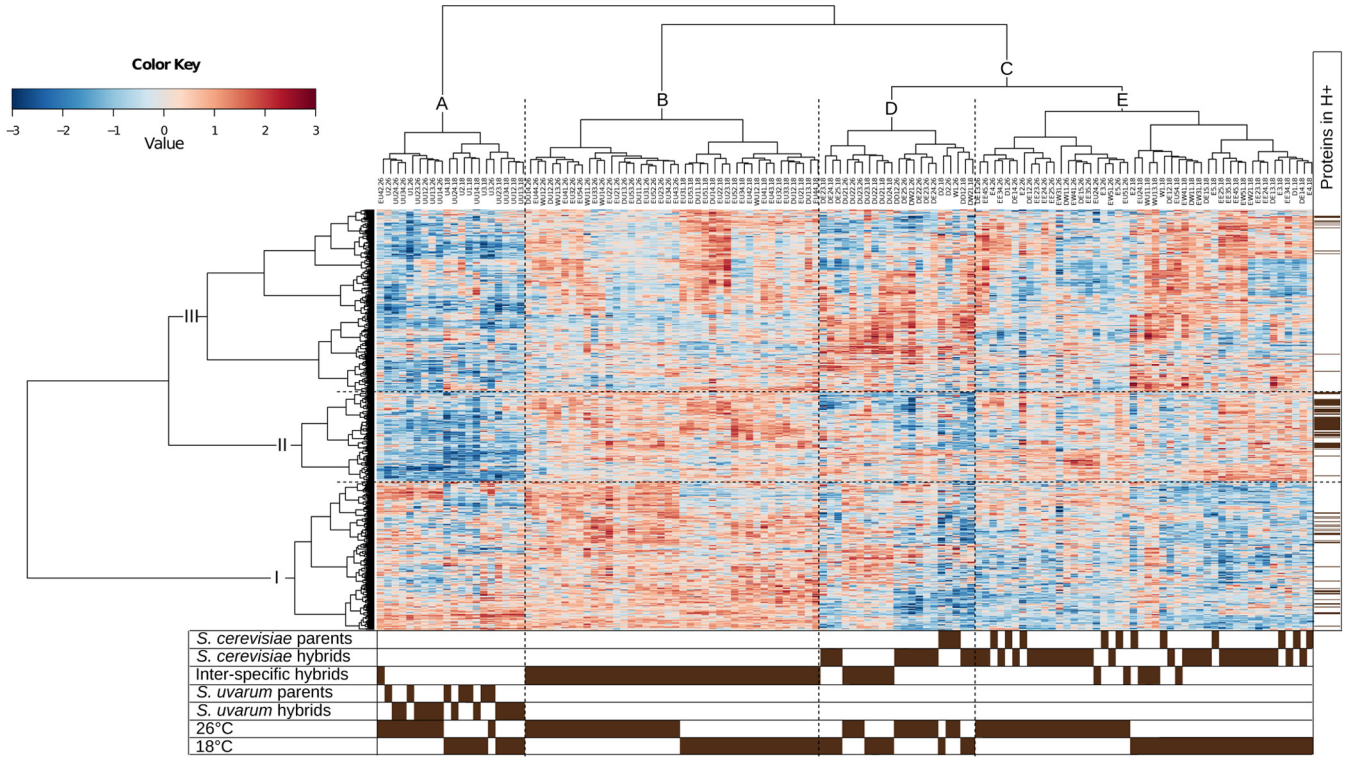


FIG. 1. **Heat map representation of the estimated protein abundances.** Each line corresponds to a protein and each column to a strain × temperature combination. A total of 615 proteins that were quantified in at least 122 strains × temperature combinations are presented (see experimental procedures for details). For each protein, abundance values were scaled and represented by a color code as indicated by the color-key bar: blue for low abundances and red for high abundances. Hierarchical clusterings of the strains (*top*) and of the proteins (*left*) were built by using Euclidean distances and Ward aggregation method. Letters on the top indicate clusters of strain × temperature combinations presenting similar proteomes. Roman numerals on the left indicate clusters of proteins exhibiting similar abundance patterns. Membership of a protein to the set H+ (see results and Fig. 6) is shown in brown on the right. The type of strain and the growth temperature is indicated in brown at the bottom.

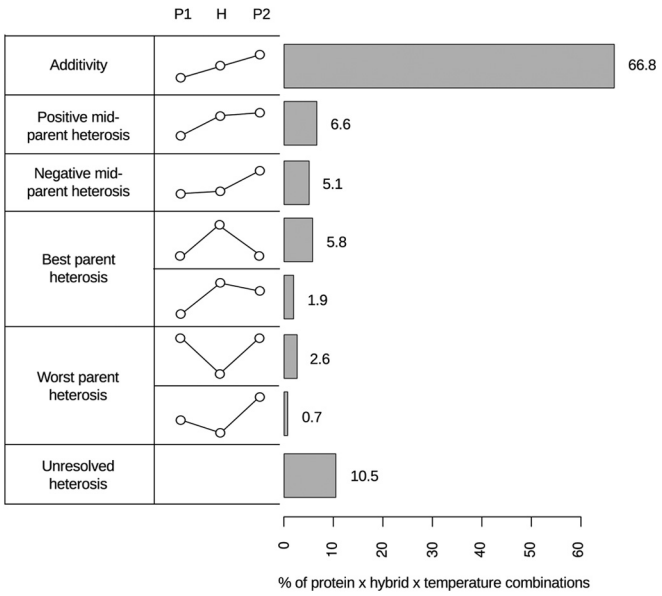


FIG. 2. **Inheritance pattern of the proteins exhibiting abundance variation between hybrid and parental strains.** P1, parent 1; H, hybrid; P2, parent 2.

because statistical tests did not allow us to distinguish between mid-parent and best/worst-parent heterosis.

The proportion of heterotic proteins per hybrid × temperature combination (invariant proteins omitted) was highly variable, ranging from 8.4 to 61.2% with a median at 31.4% (Table II). Globally, hybrids having at least one *S. cerevisiae* strain as a parent showed more heterotic proteins at 18 °C than at 26 °C (Fig. 3). On the contrary, *S. uvarum* intraspecific hybrids showed slightly more heterotic proteins at 26 °C than at 18 °C (Fig. 3).

*Interspecific Hybrids Exhibit Specific Characteristics Regarding Protein Abundance Inheritance*—We further analyzed heterosis for protein abundance in inter- *versus* intraspecific hybrids. By examining the distribution of relative additivity deviation (computed as $d/m$, where $m$ is the parental mean), we showed that $d/m$ was globally higher in inter- than in intraspecific hybrids (Fig. 4*A*, Fig. S2). In addition, the proportion of heterotic proteins with positive $d$ values was, on average, much higher in inter- than intraspecific hybrids (78.8%, 52.3%, and 42.6% in interspecific, *S. cerevisiae* and *S. uvarum* hybrids, respectively; Fig. 4*B*). This indicates a strong bias toward positive heterosis in interspecific hybrids.



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TABLE II

*Counting of quantified proteins, invariant proteins and heterotic proteins in each hybrid x temperature combination at 18 °C, 26 °C, and at both temperatures*

| Hybrid | 18 °C | | | 26 °C | | | Both 18 and 26 °C | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total nb of proteins | % of invariant proteins (a) | % of heterotic proteins (b) | Total nb of proteins | % of invariant proteins (a) | % of heterotic proteins (b) | Total nb of proteins | % of invariant proteins (a) | % of heterotic proteins (b) |
| DD12 | 1,189 | 64.5 | 29.6 | 1,210 | 62.8 | 41.1 | 1,174 | 48.5 | 10.1 |
| DE12 | 1,217 | 68.4 | 39.5 | 1,178 | 65.9 | 30.6 | 1,164 | 51.6 | 11.4 |
| DE13 | 1,212 | 85.2 | 23.5 | 1,184 | 80.8 | 33.9 | 1,174 | 72.1 | 0.9 |
| DE14 | 1,222 | 81.8 | 17.9 | 1,210 | 77.0 | 37.8 | 1,202 | 68.3 | 2.1 |
| DE15 | 1,190 | 73.6 | 25.8 | 1,221 | 72.3 | 37.0 | 1,182 | 59.8 | 7.8 |
| DE22 | NA | NA | NA | 1,127 | 65.7 | 30.2 | NA | NA | NA |
| DE23 | 1,178 | 56.9 | 51.4 | 1,208 | 70.1 | 30.5 | 1,163 | 47.7 | 9.5 |
| DE24 | 1,187 | 63.5 | 48.0 | 1,216 | 62.7 | 17.0 | 1,177 | 47.6 | 5.7 |
| DE25 | 1,187 | 60.7 | 37.9 | 1,209 | 58.6 | 43.1 | 1,170 | 43.1 | 10.4 |
| DW11 | 1,214 | 69.9 | 20.2 | 1,216 | 71.2 | 36.9 | 1,198 | 57.0 | 7.2 |
| DW21 | 1,175 | 64.2 | 31.4 | 1,212 | 61.5 | 46.3 | 1,162 | 47.3 | 12.4 |
| EE23 | 1,203 | 71.0 | 39.8 | 1,178 | 69.9 | 29.1 | 1,156 | 54.2 | 4.2 |
| EE24 | 1,211 | 69.9 | 46.8 | 1,168 | 65.8 | 20.1 | 1,153 | 51.4 | 2.9 |
| EE25 | 1,205 | 59.6 | 61.2 | 1,176 | 70.3 | 25.8 | 1,157 | 47.2 | 7.4 |
| EE34 | 1,207 | 83.8 | 41.5 | 1,213 | 77.4 | 25.9 | 1,193 | 70.7 | 6.9 |
| EE35 | 1179 | 73.8 | 46.3 | 1,215 | 80.1 | 38.0 | 1,169 | 63.8 | 8.7 |
| EE45 | 1,223 | 76.6 | 36.7 | 1,219 | 68.1 | 33.7 | 1,209 | 59.5 | 7.8 |
| EW21 | 1,200 | 61.7 | 55.0 | NA | NA | NA | NA | NA | NA |
| EW31 | 1,197 | 70.7 | 23.1 | 891 | 67.0 | 33.3 | 884 | 54.2 | 5.2 |
| EW41 | 1,211 | 67.2 | 28.5 | 1,214 | 60.1 | 25.0 | 1,194 | 47.1 | 6.3 |
| EW51 | 1,215 | 69.4 | 44.9 | 1,217 | 66.0 | 21.7 | 1,200 | 50.8 | 4.7 |
| DU11 | 860 | 60.3 | 35.5 | 870 | 56.4 | 24.0 | 841 | 42.7 | 6.2 |
| DU12 | 883 | 58.9 | 25.9 | 869 | 59.4 | 34.3 | 844 | 42.5 | 9.5 |
| DU13 | 886 | 52.8 | 38.0 | 878 | 58.4 | 20.5 | 857 | 41.1 | 7.1 |
| DU14 | 820 | 58.8 | 49.7 | NA | NA | NA | NA | NA | NA |
| DU21 | 813 | 59.0 | 30.9 | 837 | 59.1 | 21.3 | 790 | 43.4 | 5.1 |
| DU22 | 821 | 56.3 | 40.4 | 851 | 54.2 | 39.7 | 795 | 39.0 | 11.1 |
| DU23 | 804 | 55.0 | 24.0 | 854 | 55.9 | 23.3 | 794 | 39.7 | 6.1 |
| DU24 | 779 | 61.6 | 34.8 | NA | NA | NA | NA | NA | NA |
| EU21 | 833 | 61.3 | 32.9 | 806 | 57.7 | 15.5 | 778 | 42.4 | 4.5 |
| EU22 | 827 | 54.5 | 39.9 | 807 | 58.9 | 27.1 | 772 | 38.0 | 3.8 |
| EU23 | 836 | 52.3 | 46.4 | 813 | 59.7 | 20.7 | 778 | 39.2 | 5.9 |
| EU24 | 781 | 71.3 | 19.6 | NA | NA | NA | NA | NA | NA |
| EU31 | 841 | 61.1 | 37.3 | 865 | 63.7 | 31.2 | 822 | 49.0 | 8.8 |
| EU32 | 833 | 61.1 | 27.8 | 874 | 66.2 | 44.7 | 812 | 49.9 | 9.3 |
| EU33 | 841 | 64.4 | 21.7 | 856 | 62.1 | 42.9 | 812 | 48.8 | 7.0 |
| EU34 | 795 | 64.3 | 25.4 | NA | NA | NA | NA | NA | NA |
| EU41 | 877 | 58.3 | 37.4 | 820 | 57.3 | 16.9 | 811 | 41.3 | 5.0 |
| EU42 | 878 | 56.0 | 44.0 | 834 | 65.9 | 24.6 | 822 | 45.3 | 8.9 |
| EU43 | 880 | 56.5 | 32.9 | 868 | 56.0 | 22.8 | 845 | 41.4 | 6.9 |
| EU44 | 822 | 57.5 | 35.5 | NA | NA | NA | NA | NA | NA |
| EU51 | 870 | 60.3 | 46.7 | 843 | 66.0 | 8.4 | 826 | 48.9 | 0.9 |
| EU52 | 871 | 53.5 | 44.2 | 874 | 64.6 | 21.7 | 844 | 43.1 | 5.4 |
| EU53 | 871 | 50.4 | 36.1 | 869 | 60.8 | 25.2 | 844 | 39.1 | 6.4 |
| EU54 | 816 | 65.0 | 36.4 | NA | NA | NA | NA | NA | NA |
| WU11 | 804 | 63.8 | 32.3 | 803 | 58.5 | 28.5 | 783 | 44.6 | 4.8 |
| WU12 | 803 | 60.6 | 33.9 | 803 | 59.9 | 28.9 | 780 | 43.3 | 6.1 |
| WU13 | 809 | 56.4 | 39.9 | 800 | 54.5 | 28.3 | 782 | 42.7 | 13.2 |
| UU12 | 1,050 | 77.1 | 23.3 | 1,038 | 67.3 | 27.1 | 1,031 | 59.6 | 3.8 |
| UU13 | 1,052 | 72.3 | 27.1 | 1,044 | 66.1 | 39.5 | 1,036 | 56.9 | 8.9 |
| UU14 | 1,047 | 72.0 | 49.8 | NA | NA | NA | NA | NA | NA |
| UU23 | 1,038 | 62.3 | 30.7 | 1,036 | 72.2 | 30.6 | 1,023 | 51.9 | 4.7 |
| UU24 | 1,053 | 76.3 | 28.4 | NA | NA | NA | NA | NA | NA |
| UU34 | 1,050 | 66.6 | 24.2 | NA | NA | NA | NA | NA | NA |

(a) proteins whose abundance did not vary neither between a hybrid and its parent nor between parents.

(b) invariant proteins omitted.

We next looked whether the temperature affected protein inheritance similarly in interspecific hybrids compared with intraspecific hybrids. For the majority of the protein × hybrid combinations (82.5%), the protein was heterotic at only one temperature. This indicates that heterosis for protein abundance is generally dependent on the temperature. For the remaining 17.5%, four scenarios were possible depending on the sign of *d*: positive at both 18 °C and 26 °C (+/+), negative at both 18 °C and 26 °C (−/−), positive at 18 °C and negative at 26 °C (±), negative at 18 °C and positive at 26 °C (−/+).



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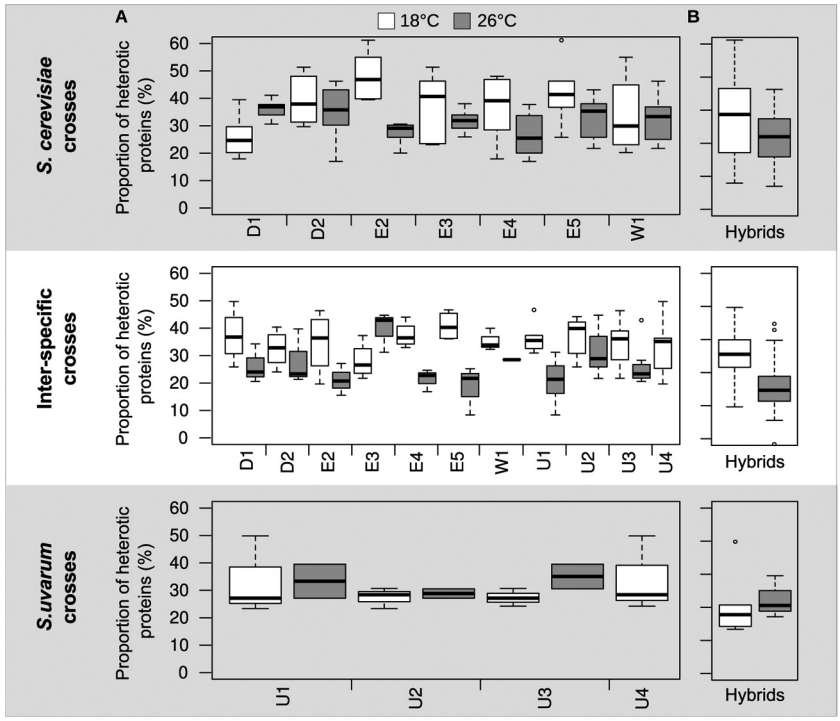


FIG. 3. **Relationships between the proportion of heterotic proteins, the parental strains and the temperature.** (*A*) Distribution of the proportion of heterotic proteins according to parental strains and temperature. (*B*) Distributions of the proportion of heterotic proteins among *S. cerevisiae* hybrids, interspecific hybrids and *S. uvarum* hybrids at the two temperatures.

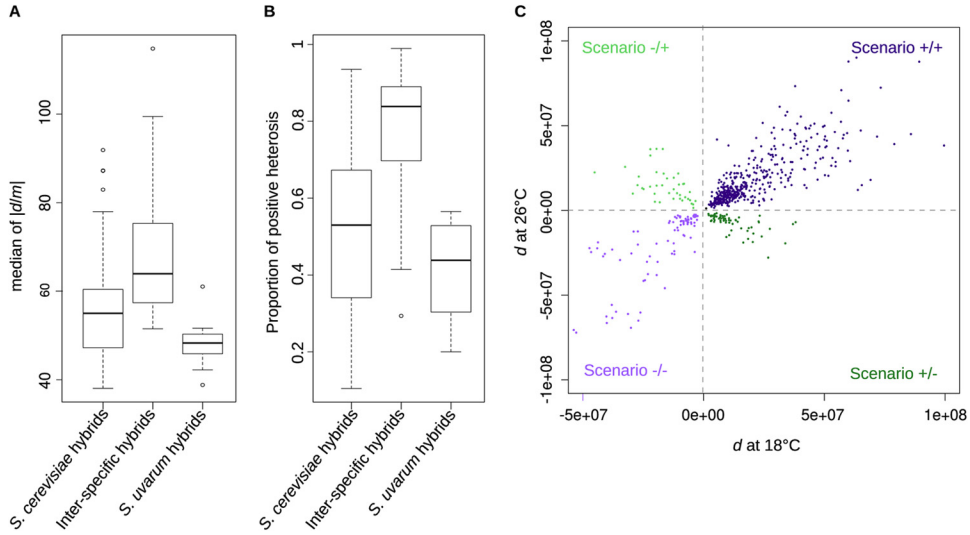


FIG. 4. **Comparing additivity deviations in inter- and intra-specific hybrids.** (*A*) Distributions of the medians of absolute values of relative additivity deviation (*d*/*m*) computed from all the proteins analyzed in the hybrid × temperature combinations. (*B*) Distribution of the proportions of proteins showing positive heterosis in the hybrid ×x temperature combinations. (*C*) Relationships between additivity deviation (*d*) at 18 °C and at 26 °C for the proteins exhibiting heterosis at the two temperatures in interspecific hybrids. The same representation for intra-specific hybrids is shown in Fig. S4.

Globally, interspecific hybrids presented an excess of +/+ scenarios (451 over 656, $\chi^2$ test, $p = 7.6 \times 10^{-22}$; Fig. 4*C*). This result holds true for nearly all interspecific hybrids (Fig. S3*A*). Regarding intraspecific hybrids, *S. cerevisiae* hybrids presented an excess of ± scenarios (252 over 719, $\chi^2$ test, $p = 1.1 \times 10^{-15}$; Fig. S4*A*), while *S. uvarum* hybrids lacked ± scenarios (6 over 79, $\chi^2$ test, $p = 4.8 \times 10^{-14}$; Fig. S4*B*). However, this result largely depended on the hybrid considered (Fig. S3*B*).

*The Remodeling of the Proteome of Interspecific Hybrids Predominantly Affects Particular Categories of Proteins*—Principal component analysis based on the estimated protein abundances was performed in order to visualize the effects of the strains and of the temperature on the proteome (Fig. 5). The first axis (PC1, 15% of the total variance) separated the parental and hybrid strains of *S. cerevisiae* from those of *S. uvarum,* with interspecific hybrids located between the two species. Interestingly, within each type of hybrid (*S. cerevi-*

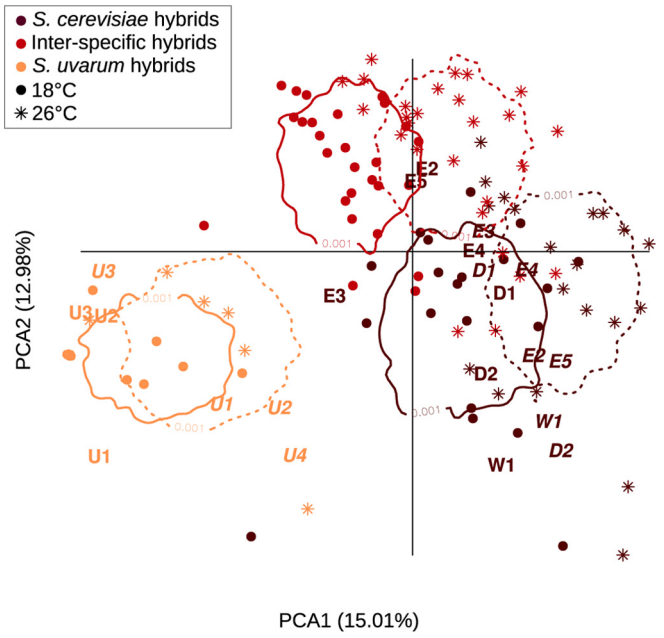


FIG. 5. **Principal component analysis based on the estimated abundances of 615 proteins quantified in at least 122 strains x temperature combinations (see experimental procedures for details).** Parental strains are written in upright (18 °C) or italics (26 °C) characters. Plain and dotted lines represent the limits that contain 99.9% of the distribution of the PC1 and PC2 coordinates of strain × temperature combinations for each group. They were obtained by simulating the kernel densities from group's means and variances assuming bivariate normal distributions and using the R package MASS.

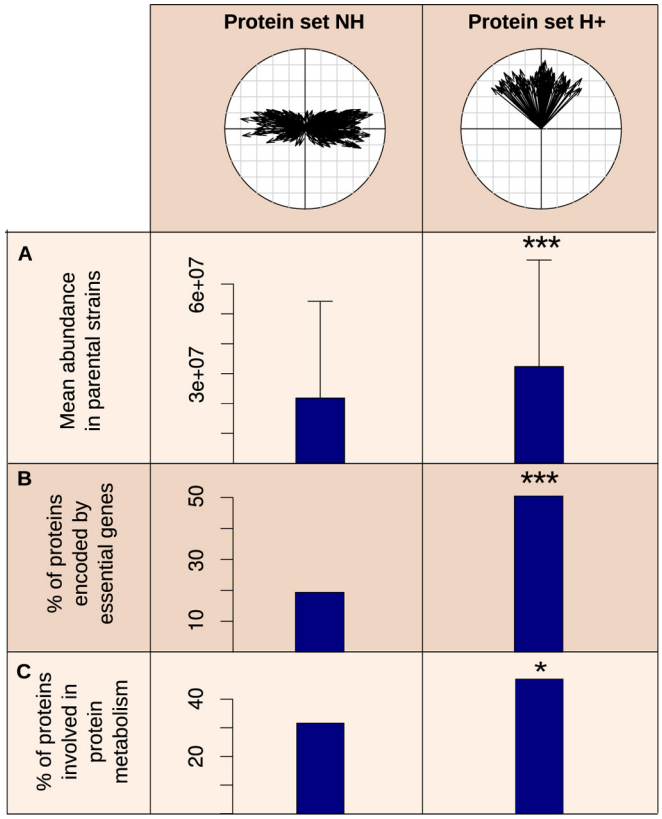


FIG. 6. **Characteristics of the proteins in the set H+.** The proteins correlated with $r > 0.5$ to the second axis of the PCA shown Fig. 5 (Pearson correlation test, set H+) were compared with those that were not correlated (set NH) for: (*A*) the mean abundance in parental strains (Student test); (*B*) the proportion of proteins encoded by essential genes ($\chi^2$ test); (*C*) the proportion of proteins involved in protein metabolism ($\chi^2$ test). Symbols: . $6.10^{-2} > p \geq 5.10^{-2}$; * $5.10^{-2} > p \geq 5.10^{-3}$; ** $5.10^{-3} > p \geq 5.10^{-4}$; *** $5.10^{-4} > P$.

*siae*, *S. uvarum,* and interspecific), PC1 also separated hybrid × temperature combinations according to the temperature. Globally, the effect of temperature on the proteome was similar for all the genotypes: *S. uvarum*, *S. cerevisiae,* and interspecific strains grown at 26 °C were shifted to the right of PC1. Consequently, *S. uvarum* strains moved along PC1 toward *S. cerevisiae* when temperature changed from 18 °C to 26 °C, and reciprocally, *S. cerevisiae* strains moved along PC1 toward *S. uvarum* when temperature changed from 26 °C to 18 °C. This result shows that, when a species is grown at nonoptimal temperature, its proteome tends to resemble that of the other species for which the temperature is optimal.

The second axis (PC2, 13% of the total variance) separated interspecific hybrids from the other strains. PC2 contributed nearly as much as PC1 to the total variance, indicating that interspecific hybridization has extensively remodeled the proteome. To characterize the proteins involved in the differentiation of interspecific hybrids, we analyzed the proteins significantly correlated to PC2 (Pearson correlation test, adjusted $p < 0.01$) with $r > 0.5$ (set H, 104 proteins; Table S9). For all of them but one, $r$ was positive, which indicates that these proteins contributed positively to a greater abundance in interspecific hybrids, regarding the part of variation represented by PC2. This is in agreement with Fig. 1, showing that the majority of the proteins in the set H+ (*i.e.* the set H without the protein negatively correlated to PC2) were included in cluster II. These proteins contributed poorly to PC1, which is consistent with the fact that they displayed little abundance variation between the parents of interspecific hybrids and between temperatures (Fig. S5). Compared with the proteins that were not correlated to PC2 (set NH, 253 proteins; Table S9), these proteins exhibited other specific characteristics: They were more abundant than other proteins (average abundance in parental strains: $2.2 \times 10^7$ in NH *versus* $3.1 \times 10^7$ in H+; Student test, $p = 2.5 \times 10^{-64}$, Fig. 6*A*); they were significantly enriched in proteins encoded by essential genes, *i.e.* genes that are required for viability of *S. cerevisiae* under standard laboratory conditions (69, 70) (22,6% in NH *versus* 55.3% in H+; $\chi^2$ test, $p = 1.6 \times 10^{-7}$, Fig. 6*B*); and they were slightly enriched in proteins involved in protein metabolism (protein synthesis and protein fate; 32.0% in NH *versus* 49.0% in H+; $\chi^2$ test, adjusted $p = .029$; Fig. 6*C*).

Altogether, these results show that interspecific hybridization caused BPH for a defined portion of the proteome that

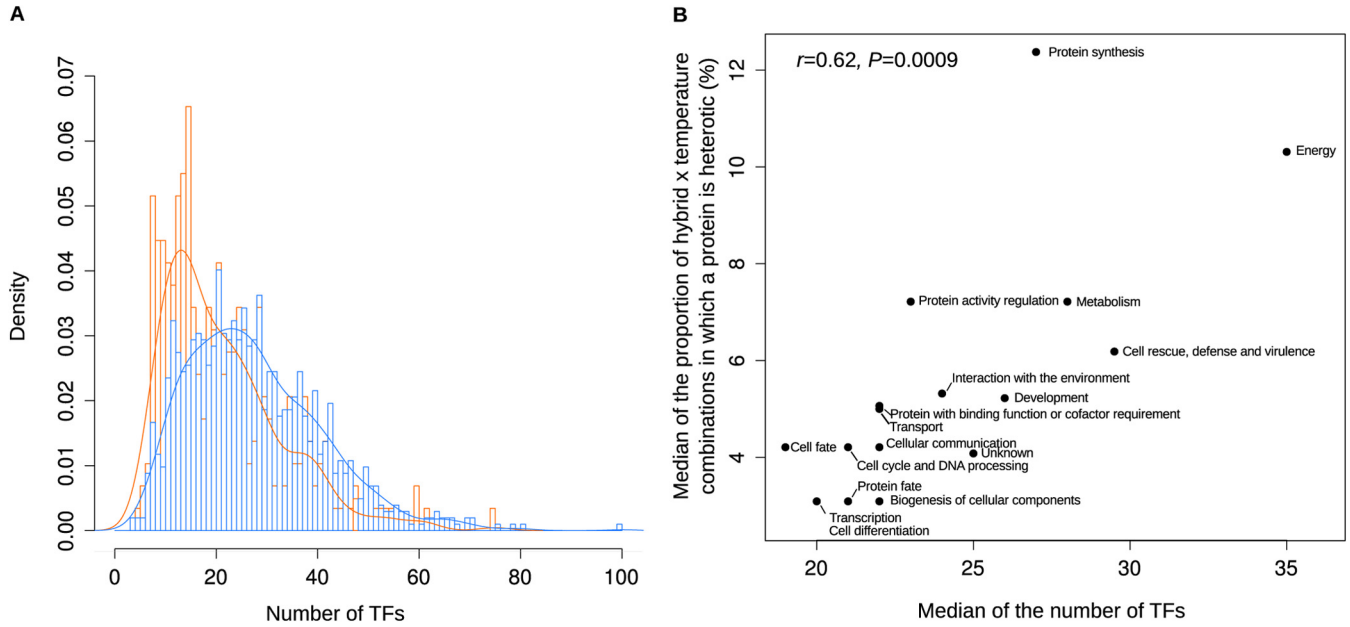


FIG. 7. **Relationships between the frequency at which a protein is heterotic and the number of putative transcription factors (TFs) of a gene.** (*A*) Distributions of the number of putative TFs of a gene for proteins that were heterotic in at least one hybrid × temperature combination (blue) and proteins never observed as heterotic (orange). (*B*) Relationship between the proportion of strain × temperature combinations in which proteins were heterotic and the number of putative TFs of the encoding genes for data organized by functional category.

contains proteins characterized by the stability of their abundances toward genetic and environmental changes, by their high abundances, and by their importance for the cell viability.

*Heterosis for Protein Abundance Is Partly Related to the Complexity of Transcriptional Regulation*—To determine the extent to which the factors controlling protein abundances could be involved in heterosis for protein abundance, we focused on the transcription factors (TFs) possibly involved in the regulation of the genes encoding the proteins quantified in our study. A total of 162 TFs with a consensus DNA-binding sequence were retrieved from the Yeastract database (www.yeastract.com; 71–74). On average, the genes encoding proteins that were heterotic in at least one hybrid × temperature combination were putative targets of a higher number of TFs than the genes encoding non-heterotic proteins (27.7 *versus* 21.4; Mann–Whitney test $p = 1.96 \times 10^{-15}$; Fig. 7*A*). In addition, a significant correlation was found between the number of putative TFs of a gene and the proportion of hybrids × temperature combinations in which the encoded protein was heterotic (Kendall correlation test, $r = 0.18$, $p < 2.2 \times 10^{-16}$, Fig. S6).

The number of putative TFs of a gene depended significantly on the functional category of the gene (generalized linear model, ANOVA $p < 2.2 \times 10^{-16}$). As a consequence, the frequency at which a protein was heterotic was also dependent on its functional category. For example, the genes involved in metabolism, energy and cell rescue, defense, and virulence had, on average, more putative TFs, and their proteins were more frequently heterotic than those involved in cell differentiation (Fig. 7*B*). Note that the protein synthesis category appeared as an outlier, containing proteins that were heterotic in a high proportion of hybrids but not presenting a very high number of putative TFs.

Altogether, these results suggest that the number of factors involved in transcriptional regulation may have an influence on heterosis for protein abundance, which may also explain why some functional categories are more prone to heterosis than others.

*Predicting Protein Inheritance According to a Nonlinear Model*—A general property of metabolic systems is the nonlinear response of the fluxes to genetic variations of enzyme concentrations and/or activity parameters (75). This relationship allowed Kacser and Burns (76) to propose a metabolic basis for dominance. In addition, Fiévet *et al.* (7) showed that when two or more enzymes are variable, the concave relationship between a flux and its parameters necessarily results in positive MPH or in BPH for the flux when the enzyme parameters, *i.e.* any genetic parameter that determines the enzymatic activity, are additively inherited (Fig. 8*A*).

Interestingly, the protein synthesis rates seem also to be a concave function of various factors, such as mRNA amount (77), translation factor abundance (78), ribosomal initiation rate, and elongation rate (79). Therefore, the basis of heterosis put forward for metabolic fluxes could apply for protein abundances, even though the relationship is mathematically different. In order to test this hypothesis and interpret our results, we used a simple nonlinear function for modeling and simulating the consequences of concavity on protein heterosis.



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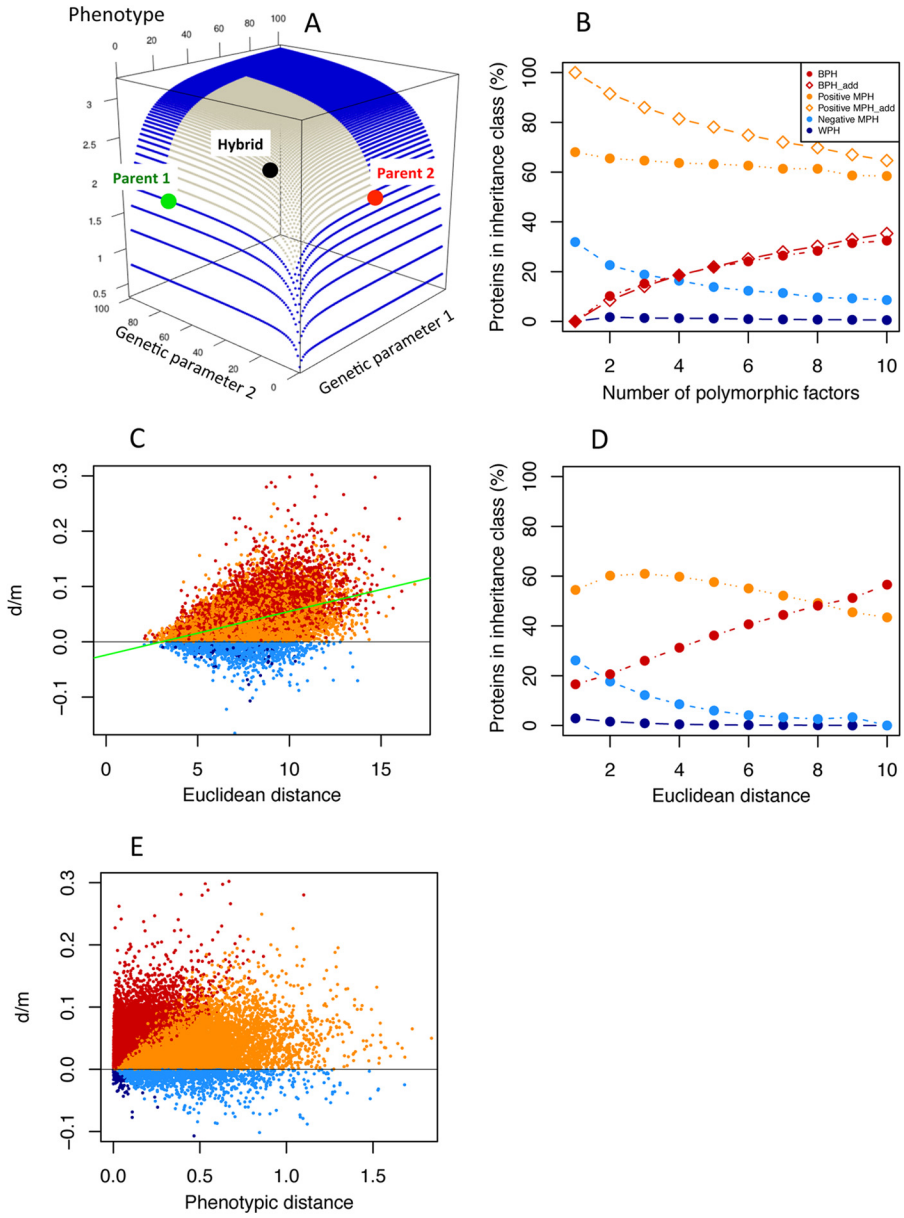


FIG. 8. ***In silico* simulation of heterosis.** (*A*) Phenotypic response with respect to the variation of two genetic parameters in the framework of a concave genotype-phenotype relationship. Parent 1 (green filled circle) and parent 2 (red filled circle) have close phenotypic values. In case of additivity of the values of both genetic parameters, there is BPH (black filled circle). Otherwise the hybrid value can occupy any point of the gray sub-surface, depending on the values of the inheritance parameters (see experimental procedures). (*B*) Effect of the number of polymorphic factors on the percentages of proteins in the different classes of inheritance. Empty diamonds: additivity of the values of the factors. Filled circles: general case, with inheritance parameters of the factors drawn in normal distributions. In case of additivity of the factors, only BPH and positive MPH can be observed. If there is only one polymorphic factor, BPH and WPH cannot be observed, whatever the inheritance of the factors. (*C*) Relationship between *d*/*m* and Euclidean distance between parents, for 10 polymorphic factors ($R^2 = 0.20$). (*D*) Relationship between the Euclidean distance between parents and the percentages of proteins in the different classes of inheritance for 10 polymorphic factors. (*E*) Relationship between the phenotypic distance between parents and *d*/*m*, for 10 polymorphic factors.

We analyzed the relationships between heterosis for protein abundance and (i) the type of inheritance of the genetic factors controlling abundance; (ii) the number of polymorphic factors controlling a protein, which is an indicator of the complexity of its genetic control; (iii) the Euclidean distance between parents computed from the values of the factors controlling protein abundances; and (iv) the phenotypic distance, *i.e.* the difference in protein abundance between parents.

When there is only one polymorphic factor, only positive MPH can be observed if there is additivity of the factor and positive and negative MPH in case of nonadditivity (Fig. 8*B*).

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When two or more factors are polymorphic, positive MPH *and* BPH are possible if there is additivity of the factor, and if there is nonadditivity, the four types of heterosis are possible. When the number of polymorphic factors increases, BPH proportion increases at the expense of the other types of heterosis (Fig. 8*B*).

The Euclidean distance between parents was positively correlated with *d*/*m* (Fig. 8*C*), which is consistent with the well-known relationship between genetic distance and heterosis. More interestingly, the proportions of the different types of heterosis depended on the distance. For the smallest distances, all the types of heterosis were observed and all *d*/*m* values were small, while for the largest distances almost exclusively BPH and positive MPH were observed and their *d*/*m* values were high (Figs. 8*C* and 8*D*). This observation was valid whatever the number of polymorphic factors (Fig. S7). As expected from the concavity of the function, the distribution of the four types of heterosis tightly depended also on the phenotypic distance between parents. For the closest parents, there was a majority of BPH cases and few WPH, while the hybrids between distant parents displayed mainly positive MPH and to a lesser extent negative MPH (Figs. 8*A* and 8*E* and Fig. S7).

## DISCUSSION

We used label-free quantitative proteomics in yeast to perform a large-scale study of heterosis for protein abundance. In agreement with previous results (58), we confirmed that the proteomes of *S. cerevisiae* and *S. uvarum* were highly differentiated. Interestingly, this differentiation is partly related to the adaptation of these species to their optimal temperatures (18 °C for *S. uvarum* and 26 °C for *S. cerevisiae*), as evidenced by the fact that lower temperatures drive *S. cerevisiae*'s proteome close to that of *S. uvarum*, while the higher temperature drives *S. uvarum*'s proteome close to that of *S. cerevisiae*.

*Heterosis for Protein Abundance Is Subject to Genotype × Environment Interactions*—Heterotic proteins were detected in every hybrid × temperature combinations analyzed. This is in line with previous results showing that heterosis for gene expression and protein abundance is a common occurrence, regardless the species or genotypes considered (reviewed in (11)). The proportion of heterotic proteins varied from 8.4 to 61.2% depending on the hybrid × temperature combination considered. Comparatively, Khan *et al.* (80) found 85.9% of heterotic proteins (342 out of 398) in one *S. cerevisiae* × *S. uvarum* cross. However, these authors used an arbitrary threshold without statistical test to decide on the inheritance of the proteins, which may explain the discrepancy with our results. In any case, our study is much more representative of both the proteome and the genetic diversity of *S. cerevisiae* and *S. uvarum* since we examined 1,396 proteins quantified in 55 crosses and at two temperatures. This allowed us to show that there were genotype × environment interactions for heterosis since the temperature did not affect protein inheritance similarly in the different types of hybrid examined. Indeed, the proportion of heterotic proteins was higher at 18 °C for *S. cerevisiae* and interspecific hybrids and at 26 °C for *S. uvarum* hybrids. Note that in the case of intraspecific hybrids, these temperatures were nonoptimal, suggesting that there may be a relationship between the proportion of heterotic proteins and stressful growth conditions. In addition, the sign of *d* was little affected by temperature in interspecific hybrids, which was not the case in intraspecific hybrids.

*Heterosis for Protein Abundance Primarily Affects Highly Regulated Proteins*—Our results suggest that the number of putative TFs of a gene is related to the heterosis for the abundance of the encoded protein. Regulation of transcription is complex, involving a combination of several TFs individually acting as activator and/or repressor (81). Previous studies have shown that genetic polymorphism in *cis* and *trans* regulators can influence the inheritance pattern of gene expression level, polymorphism of trans regulators being preferentially associated to heterotic patterns (39, 82–84). If the number of polymorphic TFs increases with the number of TFs, the relationship between the number of putative TFs of a gene and the frequency at which the encoded protein is heterotic is consistent. Conceptually, our results are similar to what has been observed for agronomic traits in plants. Indeed the results obtained from previous studies show that highly complex, polygenic traits such as yield are more prone to heterosis (23, 31). By analyzing a very high number of traits, we show here that the relationship between genetic complexity and heterosis is robust, even for less-integrated traits such as protein abundance.

The number of putative TFs of a gene depended significantly on the functional category of the gene, explaining why the proteins from some functional categories showed more heterosis than others. Among the functional categories containing genes putatively regulated by a high number of TFs and showing frequently heterotic proteins, we found energy, metabolism, and cell rescue, defense, and virulence. This result is consistent with many studies in plants that showed that these categories were involved in heterosis for gene expression (reviewed in (22, 24)). In addition, since these categories are generally involved in response to environmental changes (85), they were expected to be highly regulated.

The proteins involved in protein synthesis appeared as outliers regarding the relationship between the number of putative TFs of a gene and heterosis for protein abundance, presenting frequencies of heterosis higher than expected based on the number of putative TFs of their encoding genes. To explain the peculiar behavior of these proteins, we assume that factors other than TFs are involved in heterosis for protein abundance as, for example, posttranslational modifications, that were recently shown to be related to the variations of phenotypic traits (86).

*Best-Parent Heterosis Is Related to Proteins Under Evolutionary Constraints*—BPH in interspecific hybrids was more

particularly related to a particular group of proteins (set H+) that were highly abundant and exhibited little abundance variations between temperatures and between *S. cerevisiae* and *S. uvarum*, yet two distantly related species (87). Observation of interspecific heterosis for these proteins necessarily implies that the two species are genetically contrasted at the loci controlling protein abundances (Protein Quantity Loci (33)). This is in agreement with dominance hypothesis, which attributes heterosis to allele complementation. In addition to these results, we also showed that the set H+ was enriched in proteins encoded by essential genes and in proteins involved in protein metabolism. Essential genes are thought to be under strong purifying selection since they are highly conserved across large evolutionary distances in yeasts and mammals (88, 89). Moreover, protein metabolism includes proteins of ribosomes and proteasome that are structurally and functionally conserved (90, 91). This suggests that the proteins of the set H+ are under evolutionary constraint. However, we have currently no hypothesis to establish a relationship between evolutionary constraint and heterosis.

*Heterosis for Protein Abundance Is Consistent with a Model of Nonlinear Genotype–Phenotype Relationship*—It has been observed from numerous experiments that heterosis is generally biased toward positive values (for example, (3, 9)). This bias is accounted for in the dominance hypothesis, where recessive deleterious alleles are complemented by dominant superior alleles (26, 27). In the context of metabolic systems, dominance of the high over the low allele is explained by the hyperbolic response of fluxes toward the variations of enzyme parameters (*e.g.* activity, concentration): Due to the concavity of the curve, the flux value in a hybrid obtained from a cross between two parents presenting contrasted enzyme parameters is systematically biased toward the highest parent, provided the value of the enzyme parameter is additively inherited (76, 92). Generalized to networks with several variable enzymes, this hyperbolic relationship generates heterosis for the metabolic flux (7, 93).

In this study, we analyzed a high number of traits in a large number of hybrids, which allowed us to examine the extent to which the bias toward positive heterosis was robust. Unexpectedly, we showed that heterosis for protein abundance was strongly biased toward positive values in interspecific hybrids but not in interspecific hybrids, where positive and negative heterosis were relatively well balanced. This result was difficult to explain from the current knowledge on heterosis, since, as far as we know, there is no model for negative heterosis. To interpret this result, we relied on previous observations showing that (i) concave genotype–phenotype relationships exist at various levels of cell organization (76, 94–97) and in particular for the protein synthesis rate (77–79) and (ii) nonadditivity can occurs at every level of cell organization, from transcript abundance to more integrated traits (24, 25).

By simulating heterosis for protein abundance using a nonlinear model of genotype–phenotype relationship, we obtained *in silico* results in agreement with those obtained from the experiments. First, we showed that negative heterosis can occur when there is nonadditive inheritance of the genetic factors, which is biologically realistic. Second, we showed that for small genetic distances positive and negative heterosis can be observed, while for large distances there is much more positive than negative heterosis. This is consistent with the bias we observed between intra- and interspecific hybrids. Third, we showed that the proportion of BPH was maximal for hybrids obtained from distant parents *and* for proteins displaying similar abundances in the parents. This is consistent with the frequent BPH observed in interspecific hybrids for the proteins of set H+. Finally, we showed that heterosis was related to the number of polymorphic factors controlling a protein. This is consistent with the observation that the proteins regulated by a high number of TFs were more prone to heterosis.

To conclude, we performed a large-scale study of heterosis, which allowed us to obtain original results: (i) heterosis was strongly biased toward positive values in interspecific hybrids but not in intraspecific hybrids and (ii) BPH in interspecific hybrids occurred preferentially for a special group of proteins assumed to be under evolutionary constraint. These results shed new light on heterosis by supporting a model where protein abundances would be related to transcriptional and translational parameters by concave relationships. In agreement with this hypothesis, we also showed that the complexity of transcriptional regulation, estimated through the number of putative TFs of a gene, is related to heterosis for protein abundance, which supports a general relationship between heterosis and trait complexity. Taken together, our results show the interest of high-throughput technologies to provide a more comprehensive view of complex biological phenomena such as heterosis.

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[S] This article contains supplemental material Tables S1 to S9 and Figs. S1 to S7.

[K] To whom correspondence should be addressed: PAPPSO, Plateforme d'Analyse Protéomique de Paris Sud-Ouest, UMR de Génétique Végétale, INRA-Univ Paris Sud-CNRS, Ferme du Moulon, F-91190 Gif-sur-Yvette, France. Fax: +33 1 69 33 23 40; E-mail: zivy@moulon.inra.fr.

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# *Brettanomyces bruxellensis* population survey reveals a diploid-triploid complex structured according to substrate of isolation and geographical distribution

Marta Avramova[1,8], Alice Cibrario[1], Emilien Peltier[1], Monika Coton[2], Emmanuel Coton[2], Joseph Schacherer[3], Giuseppe Spano[4], Vittorio Capozzi[4], Giuseppe Blaiotta[5], Franck Salin[6], Marguerite Dols-Lafargue[1,7], Paul Grbin[8], Chris Curtin[9], Warren Albertin[1,10] & Isabelle Masneuf-Pomarede[1,11]

***Brettanomyces bruxellensis*** **is a unicellular fungus of increasing industrial and scientific interest over the past 15 years. Previous studies revealed high genotypic diversity amongst *B. bruxellensis* strains as well as strain-dependent phenotypic characteristics. Genomic assemblies revealed that some strains harbour triploid genomes and based upon prior genotyping it was inferred that a triploid population was widely dispersed across Australian wine regions. We performed an intraspecific diversity genotypic survey of 1488 *B. bruxellensis* isolates from 29 countries, 5 continents and 9 different fermentation niches. Using microsatellite analysis in combination with different statistical approaches, we demonstrate that the studied population is structured according to ploidy level, substrate of isolation and geographical origin of the strains, underlying the relative importance of each factor. We found that geographical origin has a different contribution to the population structure according to the substrate of origin, suggesting an anthropic influence on the spatial biodiversity of this microorganism of industrial interest. The observed clustering was correlated to variable stress response, as strains from different groups displayed variation in tolerance to the wine preservative sulfur dioxide ($SO_2$). The potential contribution of the triploid state for adaptation to industrial fermentations and dissemination of the species *B. bruxellensis* is discussed.**

Grape derived wine is one of the most popular alcoholic beverages and has been produced by humans since ancient times. It is the result of grape juice fermentation by yeasts which consume the fruit sugars and mainly release ethanol and carbon dioxide. Even though microorganisms are an essential part of the winemaking process, they must cope with a very hostile and variable environment, characterised by high initial sugar content and subsequent high ethanol content, low pH, presence of antimicrobial agents and lack of nutrients. Despite

[1]Univ. Bordeaux, ISVV, Unité de recherche Œnologie EA 4577, USC 1366 INRA, Bordeaux, INP, 33140 Villenave d'Ornon, France. [2]Université de Brest, EA 3882, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB, Technopôle Brest-Iroise, 29280, Plouzané, France. [3]Université de Strasbourg, Centre National de la Recherche Scientifique, Génétique Moléculaire, Génomique, Microbiologie, Unité Mixte de Recherche, 7156, Strasbourg, France. [4]Department of the Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy. [5]Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, Viale Italia, 83100, Avellino, Italy. [6]INRA, UMR Biodiversité Gènes et Ecosystèmes, PlateForme Génomique, 33610, Cestas, France. [7]Bordeaux INP ISVV EA 4577, F-33140, Villenave d'Ornon, France. [8]School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia. [9]Department of Food Science and Technology, Oregon State University, 100 Wiegand Hall, Corvallis, Oregon, 97331-6602, USA. [10]ENSCBP, Bordeaux INP, 33600, Pessac, France. [11]Bordeaux Sciences Agro, 33170, Gradignan, France. Correspondence and requests for materials should be addressed to M.A. (email: avramova.marta@gmail.com)

these stressful conditions, some opportunistic microorganisms manage to survive and multiply during and after alcoholic fermentation. A striking example is the wine spoilage yeast *Brettanomyces bruxellensis* (teleomorph *Dekkera bruxellensis*) that is typically detected during wine aging but also at lower frequency during the early stages of the winemaking process (grapes and must)[1,2]. When it grows in wine, *B. bruxellensis* produces odorant molecules (namely volatile phenols), which are associated with unpleasant aromas described as barnyard, horse sweat, Band-aid®[3–5]. Therefore, the presence of *B. bruxellensis* in wine often provokes rejection by consumers and serious economic losses for winemakers[6].

The wider industrial relevance of this yeast is highlighted by the fact that it is isolated from various fermented beverages and products. For example, *B. bruxellensis* is an essential contributor to the elaboration of some specialty Belgian and American beers, which are the result of complex spontaneous fermentations performed by various genera of bacteria and yeasts[7,8]. Indeed, *B. bruxellensis* was the first microorganism to be patented for its contribution to English 'stock' ales[9], in 1904. This yeast has also been isolated from other fermented beverages and food like kombucha, kefir, cider, and olives[7,10,11]. Interestingly, *B. bruxellensis* was reported to be a common contaminant in bioethanol production plants[12,13], and under the right conditions can take the place of the industrial *Saccharomyces cerevisiae* strains and perform molasses fermentation[13].

The recurrent problem of *B. bruxellensis* in wine and its potential use for beer and bioethanol industrial fermentations has led to high and rising interest in this yeast species. Various studies highlighted great phenotypic diversity of *B. bruxellensis* regarding growth capacity[14–19], sugar metabolism[20–23], nitrogen source utilisation[21,24], volatile phenols production[5,14,18,20,23,25,26], behaviour in viable but not cultivable state[27], and response to abiotic factors like temperature[20,28], pH[20,29], oxygen availability[30–32] and sulfur dioxide ($SO_2$)[20,23,28,33–35]. This phenotypic variation makes it difficult to predict the spoilage potential of *B. bruxellensis* and is therefore a major concern for winemakers. For example, across several studies the concentration of molecular $SO_2$ ($mSO_2$) required to stop *B. bruxellensis*' growth ranged from 0.2 to 1.0 $mg.L^{-1}$ [36]. This observed variability was at least partly due to the use of different strains. However, only a few studies have attempted to correlate $SO_2$ tolerance to a genotypic profile[20,34]. A striking example is a study of 41 *B. bruxellensis* wine isolates from Australia showing that the most common genotype (92% of studied isolates) was correlated with $SO_2$ tolerance, thus suggesting that $SO_2$ usage patterns may have created a selective pressure on this population[34].

Despite several studies that have explored genetic diversity of this species using fingerprinting techniques such as Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), pulsed field electrophoresis (REA-PFGE), and mtDNA restriction analysis[14,17,20,25,26,34,37–40], our understanding of the *B. bruxellensis* global population structure and the factors that drive it remains limited. Several studies highlight an important intraspecific diversity of *B. bruxellensis*[14,20,38,40] which makes the prediction of its occurrence and behaviour in industrial fermentations difficult. Further, recent genetic studies on a limited number of strains[24,41,42] have suggested that polyploidy and hybridisation may play a significant role in microevolution of the species, along with plasticity in chromosomal structure due to "untraditional" centromeres[43]. The role of polyploidy in adaptive changes to suit environment and/or lifestyle has been observed in other organisms[44–47], notably for *S. cerevisiae* which shares similar fermentation niches to those occupied by *B. bruxellensis*.

To enhance our knowledge of the global *B. bruxellensis* population, here we used a recently developed microsatellite profiling method[42] to genotype 1488 isolates from various fermentation niches across five continents. Typing based on microsatellite markers is a rapid, reliable and discriminant genotyping approach that has been successfully used to decipher complex population structures[48,49] and provide insight into the ploidy-state[42]. The performed research work aimed to determine the population structure of a large *B. bruxellensis* collection and test for a link between the identified subpopulations and their adaptive ability, with a focus on tolerance to sulfur dioxide.

## Results

***B. bruxellensis* genotyping analysis and population structure.** The *B. bruxellensis* collection used in this study comprised 1488 isolates from 29 countries and 9 different substrates, the majority of strains (87%) originating from wine (Supplementary Table S1). The 1488 isolates were genotyped with 12 primer pairs amplifying microsatellite regions, including four new loci in addition to the eight previously published[42]. Characteristics of the different loci and number of alleles are given in Supplementary Table S2. One locus out of the four additional loci (D1) displayed a high allelic diversity, presenting 18 different alleles. All isolates were shown to be heterozygous for at least one locus. Many isolates were shown to have more than 2 alleles per locus. About half of the isolates had up to 3 alleles per locus (792 isolates) and some had up to 4 and 5 alleles per locus (67 and 1 isolates, respectively). The high number of isolates with up to 3 alleles per locus suggests the existence of triploidy in the studied population. Similar observation was reported previously by Curtin *et al.*[41] and Borneman *et al.*[24] who performed *de-novo* sequencing and comparative genomics respectively, highlighting two triploid strains having core diploid genome and additional sets of chromosomes resulting from different triploidisation origins for the two strains. Based on those observations and the occurrence among the isolates of genotypes presenting more than two alleles/locus we extend this hypothesis to the latter.

The raw data obtained by the microsatellite analysis corresponds to the alleles (*i.e.* the size of the amplified microsatellite sequences) per locus and per strain (Supplementary Table S3). This data was further used for the construction of a dendrogram reflecting the genetic proximity between strains (Fig. 1A). The method was based on Bruvo's distance and Neighbour Joining (NJ) and was chosen for being reliable and suitable for populations with mixed ploidy levels. The population clusters in 3 main genetic groups (Fig. 1A). Additional methods, including complementary tests and Bayesian approaches were applied to verify the reliability of the clustering obtained by NJ (Fig. 1). The NJ tree showed three main branches that were almost perfectly conserved with UPGMA method (Fig. 1A and B). Then, a multidimensional scaling was performed with Bruvo's distance matrix on the same dataset and using the *cmdscale* function on R (Fig. 1C). The multidimensional scaling analysis showed

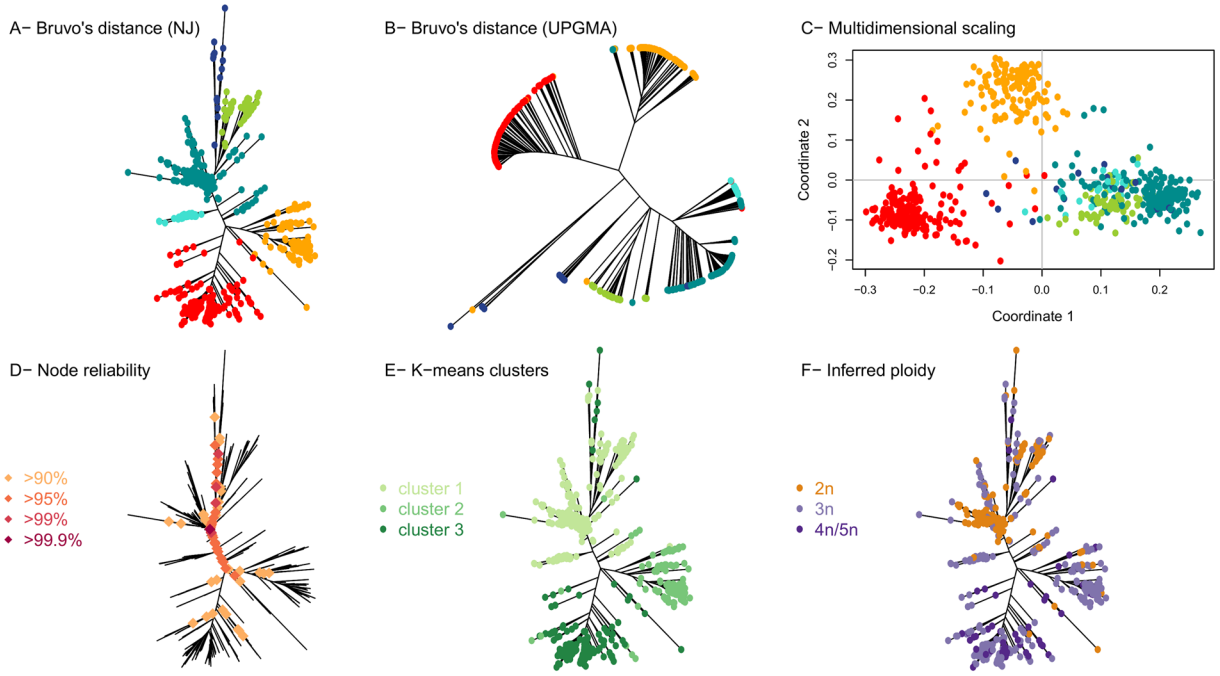


**Figure 1.** *B. bruxellensis* population clusters identification by combining different tools and parameters. (**A**) Dendrogram using Bruvo's distance and NJ clustering. The figure was produced using the *poppr* package in R. (**B**) Dendrogram using Bruvo's distance and UPGMA clustering. The figure was produced using *poppr*. Isolates are shown in the same colours as in A. (**C**) Multidimensional scaling performed with Bruvo's distance matrix on the same dataset and using the *cmdscale* function on R. For isolates with incomplete genotyping, the missing data was inferred from the closest neighbour using Bruvo's distance. Isolates are shown with the same colours as in A. (**D**) Node reliability using the partition method[50]. Only the nodes with reliability >90% are shown on the NJ tree. (**E**) Cluster identification using successive K-means. The *find.cluster* function from the *adegenet* package in R was applied, using within-groups sum of squares (WSS) statistics and the default criterion *diffNgroup*. This tool identifies an optimal number of 3 clusters, represented on the NJ tree using different arbitrary colours. (**F**) Inferred ploidy. The maximum number of alleles per locus was computed. Isolates with up to 2 alleles/locus were considered as diploid (2n). Isolates with up to 3 alleles/locus were considered as triploid (3n), and the number of loci showing up to 3 alleles was recorded (1–2 loci, or more than 2 loci showing up to three alleles). Finally, isolates with up to 4 or 5 alleles/locus were noted as 4n/5n. The inferred ploidy is represented on the NJ tree.

that the three main groups were almost identical to the clusters previously defined. Furthermore, the partition method[50] was applied on the same dataset. This algorithm identifies monophyletic clusters for which the individuals are more closely related than randomly selected individuals. The reliability of the node is then computed and nodes with reliability higher than 90% are considered (Fig. 1D). The partition method also confirmed the three main clusters obtained with NJ as reliable. Finally, clusters were identified using successive K-means (adegenet package, function '*find.clusters*'). This function implements the clustering procedure used in Discriminant Analysis of Principal Components (DAPC)[51], where successive K-means are run with an increasing number of clusters (k), associated with a statistical measure of goodness of fit. This approach identified 3 clusters, once again very similar to those obtained by NJ (Fig. 1E). Overall, the five approaches taken together confirmed the reliability of the three main clusters observed in the studied *B. bruxellensis* population.

Since *B. bruxellensis* is known to exhibit different ploidy levels[24,41], we inferred putative ploidy level based on the microsatellite genotyping. Isolates with up to 2 alleles per locus were considered diploid and noted 2n (Fig. 1F). Isolates with up to 3 alleles/locus were considered triploid (3n). Finally, isolates with up to 4–5 alleles/locus were noted as 4n/5n. The ploidy level coincided clearly with the three main branches of the dendrogram, the red and orange groups being mostly triploid and the blue-green mostly diploid. Within this last cluster, two triploid sub-groups based on the substrate origin and ploidy level of the strains were defined, marked with blue and cyan colours. Finally, the combination of different methods and factors defined of 3 main groups, the 'diploid' one being further divided into 3 subgroups (Table 1 and Fig. 2).

To assess the relative importance of geographical localisation, substrate origin and ploidy level on *B. bruxellensis*' population structure, an analysis of molecular variance (AMOVA) was performed. The three factors were shown to be significant (p-value $< 0.0001$). Ploidy level explained 46.9% of the variance, whereas the geographical origin and substrate factors explained only small proportions of the total variation (around 5% for each) (Table 2). However, when considering non-wine isolates, the geographical origin explains 54.8% of the total variance, suggesting that wine genotypes are highly disseminated across the regions studied in comparison with other substrates. The correlation between genetic and geographic distance matrix (MANTEL test) was also

| Group name | Number of isolates | Number of genotypes | Putative ploidy (for most of the isolates in the group) | Substrate |
|---|---|---|---|---|
| AWRI1499-like | 548 | 197 | Triploid | Mostly from wine |
| AWRI1608-like | 210 | 127 | Triploid | Beer and Wine |
| CBS 2499-like | 573 | 208 | Diploid | Wine |
| L0308-like | 37 | 26 | Triploid | Wine |
| CBS 5512-like | 18 | 16 | Triploid | Bioethanol and tequila |
| L14165-like | 108 | 58 | Diploid | Kombucha |

**Table 1.** Clusters considered as a result of the microsatellite analysis and cluster validation with five different clustering methods.

significant (p-value = 0.0009), confirming that the genetic variation of the total population is significantly related to geographical localisation. The MANTEL test, performed only on the wine strains (p-value = 0.0040), also confirmed the results obtained with AMOVA, suggesting a different population structure amongst wine strains compared to those from the other niches.

**Core genotype analysis.** *Core diploid data subset.* Most classical population genetic analyses cannot be performed using our initial microsatellite dataset since *B. bruxellensis* population include diploid and polyploid isolates, and most traditional analyses are not available for mixed ploidy levels. To overcome such difficulties, we excluded the alleles identified as specific to the isolates showing more than 3 alleles for at least one locus. Among the 124 alleles included in the initial dataset, 70 were found to be significantly associated with the triploid isolates ($\chi^2$ test, p-value < 0.01), and were excluded to create a new dataset comprising alleles representative of the core genotype (*i.e.* the genotype common to all groups). This approach is justified as previous comparative genomics studies showed that *B. bruxellensis* isolates shared a core diploid genome[24].

The obtained core genotype dataset showed up to 2 alleles per locus for most individuals (1350 out of 1488) and only 138 remaining individuals had loci with 3 or 4 alleles. This indicates that the removal of specific triploid alleles allowed us to have access to the core diploid genome common to all *B. bruxellensis* isolates. Loci with more than 2 alleles were considered as missing data and only concerned 138 individuals, of which 130 only had one locus with 3 alleles.

*Ancestral populations and inference of population structure.* LEA package and the *snmf* function in R were used to infer population structure for the 'core diploid' dataset. The number of ancestral populations tested ranged from K = 1 to K = 15 (100 repetitions), and entropy criterion was computed to choose the number of ancestral populations explaining the genotypic data in the best way (Supplementary Fig. S1). Entropy was minimal for K = 5 ancestral populations (K = 3, 4, 5, 6 shown on Supplementary Fig. S2). Such Bayesian analysis shows that these 5 ancestral populations are congruent with previous analyses that considered the complete dataset (Fig. 3): the AWRI1499-like (wine, red) and AWRI1608-like (beer, orange) groups were associated with only one ancestral population. Likewise, most of the blue-green subgroups (wine CBS 2499-like, wine L0308-like, kombucha L14165-like) previously defined were associated with only one ancestral population. Finally, only the tequila/ethanol group (CBS 5512-like) seemed to be associated with more than one ancestry. Altogether, the population structure analysis on the core diploid genotype confirmed the previous clustering and suggested the existence of only one ancestral population for each current population.

*Population differentiation analysis.* A population differentiation analysis was performed by calculating the fixation index ($F_{ST}$) on the core diploid genotype dataset (Fig. 4). The wine AWRI1499-like population is highly differentiated from beer AWRI1608-like and wine CBS 2499-like groups (with $F_{ST}$ 0.36 and 0.39 respectively). This confirms the grouping obtained by the previous analyses. In addition, the pairwise $F_{ST}$ values showed high differentiation between beer AWRI1608-like and wine CBS 2499-like populations ($F_{ST}$ 0.28). The L14165-like kombucha population seems to be mostly differentiated from the 1608-like beer population and is closer to CBS 5512-like tequila/ethanol group. Finally, it is interesting to point out that the CBS 5512-like group is not highly differentiated from all other groups, which is congruent with the fact that population structure analysis inferred multiple ancestries populations for that group.

**Sulfite tolerance.** Sulfur dioxide tolerance was assayed for a subset of *B. bruxellensis* (a total of 39 strains). The chosen strains were selected according to their various geographical origins, substrates and different genetic groups. Some isolates showing identical microsatellite genotypes were included to evaluate possible sulfur tolerance variation between strains with undifferentiated genotypic patterns (13-EN11C11 = L0417 = L0424; UWOPS 92–244.4 = UWOPS 92–262.3; L0469 = L14186). Each strain was grown in medium with increasing $SO_2$ concentration (ranging from 0 to 0.6 mg.$L^{-1}$ molecular $SO_2$) in biological triplicates, so that more than 480 fermentations were monitored.

Three growth parameters (lag phase, maximum growth rate, maximal OD) in the presence of four different concentrations of $mSO_2$ were followed until stationary phase was reached or for a maximum of 300 h when growth was slow or absent. The isolates presented different behaviour according to $mSO_2$ concentration (Fig. 5). Based on the growth parameters of the strains when exposed to increased concentrations of $mSO_2$, two main

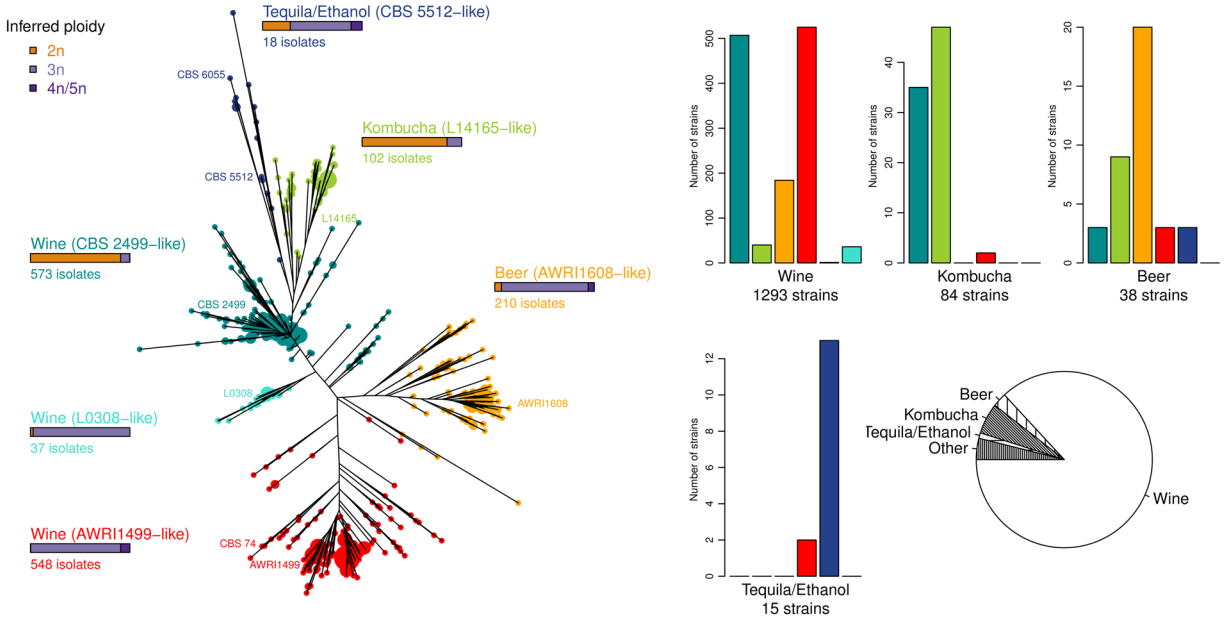


**Figure 2.** Dendrogram of 1488 isolates of *B. bruxellensis* using 12 microsatellite markers. The dendrogram was drawn *via* the *poppr* package, using Bruvo's distance and NJ clustering. Five clusters were considered and are represented by different colours. Isolates displaying identical genotypes are represented by a unique tip whose size is proportional to the number of isolates. Inferred ploidy was made as described in Fig. 1F. The histograms represent the distribution of isolates depending on the substrate and the five considered clusters. The pie chart illustrates the proportion of the strains originating from different types of sources.

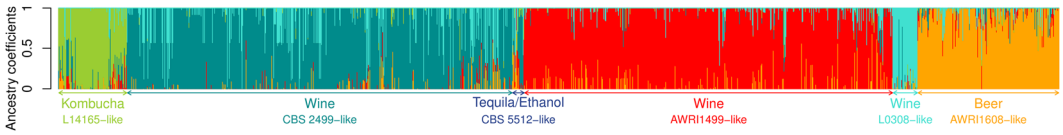


**Figure 3.** Ancestral populations of 1488 *B. bruxellensis* strains STRUCTURE plots for K = 5 (the number of ancestral population with lowest entropy, see Supplementary Fig. S1). Each bar represents an isolate and the colour of the bar represents the estimated ancestry proportion of each of the K clusters. The same colour code is kept as in Figs 1 and 2.

| Factor | %Variance | p-value |
|---|---|---|
| Country | 4.89 | <0.0001 |
| Country (wine isolates) | 3.7 | <0.0001 |
| Country (non-wine isolates) | 54.8 | <0.0001 |
| Substrate | 5.93 | <0.0001 |
| Ploidy | 46.9 | <0.0001 |

**Table 2.** Impact of geographical localisation, substrate origin and ploidy on the population variance (AMOVA test).

groups were identified: (1) sensitive strains (S) characterised by an altered growth with (i) a significant lag phase prolongation, (ii) a significant decrease in maximum growth rate, and/or (iii) significant decrease in maximum $OD_{600}$ (e.g. the sensitive strain L0422 had a lag phase of 17.2 h, 40.7 h, 255.8 h and growth absence, growth rate values were 0.11, 0.07, 0.02 divisions/h and growth absence for and $OD_{600}$ 2, 1.9, 0.8 and no growth at 0, 0.2, 0.4 and 0.6 $mg.L^{-1}$ $mSO_2$ respectively); (2) tolerant strains (T) that showed unmodified growth rate and maximum $OD_{600}$ but sometimes a significant prolongation of lag phase was observed (e.g. the tolerant strain AWRI1499 had a maximal growth rate of 0.07, 0.09, 0.08 and 0.07 divisions/h, $OD_{600}$ 1.9, 2.0, 1.9 and 1.9, lag phase of 75, 56.5, 91.5 and 110.3 h at 0, 0.2, 0.4 and 0.6 $mg.L^{-1}$ $mSO_2$ respectively for the same strain) (mean values of those parameters for each strain are shown in Supplementary Table S4). A clear relation between genetic group and $SO_2$

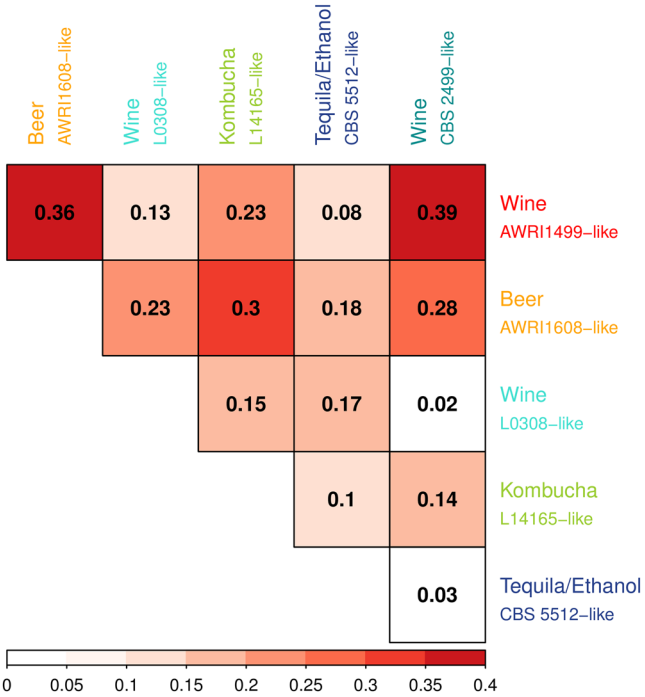


**Figure 4.** Population differentiation represented by fixation index ($F_{ST}$) of *B. bruxellensis* genetic groups between each other. The range of $F_{ST}$ is from 0 to 1, 1 meaning that the two populations do not share any genetic diversity.

tolerance was highlighted (Fig. 5). The isolates from groups AWRI1608-like, CBS 5512-like, CBS 2499-like and L14165-like were mostly identified as sensitive (S), whereas the triploid AWRI1499-like and triploid L0308-like groups were mostly classified as tolerant (T). Furthermore, the isolates with an identical microsatellite profile presented similar behaviour in means of growth parameters in the different conditions studied here (Fig. 5 and Supplementary Table S4).

## Discussion

The yeast *B. bruxellensis* has gained importance for its impact not only in wine industry, but also in beer- and bioethanol-associated fermentation processes. Subsequently, many independent studies were held and results were obtained on different *B. bruxellensis* collections but without leading to a holistic picture of the *B. bruxellensis* species. In this study, a large collection of *B. bruxellensis* strains (1488 isolates) from various substrates (9, the majority of strains (87%) being isolated from wine) and geographic origins (5 continents) was genotyped. The use of a reliable and robust method (microsatellite analysis) determined a general picture of the species' genetic diversity and population structure. The analysis of the complete genotype dataset highlighted 3 main genetic clusters in the *B. bruxellensis* population represented by the AWRI1499-like group, AWRI1608-like and CBS 2499-like group correlating with ploidy level and substrate of isolation. Three sub-clusters were also defined for their ploidy level and substrate of isolation, namely tequila/ethanol CBS 5512-like group, wine L0308-like, and kombucha L14165-like group. Our results are consistent with comparative genomics analysis showing that the AWRI1499, AWRI1608 and AWRI1613 (genetically close to the strain CBS 2499) strains are genetically distant and that the AWRI1499 and AWRI1608 strains are triploid while AWRI1613 is diploid[24].

Heterozygosity for at least one out of the 12 microsatellite loci was shown for all *B. bruxellensis* isolates. This observation supports the assumption that a simple haploid organisation of the genome is excluded, which is congruent with previous results based on the Southern analysis of single gene probes of 30 *B. bruxellensis* strains from different geographical origins[52]. In comparison, using microsatellite analysis, Legras *et al.* (2007) reported 102 out of 410 *S. cerevisiae* isolates (about 25%) and 75% of *Saccharomyces uvarum* strains (among 108 isolates from various geographical and substrates origins) to be homozygous[53]. In general, highly homozygous strains are associated with sporulation and selfing phenomena[54]. So, this could suggest that in the case of *B. bruxellensis* these mechanisms are non-existent or very rare amongst isolates from industrial fermentation environments. Indeed, there is only one study to our knowledge[55], which reports spore formation for *B. bruxellensis* (and therefore its teleomorph form *Dekkera bruxellensis*). In the scenario of rare or non-existent sexual reproduction, a large proportion of heterozygous strains would promote higher phenotypic diversity and therefore colonisation of new niches and adaptation to new environments[56].

Our results confirm on a large scale the assumption that the *B. bruxellensis* population is composed of strains with different ploidy level[24,41,42,52], as 57.8% of the isolates were shown to have more than 2 alleles for at least one locus. Moreover, polyploid strains were associated with various fermentation niches and geographical regions. A strong correlation between genetic clustering and ploidy level was highlighted, with some clusters predicted

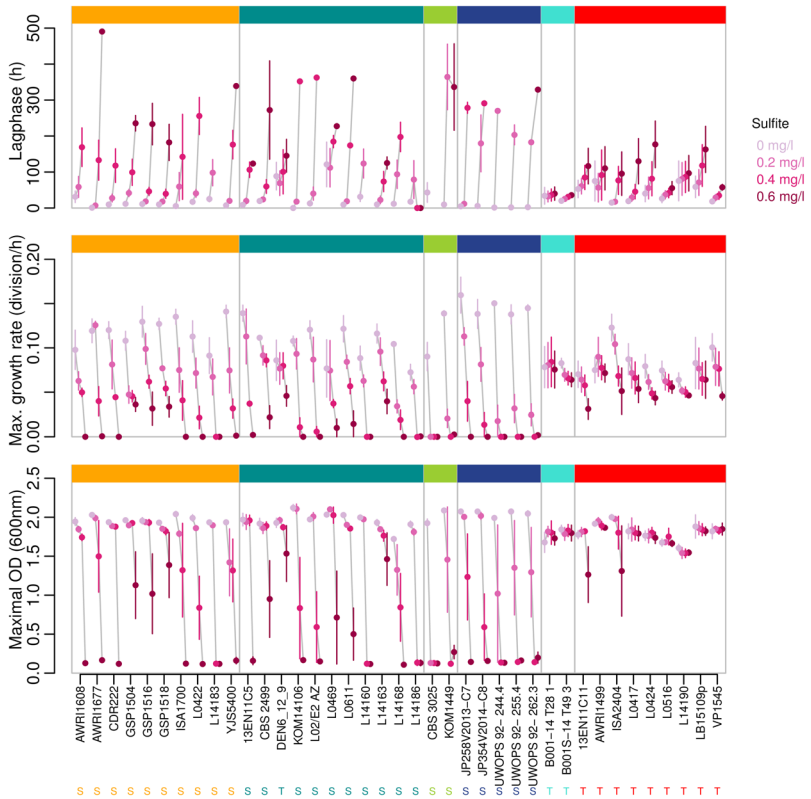


**Figure 5.** Growth parameters of *B. bruxellensis* strains at different concentrations of $SO_2$. 39 strains belonging to the 6 genetic groups defined previously were tested in small scale fermentations and growth ($OD_{600}$) was measured in media containing different concentrations of sulfur dioxide (0, 0.2, 0.4, and 0.6 mg.$L^{-1}$ $mSO_2$) and in biological triplicates. Three parameters were considered: lag phase (h): end of lag phase considered when OD above initial OD*5%; maximal growth rate (r) = number of cellular divisions per hour; maximal OD; S and T stand for sensitive and tolerant (Kruskal-Wallis test, $\alpha = 5\%$). Genetic groups are represented in the same colours as on Fig. 2.

to be diploid (CBS 2499-like) while others were composed of mainly triploid isolates (e.g. AWRI1499- and AWRI1608-like). The latter two clusters derive from distinct ancestral populations and thus, presumably from different triploidisation events. The polyploid state typically has a high fitness cost on the eukaryote cell due to the difficulty to maintain imbalanced number of chromosomes during cell division as well as other effects caused by nucleus and cell enlargement[45]. Thus, it is presumed that a stable polyploid or aneuploid state is maintained when it confers advantage for the survival of the cell in particular conditions[47]. Indeed, aneuploidy and polyploidy contribute to genome plasticity and have been shown to confer selective and fitness advantages to fungi in extreme conditions, such as the presence of high concentrations of drugs, high osmotic pressure, low temperature, and others (see[44,47,57] for review). Similar observations have been made in clinical microbiology, for example, 70% of 132 completely sequenced *S. cerevisiae* clinical isolates with different geographic origins were shown to be poly- or aneuploid[58]. It has been suggested that the aneuploid state contributes to the transition from commercial (industrial fermentations) to clinical (human pathogen lifestyle) environments. Aneuploidy was also reported for another human pathogen – *C. albicans*, for which an aneuploidy of an isochromosome [i(5 L)] is shown to confer resistance to fluconazole[59]. In the industry, stable autotetraploid *S. cerevisiae* strains have been described among isolates from a bakery environment and it was suggested that their prevalence in sour dough fermentation could be the result of human selection for tolerance to high osmotic pressure and high metabolic flux – highly favourable characteristics for baking[60]. In the case of *B. bruxellensis*, however, polyploidy seems to be not only due to a "simple" duplication of chromosomes and/or regions of chromosomes but is the result of independent hybridisation events with closely or distantly related unknown species[24], which result in allotriploid strains. Efficient hybrid species are not rare in human related fermentations[44,61,62] and often the hybridisation with a genetically close species is believed to confer tolerance to specific stress factor in a given environment. This is the case of *S. pastorianus*, used for lager beer fermentations characterised with low temperatures. This yeast has recently been shown to be a hybrid between *S. cerevisiae* and *S. eubayanus* – a cryotolerant species isolated from forests in Patagonia[63], Tibet[64] and recently from New Zealand[65]. Thus, presumably sterile hybrids were naturally generated and they multiplied clonally, accumulating mutations which enhanced the adaptability of the new "species"[63]. Hybrids are also a widespread state among wine yeast, where natural or laboratory obtained combinations between two species could have interesting technological properties[62,66–69]. Other form of genome dynamics was also highlighted for the diploid CBS 2499 strain possessing specific centromeric loci configuration that enables genome rearrangements and ploidy shifts[43]. Based on the body of knowledge concerning other polyploid

micro- and macro-organisms and the prevalence of polyploid strains highlighted in this study, we assume that *B. bruxellensis* has adapted to environmental stress factors by the means of genome plasticity, namely polyploidy.

Our study showed that at least one group, the AWRI1499-like triploid wine group, is composed of wine isolates that are highly tolerant to $SO_2$ and that are clearly divergent from other *B. bruxellensis* clusters ($F_{ST}$ higher than 0.35 when compared with AWRI1608-like and CBS 2499-like groups). Nevertheless, for some wine samples, isolates from both AWRI1499-like triploid group and the CBS 2499-like diploid group were identified. Coexistence of diploid and polyploid (auto- and allopolyploid) "microspecies" has often been reported for plants, in which the polyploids are widely distributed as opposed to the diploids that have a more restricted distribution[70]. Babcock and Stebbins were the first to name this coexistence of populations a diploid-polyploid complex[71] for a *Crepis* species defined as a group of interrelated and interbreeding species that also have different levels of ploidy. These authors claimed that such polyploid complex can arise when there are at least two genetically isolated diploid populations and auto- and allopolyploid derivatives that coexist and interbreed. In the case of *B. bruxellensis*, the sexual cycle of this yeast is not yet elucidated and interbreeding remains to be evidenced. However, we propose that *B. bruxellensis* could be described as a diploid-triploid complex, in which sub-populations with different ploidy levels coexist.

To obtain a deeper understanding of the factors shaping *B. bruxellensis* population structure, we explored the impact of geographical localisation and industrial fermentation environment of origin on the total genetic variance of the studied population. Contribution of the "geographic origin" factor to the population structure was shown to be significant yet only explained a relatively small proportion of variation. However, the variance proportion explained by this factor is much higher when considering non-wine isolates, suggesting that wine strains are highly dispersed worldwide. This dispersal could easily reflect exchange of material and human transport associated with winemaking, followed by adaptation to local winemaking practices[38]. Exchange of material also happens between different industries, which would facilitate local transfer of microorganisms between beverages. For example, some beers are aged in oak barrels previously used for winemaking[72]. Also, in the past, beer fermentation is thought to have been initiated by the addition of a small amount of wine[73]. Such exchanges could be a possible explanation for the low (but significant) contribution of the "substrate of isolation" factor to the total genetic variance in the studied population (5.93%, p-value $< 0.0001$). Substrate of isolation and geographic origin contributed to a similar extent to the total genetic variance of the population. However, this percentage remained low (5%) compared to *S. cerevisiae* for which geographic origin was shown to contribute to 28% of the genetic variance[53], and *Candida albicans* for which 39% were reported[74]. For *S. cerevisiae*, a significant contribution of geographic origin to the genetic variance is often perceived as a sign of local domestication[53,75]. Like *S. cerevisiae, B. bruxellensis* is isolated from human-conducted fermentations including beer and wine. However, until now there are no *B. bruxellensis* isolates from "natural" non-human related habitats contrary to the case of *S. cerevisiae*[76–78]. A recent comparative study of strains with different industrial origins and their growth capacities in various type of media (wine, beer, and soft drink) suggests adaptation of *B. bruxellensis* strains to different fermented beverages[23]. In our study, a low but significant contribution of substrate of isolation to the total genetic variance of the species was highlighted (5.93%, p-value $< 0.0001$), which is an indicator for the adaptation of certain sub-groups to different human-related niches (e.g. winemaking conditions, kombucha fermentation, and others). This structuration is further accompanied by a specific genetic configuration, some groups being mostly diploid and others polyploid.

The hypothesis that the triploid state of *B. bruxellensis* is maintained for some genetic groups because of its contribution to adaptation to a certain type of environment or stress factors is strongly supported by the sulfite tolerance assay performed in our study. This indicated that strains representative of the globally dispersed wine triploid AWRI1499-like group are highly tolerant to $SO_2$. Sulfur dioxide is the most common antimicrobial agent used in winemaking. However, very tolerant *B. bruxellensis* strains have been reported[36]. Particularly, in Australia 92% of the isolates are genetically close to a strain that has be shown to be triploid by genome sequencing and highly tolerant to $SO_2$ (normal growth at more than 0.6 $mg.L^{-1}$ $mSO_2$)[34]. Here, we show that isolates from this genetic group are highly represented worldwide, namely in France, Italy, Portugal, Southern Argentina and Chile. Furthermore, we confirmed on a larger scale (39 strains from different geographical and fermentation niches) that even high $SO_2$ doses could not guarantee the absence of growth of these strains and therefore their potential to spoil wine. In this context, it is worth noting that isolates from substrates other than wine, were all sensitive to $SO_2$ which suggests a direct link between $SO_2$ exposure in wine and tolerance to this compound. Survival in the presence of $SO_2$ has been broadly studied in *S. cerevisiae* but is still not fully elucidated. Molecular $SO_2$ was reported to be the major active antiseptic species of $SO_2$ in wine by different authors (see review of Divol *et al.*, 2012) whereas bisulfites species could also play a role at minor level, in the biocidic effect of PMB[79]. Molecular $SO_2$ could enter the cell passively or *via* selective transport[80]. Once inside the cell, molecular $SO_2$ at approximate intracellular pH 5.5–6.5, rapidly dissociates into bisulphite and sulphite anions. Then, bisulphite is the dominant and main antimicrobial species of $SO_2$ inside the cell that can interact with different enzymes and molecules thus having an impact on the basic metabolic pathways of the cell, such as glycolysis. Strategies to tolerate $SO_2$ are also numerous, like its action on the cell: through the production of molecules that bind $SO_2$ (acetaldehyde, pyruvate, and others), $SO_2$ oxidation and $SO_2$ active efflux by sulfite pump (*SSU1*)[80]. Even if in *B. bruxellensis* these mechanisms are not elucidated, $SO_2$ tolerance could be linked to different aspects – presence of gene(s) coding for a sulfite transporter or presence of this gene (or genes) in multiple copies and therefore overexpression, differences in the gene regulation leading to more efficient response to $SO_2$ toxicity, or morphological and physiological state of the cell that would give it the ability to tolerate this antimicrobial agent (cell membrane structure, growth, *etc.*). The fact that all the highly tolerant *B.bruxellensis* strains are triploid indicates that this genetic configuration could contribute to $SO_2$ tolerance. As mentioned in the previous paragraphs, polyploid states are maintained when they confer a selective advantage. In this case, we can hypothesise that the allotriploid AWRI1499-like strains combine genetic and physiological characteristics from the parent genomes that confer to them the ability to survive in the presence of $SO_2$.

A possible strategy to cope with the issue of highly tolerant strains would be the increase of $SO_2$ concentration added to the must and wine. However, the strong legislation and consumer pressure to reduce any kind of wine additives makes it undesirable to produce wines with high concentrations of $SO_2$ which would be needed for the prevention of AWRI1499-like strains growth. Therefore, the genetic content of *B. bruxellensis* has to be considered when choosing spoilage prevention and treatment methods in the winery in order to obtain optimal effect with minimum intervention. Overall, our results show that polyploid strains are widely disseminated and suggest that *B. bruxellensis* is a diploid-triploid complex whose population structure has been influenced by the use of sulfur dioxide as a preservative in winemaking. Thus, we highlight the importance of *B. bruxellensis* species as a non-conventional model microorganism for the study of polyploidy as an adaptation mechanism to human-related environments.

## Materials and Methods

**Yeast strains.** *B. bruxellensis* strains used in this study were collected from different origins: (i) from CRB Oenologie collection (Centre de Ressources Biologiques Oenologie, Institut des Sciences de la Vigne et du Vin, France), (ii) sent from other laboratories, and (iii) isolated from wines for the purpose of this work. Overall, the collection of *B. bruxellensis* used in this study contained 1488 isolates (Supplementary Table S1) which were further analysed by genotyping.

Strain isolation from contaminated wines was performed by spreading 100 µL of wine sample on solid YPD medium containing 10 $g.L^{-1}$ yeast extract (Difco Laboratories, Detroit M1), 10 $g.L^{-1}$ bactopeptone (Difco Laboratories, Detroit M1), 20 $g.L^{-1}$ D-glucose (Sigma-Aldrich) and 20 $g.L^{-1}$ agar (Sigma-Aldrich). This medium was supplemented with antibiotics in order to limit the growth of bacteria (5 $g.L^{-1}$ chloramphenicol Sigma-Aldrich), moulds (7.5 $g.L^{-1}$ biphenyl, Sigma-Aldrich), and yeast of the *Saccharomyces* genus (50 $g.L^{-1}$ cycloheximide, Sigma-Aldrich). The samples were then incubated at 30 °C for 5 to 10 days. Ten colonies were then picked randomly and analysed by PCR using the DB1/DB2 primers[81] (Eurofins MWG Operon, Les Ulis, France) for species identity confirmation (DNA extraction was performed as described below for the microsatellite analysis). Putative *B. bruxellensis* colonies were streaked and grown on selective YPD medium twice consecutively in order to insure the strain purity. Colonies that gave a positive result by PCR DB1/DB2 were stored at -80 °C in 50% YPD/glycerol medium.

**Genotyping by microsatellite analysis.** *DNA extraction.* For DNA extraction, strains were grown on YPD solid medium at 30 °C for 5 to 7 days and fresh colonies were lysed in 30 µL of 20 mM NaOH solution heated at 99 °C for 10 minutes using iCycler thermal cycler (Biorad, Hercules, CA, USA).

*Microsatellite loci identification and primers design.* Twelve pairs of primers were designed on the basis of the *de-novo* genome assembly of the triploid *B. bruxellensis* strain AWRI1499[41] as previously described by Albertin *et al.*[42]. Four pairs of primers were added to the eight that were previously described in order to improve the discriminative power of the test and to insure its robustness (Supplementary Table S2).

*Microsatellites amplification.* In order to reduce the time and cost of analysis, some of the PCR reactions were multiplexed as shown in the Tm column in Supplementary Table S2. By this procedure the number of PCR reactions per sample was reduced from 12 to 9.

PCR reactions were performed in a final volume of 15 µL containing 1 µL of DNA extract (extraction performed as described above), 0.05 µM of forward primer, 0.5 µM of reverse primer and labelled primer (or 1 µL in the case of duplex PCR reactions), 1 × Taq-&GO (MP Biomedicals, Illkirch, France). The forward primers were tailed on their 5′ end with M13 sequence as described by Schuelke *et al.*[82]. Universal M13 primers were labelled with FAM-, HEX-, AT565- (equivalent to PET) or AT550- (equivalent to NED) fluorescent dies (Eurofins MWG Operon, Les Ulis, France). This method allows labelling of several microsatellite marker primers with the same fluorochrome marked primer (M13) instead of marking each of the 12 forward primers and thus reduces significantly the analysis cost.

Touch-down PCR was carried out using an iCycler thermal cycler (Biorad, Hercules, CA, USA). The program consisted of an initial denaturation step of 1 min at 94 °C followed by 10 cycles of 30 s at 94 °C, 30 s at Tm + 10 °C (followed by a 1 °C decrease per cycle until Tm is reached) and 30 s at 72 °C, then 20 cycles of 30 s at 94 °C, 30 s at Tm and 30 s at 72 °C, and a final extension step of 2 min at 72 °C.

Amplicons were first analysed by a microchip electrophoresis system (MultiNA, Shimadzu) and the optimal conditions for PCR amplifications were assessed. Then, the exact sizes of the amplified fragments were determined using the ABI3730 DNA analyser (Applied Biosystems) (a core facility of INRA, UMR Biodiversité Gènes et Ecosystèmes, PlateForme Génomique, 33610 Cestas, France). Prior to the ABI3730 analysis, PCR amplicons were diluted (1800-fold for FAM, 600-fold for HEX, 1200-fold for AT565 and 1800-fold for AT550) and multiplexed in formamide. The LIZ 600 molecular marker (ABI GeneScan 600 LIZ Size Standard, Applied Biosystems) was diluted 100-fold and added to each multiplex. Before loading, diluted amplicons were heated 4 min at 94 °C. Allele size was recorded manually using GeneMarker Demo software V2.2.0 (SoftGenetics).

*Microsatellite data analysis.* To investigate the genetic relationships between strains, the microsatellite dataset was analysed using the Poppr package[83] in R (3.1.3 version, https://www.r-project.org). A dendrogram was established using Bruvo's distance[84] and Neighbour Joining (NJ) clustering[85]. Bruvo's distance takes into account the mutational process of microsatellite loci and is well adapted to populations with mixed ploidy levels and is therefore suitable for the study of the *B. bruxellensis* strain collection used in this work. Supplementary tests were applied to the same dataset in order to confirm the clusters obtained by Neighbour Joining. First, an UPGMA

(Unweighted Pair Group Method with Arithmetic Mean) analysis was compared with NJ. Then, the partition method[50] was applied in order to confirm the reliability of the nodes obtained by NJ. Also, a multidimensional scaling was performed with Bruvo's distance matrix on the same dataset and using the *cmdscale* function on R and finally, the function '*find.clusters*' available in the adegenet R package was used to identify clusters by successive K-means[86]. Further, AMOVA (analysis of molecular variance) was used to assess the relative importance of geographical localisation and substrate origin regarding *B. bruxellensis* genetic diversity. To confirm the results obtained by the AMOVA analysis, the link between genetic divergence and geographic distance was further evaluated by MANTEL test.

*Core genotype analysis.* Among the 124 alleles included in the initial dataset, 70 were found to be significantly associated with the triploid isolates ($\chi^2$ test, $p < 0.01$) and were excluded to create a new dataset comprising alleles common to all groups and representative of the core genotype (*i.e.* the genotype common to all groups).

For the inference of population structure with this dataset, LEA package was used[87] in combination with the TESS tool to map the geographical cluster assignments of the ancestral populations as defined by Höhna *et al.*[88]. Further, a differentiation test analysis was performed by calculating the fixation index ($F_{ST}$) for the core diploid genotype.

**Sulfite tolerance assessment.** The assay was performed in liquid medium containing 6.7 $g.L^{-1}$ of YNB (Difco™ Yeast Nitrogen Base, Beckton, Dickinson and Company), 2.5 $g.L^{-1}$ D-glucose, 2.5 $g.L^{-1}$ D-Fructose, 5% (v/v) ethanol and increasing concentrations of potassium metabisulfite (PMB, $K_2S_2O_5$)(Thermo Fischer Scientific) in order to obtain 0, 0.2, 0.4 and 0.6 $mg.L^{-1}$ $mSO_2$ final concentrations. For the calculation of $mSO_2$ it was considered that $K_2S_2O_5$ corresponds to about 50% of total $SO_2$ (therefore a solution of 10 $g.L^{-1}$ $K_2S_2O_5$ corresponds to approximately 5 $g.L^{-1}$ total $SO_2$). In order to deduce the final $mSO_2$ concentration, the free $SO_2$ concentration was assessed by aspiration/titration method. Then, the $mSO_2$ was calculated by using the Henderson-Hasselbalch equation on dissociation constant p*K*1[89]. Final pH was adjusted to 3.5 (corresponding to an average value for pH generally encountered in red winemaking conditions) with phosphoric acid (1 M $H_3PO_4$) and the four media (corresponding to the 4 different concentrations of $SO_2$) were filtered separately with 0.22 µm pore filter (Millipore).

Small-scale fermentations were performed in sterile 4 ml spectrophotometer cuvettes containing a sterile magnet stirrer (Dutscher, France). The cells were grown on YPD agar and inoculated into the YNB-based medium without $SO_2$. After 96 h of pre-culture (the point at which all strains reached stationary phase), the cells were inoculated at $OD_{600}$ 0.1 in a final volume of 3 ml. The inoculated medium was then covered with 300 µL of sterile silicone oil (Sigma-Aldrich) to avoid oxidation of the medium which could favour the free $SO_2$ consumption. Then, the cuvette was capped with a plastic cap (Dutscher) and sealed with parafilm. A sterile needle was added by piercing the cap to allow $CO_2$ release. The "nano-fermenters" were then placed in a spectrophotometer cuvettes container box and on a 15 multi-positions magnetic stirrer plate at 25 °C (the final temperature in the "nano-fermenters" was therefore 29 °C due to the stirrer heating). Optical density ($OD_{600}$) was measured every 24 h during at least 300 h to follow cell population growth until stationary phase was reached.

For each growth curve, the following three parameters were calculated: maximal OD was the maximal OD reached at 600 nm, the lag phase (in hours) was the time between inoculation and the beginning of cell growth (5% maximal OD increase), and finally, the maximal growth rate was calculated (maximal number of division per hour based on the OD measurement divided by time). A non-parametric Kruskal-Wallis test was used at $\alpha = 5\%$ to identify the means that were significantly different.

**Data availability.** The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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## Author Contributions

W.A., I.M.-P., C.C., M.D.-L. conceived the experimental design; M.A., A.C., E.P., M.C., E.C., J.S., G.S., V.C., G.B., C.C. and W.A. participated in strain collection and isolation; M.A., A.C., F.S. performed genotype analysis experiments, E.P. contributed to protocol validation; M.A. performed phenotypic assay, E.P. contributed to

protocol validation; W.A., M.A., I.M.-P. and C.C. performed data analysis; I.M.-P., W.A., C.C. and P.G. supervised the experiments; M.A. wrote the paper. I.M.-P., W.A., C.C., P.G., V.C., E.P., E.C., M.C., J.S., G.B., G.S. and A.C. reviewed the manuscript.

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# *Brettanomyces bruxellensis* : diversité génétique et sensibilité aux sulfites

**Warren Albertin[1, 2], Marta Avramova[1], Alice Cibrario[1], Patricia Ballestra[1], Marguerite Dols-Lafargue[1, 2], Chris Curtin[3], [1, 4] Isabelle Masneuf-Pomarède**

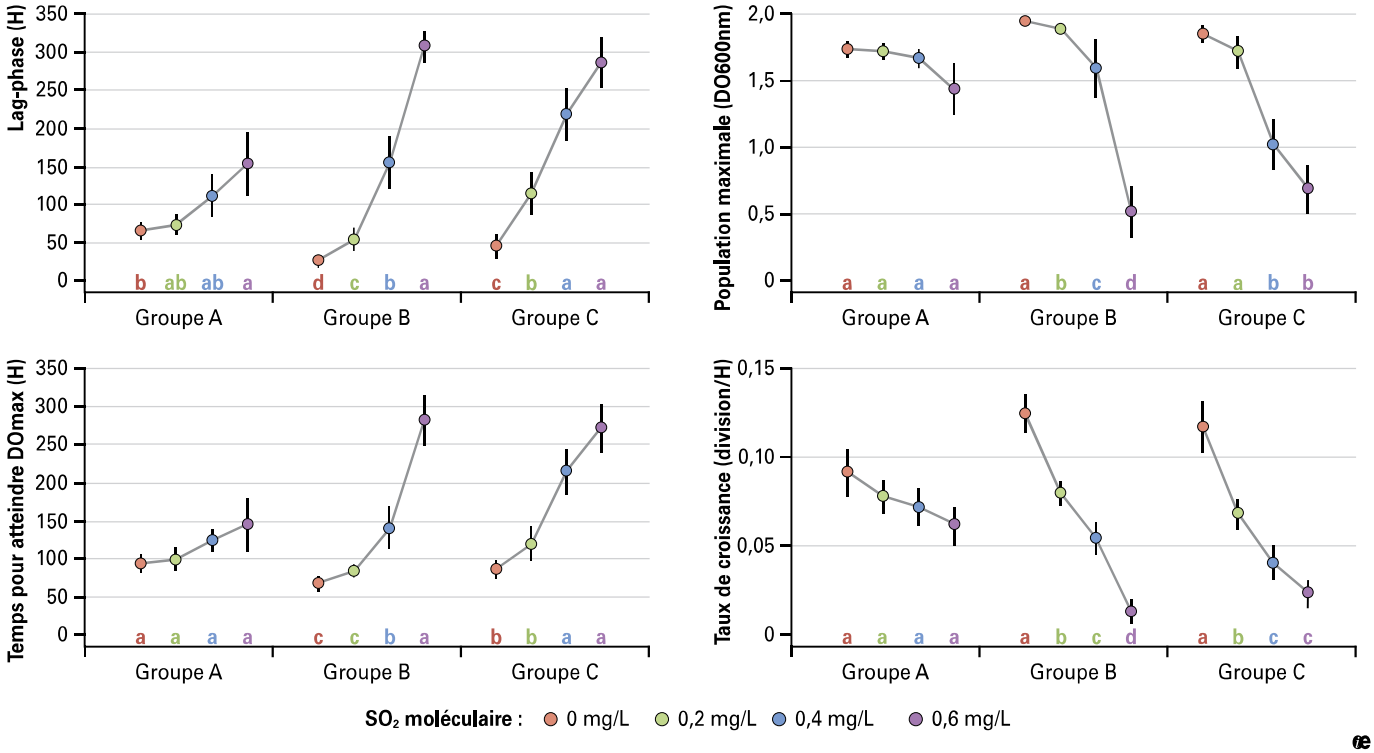
[1] *Université Bordeaux – ISVV – Unité de recherche œnologie EA 4577 – USC 1366 INRA – Bordeaux INP – Villenave d'Ornon – France.*
[2] *Bordeaux INP ISVV EA 4577 – Villenave d'Ornon – France.*
[3] *The Australian Wine Research Institute – Glen Osmond – Adelaide, SA – Australia.*
[4] *Bordeaux Sciences Agro – Gradignan – France.*

*Brettanomyces* est une levure d'altération à l'origine de défauts olfactifs majeurs, affectant près de 25 % des vins rouges *(Gerbaux, Jeudy et al., 2000, Conterno, Joseph et al., 2006)*. Le caractère *« Brett »* constitue un critère de rejet systématique des vins par les consommateurs avec pour conséquence des pertes économiques importantes. Bien que de nombreuses méthodes préventives et curatives soient proposées aux vinificateurs, le problème est parfois récurrent d'une année sur l'autre, et la maîtrise du risque *« Brett »* peut devenir prioritaire sur les choix d'itinéraire de vinification ou d'élevage. La pratique du sulfitage constitue le moyen de lutte le plus utilisé pour prévenir le risque *« Brett »* mais, du fait de la pression des consommateurs à en limiter l'usage, cette stratégie risque de poser problème à l'avenir. Par ailleurs, la mise en évidence récente de souches tolérantes aux sulfites pose la question de l'utilisation systématique du $SO_2$ à des doses élevées et de la pérennité de ce mode de prévention et de lutte à long terme. Nous présentons dans ce travail nos résultats concernant la diversité de *Brettanomyces* tant génétique que phénotypique, avec un focus particulier sur la sensibilité des souches aux sulfites.

## Une diversité génétique insoupçonnée

Actuellement, de nombreux outils de diagnostic permettent la détection et la quantification de l'espèce *Brettanomyces bruxellensis*. En revanche, peu de méthodes fiables, discriminantes et reproductibles ont été développées pour le typage génétique des souches. Ainsi, la diversité génétique et l'écologie de cette espèce sont peu documentées jusqu'à présent. Si le niveau de population dans un échantillon de moût ou de vin est déterminé de façon fiable, il était jusqu'à présent relativement difficile de connaître la nature des souches présentes et leur dangerosité vis-à-vis de la contamination des vins. À partir de la séquence du génome de *Brettanomyces bruxellensis* obtenue par différentes équipes scientifiques dans le

**Figure 1 : Paramètres de croissance de différentes souches de *Brettanomyces bruxellensis* appartenant aux trois grands groupes génétiques A** (8 souches)**, B** (8 souches) **et C** (17 souches) **pour des concentrations croissantes de $SO_2$ moléculaire. Un test de Kruskal-Wallis a été réalisé pour chaque paramètre et chaque groupe de souches, des lettres différentes indiquent des moyennes significativement différentes au seuil de 5 %.**



monde entier, notre laboratoire a développé 12 marqueurs génétiques appelés « microsatellites » pour le typage des souches de cette espèce *(Albertin, Panfili et al., 2014)*. Cette méthode, qui n'avait jusqu'à présent jamais été développée pour *Brettanomyces*, s'est révélée discriminante, permettant d'établir, contrairement aux autres approches, les liens génétiques entre souches.

Nos travaux révèlent une diversité génétique importante au sein de l'espèce. En accord avec les premiers résultats d'analyse des génomes, ils confirment l'existence de souches triploïdes, c'est-à-dire de souches possédant chaque gène en trois exemplaires au lieu de deux habituellement. Ces souches triploïdes contiennent un génome additionnel, dont l'origine reste inconnue à ce jour. Elles existent en proportion élevée dans l'espèce. L'hypothèse est que cet état « triploïde » pourrait conférer à *Brettanomyces bruxellensis* des propriétés particulières d'adaptation au milieu vin. Pour les 1 280 isolats analysés, 617 profils génétiques différents répartis en trois groupes génétiques A, B et C ont été mis en évidence. Le groupe A rassemble un premier type de souches triploïdes du vin, le groupe B un deuxième type de souches triploïdes du vin et de la bière et enfin, le groupe C des souches majoritairement diploïdes du vin et autres substrats (kombucha, tequila, bioéthanol). Cette grande diversité génétique permet de mieux comprendre la variabilité importante des propriétés technologiques des souches, révélée dans des travaux antérieurs, notamment au niveau de la capacité de croissance, de la production d'éthyl-phénols ou de la tolérance aux sulfites. Par ailleurs, dans une même propriété viticole, différents groupes génétiques peuvent coexister dans un même échantillon et être retrouvés dans des échantillons différents avec parfois, la persistance d'un même profil génétique dans des échantillons de vins pendant plusieurs dizaines d'années.

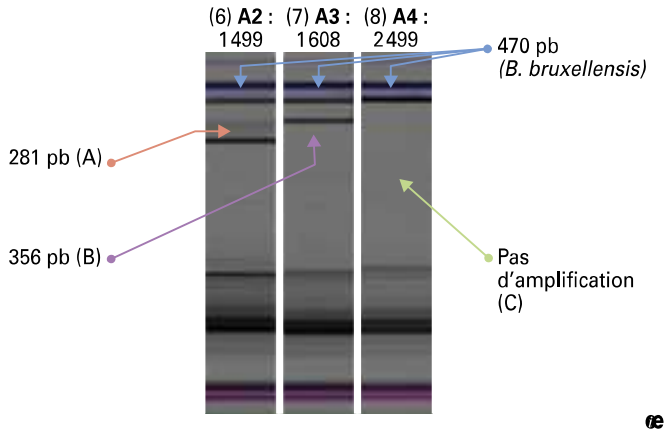
## *Brettanomyces bruxellensis* et la pratique du sulfitage

Le dioxyde de soufre est l'antiseptique le plus fréquemment utilisé en œnologie. La forme moléculaire du $SO_2$ constitue, jusqu'à présent, le principal moyen de lutte vis-à-vis de *Brettanomyces*. L'efficacité du $SO_2$ moléculaire dépend de la dose pratiquée au sulfitage et principalement du pH et de la température du milieu. Les praticiens s'accordent à reconnaître qu'une dose de 0,5 mg/L de $SO_2$ moléculaire est suffisante pour inhiber la croissance de *Brettanomyces* et qu'à partir de 0,7-0,8 mg/l, la dose est létale *(Chatonnet et al., 2012)*. Mais ce raisonnement doit prendre en compte le pH du vin ; ainsi, si 30 mg/l de $SO_2$ libre sont suffisants à pH 3,6, il faudra des teneurs de 60 mg/l à pH 3,9 pour maintenir les mêmes teneurs en $SO_2$ actif. Or, l'addition massive de $SO_2$ n'est pas toujours compatible avec la production de vins de qualité et des vins biologiques.

L'addition de $SO_2$ dans le vin peut s'accompagner de l'entrée des cellules de *Brettanomyces* dans un état physiologique qualifié de « viable mais non cultivable » *(du Toit, Pretorius et al., 2005, Serpaggi, Remize et al., 2012)*. Les cellules ne peuvent plus se multiplier sur des milieux solides classiques de laboratoire, mais ont toujours une activité métabolique. Elles peuvent par ailleurs, lorsque les conditions redeviennent favorables, sortir de cet état physiologique particulier. L'existence de souches tolérantes aux sulfites a été mise en évidence récemment. En Australie, une stratégie de contrôle de *Brettanomyces*, basée notamment sur l'utilisation de doses élevées de dioxyde de soufre, a été mise en œuvre durant une période d'une dizaine d'années. Les isolats de *Brettanomyces bruxellensis* provenant des vins sulfités ont été collectés, puis regroupés en 8 profils génétiques, dont le groupe majoritaire (85 % des isolats) s'est révélé très tolérant aux sulfites, c'est-à-dire capable de croissance à 0,6 mg/L de $SO_2$ moléculaire *(Curtin, Kennedy et al., 2012)*. Cela suggère que le dioxyde de soufre a agi comme pression de sélection, entraînant l'émergence de souches très résistantes. La prévention de la contamination aux doses habituelles d'utilisation du dioxyde de soufre n'en est que plus difficile. Le fait important est l'existence d'un lien entre la proximité génétique des souches et leurs propriétés de tolérance aux sulfites. Afin d'étendre ces observations à d'autres régions viticoles, nous avons évalué au laboratoire les sensibilités aux sulfites d'une collection de 33 souches représentatives de la diversité génétique de l'espèce (origine géographique, produits fermentés, groupe génétique A, B ou C). La croissance a été suivie dans un milieu modèle au laboratoire en triplicata en condition d'anaérobie. La multiplication en présence de concentrations de sulfites croissantes est significativement différente selon le groupe génétique auquel appartiennent les souches ***(figure 1)***. Pour les souches du groupe A, la phase de latence est faiblement mais significativement allongée, lorsque la concentration de $SO_2$ moléculaire augmente. En revanche, une fois la croissance démarrée, la dose de sulfites n'a pas d'effets significatifs sur les paramètres : taux de croissance, population maximale, et temps pour atteindre la population maximale et ce, même en présence de 0,6 mg/l de $SO_2$ moléculaire. Ces souches sont qualifiées de tolérantes aux sulfites : hormis leur phase de latence allongée, elles présentent une croissance normale de 0,2 à 0,6 mg/L de $SO_2$ moléculaire. À l'inverse, la croissance des souches des groupes B et C est très affectée par le $SO_2$ moléculaire, et ce, dès 0,4 mg/L (voire 0,2 mg/l pour certaines souches). Ces souches sont qualifiées de sensibles aux sulfites.

Ainsi, pour le suivi des vins en élevage, le maintien du $SO_2$ moléculaire à 0,6 mg/L ou plus, peut se révéler inefficace en présence de souches tolérantes aux sulfites (par exemple appartenant au groupe A).

**Figure 2 : Résultat de la PCR duplex permettant de situer une souche de *Brettanomyces* dans un des trois groupes génétiques. La bande de 470 pb est spécifique de *B. bruxellensis* *(Ibeas, Lozano et al., 1996)* ; une bande de 281 pb est obtenue pour les souches du groupe A et de 356 pb pour les souches du groupe B ; il n'y a pas d'amplification supplémentaire pour les souches du groupe C.**




## Vers le développement d'un outil de diagnostic de la sensibilité aux sulfites de *Brettanomyces bruxellensis*

Au laboratoire, nous avons développé un test moléculaire simple, permettant de mettre en évidence

l'appartenance d'un isolat à l'un des trois groupes génétiques A, B ou C et, par conséquent, de prédire la sensibilité aux sulfites des souches. Ce test, qui a fait l'objet d'une demande de dépôt de brevet, consiste en une simple analyse par PCR (Polymerase Chain Reaction) sur colonies, après isolement sur milieu sélectif. Le principe est basé sur l'amplification différentielle des souches de *Brettanomyces bruxellensis,* selon leur appartenance à un des trois groupes génétiques. Un exemple de résultat est montré sur la ***figure 2.***

En une seule analyse, il est possible de vérifier l'appartenance de la colonie à l'espèce *Brettanomyces bruxellensis* et de prédire la sensibilité aux sulfites, souches tolérantes pour le groupe A, et souches sensibles pour les groupes B et C. Sur les 1 280 isolats analysés, 435 appartiennent au groupe A, 206 au groupe B et 639 au groupe C.

Les isolats du groupe A proviennent de différentes régions viticoles françaises (Bordeaux, Bourgogne, Jura, Languedoc, Côtes du Rhône), mais aussi italiennes ou australiennes. Aucun lien n'a pu être établi entre l'appartenance à un groupe génétique et l'origine géographique d'une souche. Nous envisageons également de développer un outil de type PCR-quantitative permettant d'éviter l'étape de mise en culture sur milieu nutritif pour quantifier rapidement les populations selon leur sensibilité aux sulfites.

## Conclusion

L'étude de la diversité génétique de *Brettanomyces bruxellensis* a révélé une complexité génomique très atypique : il existe plusieurs groupes de *« Bretts »* extrêmement différents, notamment vis-à-vis de la sensibilité aux sulfites. Dans la collection analysée, 34 % d'isolats sont potentiellement très tolérants aux sulfites, ce qui prouve la capacité d'adaptation particulièrement rapide de cette espèce à l'antiseptique le plus utilisé en œnologie. Ce phénomène est répandu, puisque ces individus tolérants existent dans différentes régions en France mais également dans différents pays. Dans le contexte actuel de la réduction des intrants pour la filière vitivinicole, en particulier de l'utilisation du $SO_2$, il est intéressant de connaître la sensibilité aux sulfites des levures *Brettanomyces* qui contaminent un vin. La relation établie entre ce caractère et les différents groupes génétiques mise en évidence par PCR, montre tout l'intérêt du test.

La connaissance préalable de la capacité de résistance aux sulfites permettra au vinificateur de choisir le moyen de lutte adapté et, en particulier, de limiter l'utilisation du $SO_2$ quand celui-ci n'est pas efficace. Il s'agit d'éclairer le choix des praticiens vers la mise en place de stratégie d'utilisation raisonnée des sulfites, c'est-à-dire l'utilisation ciblée aux seuls vins ne contenant pas de souches tolérantes. Dans le cas contraire, le choix pourrait être porté sur des méthodes alternatives telles que par exemple l'utilisation de chitosane fongique ou des méthodes physiques de filtration et traitement thermique. ■

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MINI-REVIEW

# The complexity of wine: clarifying the role of microorganisms

Sophie Tempère[1] · Axel Marchal[1] · Jean-Christophe Barbe[1,2] · Marina Bely[1] · Isabelle Masneuf-Pomarede[1,2] · Philippe Marullo[1,3] · Warren Albertin[1,4]

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## Abstract

The concept of wine complexity has gained considerable interest in recent years, both for wine consumers and wine scientists. As a consequence, some research programs concentrate on the factors that could improve the perceived complexity of a wine. Notably, the possible influence of microbiological factors is particularly investigated. However, wine complexity is a multicomponent concept not easily defined. In this review, we first describe the actual knowledge regarding wine complexity, its perception, and wine chemical composition. In particular, we emphasize that, contrary to expectations, the perception of wine complexity is not related to wine chemical complexity. Then, we review the impact of wine microorganisms on wine complexity, with a specific focus on publications including sensory analyses. While microorganisms definitively can impact wine complexity, the underlying mechanisms and molecules are far from being deciphered. Finally, we discuss some prospective research fields that will help improving our understanding of wine complexity, including perceptive interactions, microbial interactions, and other challenging phenomena.

**Keywords** Wine complexity perception · Sensorial analysis · Yeast · Bacteria

## Introduction

Creating value is one of the most important challenges for wine producers. It relies largely on the ability of their wine to satisfy the consumers durably. Thus, a wine must seduce immediately but also in the long term, from the first sip to the last one, without boring the drinkers. Sensory complexity is a crucial quality to avoid weariness (Dubourdieu 2011), and the notion of wine complexity arouses more and more interest from both wine drinkers and wine scientists: Fig. 1a represents the number of scientific publications recorded by Google Scholar including the words "wine" and "complexity." The number of publications with only wine was also shown as a control. The number of publications related to wine has increased drastically over the last centuries: from around 2000 papers/year at the beginning of the twentieth century and up to > 80,000 publications/year from the last decades. Interestingly, while the proportion of the papers referring to "complexity" represented only 5% between 1900 and 1950, it now represents 30–40% of the wine publications, confirming the large attraction for wine complexity. While being commonly considered an intrinsic subdimensions of quality (Charters and Pettigrew 2007), wine complexity has been however poorly defined on an academic point of view. On the contrary, wine journalists often use this term and consider complexity as one of the greatest characteristics of wine. In an article for The Wine Spectator, the most read wine-tasting magazine, Matt Kramer explained that "The more times you can return to a glass of wine and find something different in it—in the bouquet, in the taste—the more complex the wine. The very greatest wines are not so much overpowering as they are seemingly limitless" (Kramer 2012). This opinion points out the ambiguity associated to wine complexity since it refers to a global perception and not directly to one or few sensory characters exhibited by limited chemical markers. The link between wine composition and complexity perception does not appear so obvious. In a recent work, Schlich et al. (2015) suggested to distinguish the objective chemical complexity of wine that is based on the diversity of compounds present in wine, from its more subjective perceived complexity.

✉ Warren Albertin
warren.albertin@u–bordeaux.fr

[1] Unité de recherche Œnologie EA 4577, USC 1366 INRA, Bordeaux INP, Univ. Bordeaux, ISVV, 33140 Villenave d'Ornon, France

[2] Bordeaux Sciences Agro, 33170 Gradignan, France

[3] Biolaffort, 33100 Bordeaux, France

[4] ENSCBP, Bordeaux INP, 33600 Pessac, France

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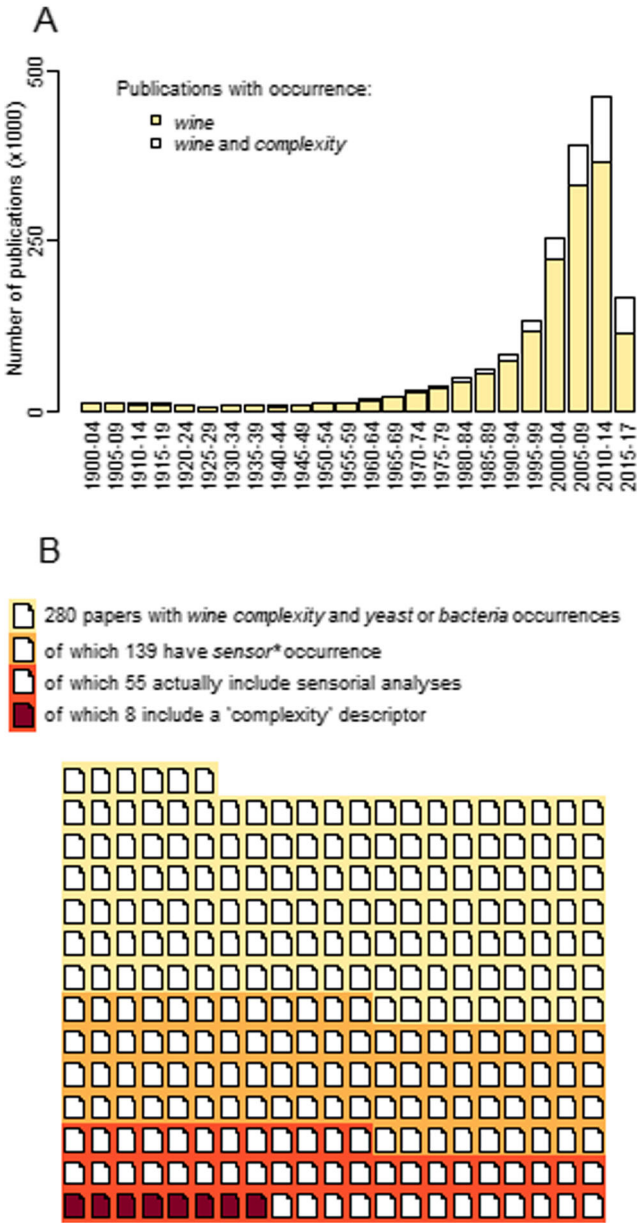


**Fig. 1** Bibliometric overview of wine complexity and sensorial analysis. **a** Number of publications with "wine" or "wine complexity" occurrence. **b** Number of publications related to wine complexity and to microorganisms ("yeast" or "bacteria"), with or without "sensor*" occurrence. Bibliometry was performed using Harzing's Publish or Perish software and Google Scholar searching (Harzing and van der Wal 2008). Manuscripts were manually checked to identify those evaluating the impact of microorganism through an actual sensory analysis

## Defining wine complexity

In 2011, to better understand the mental representations of wine complexity, Parr et al. (2011) realized comparative interviews between experts and novices. They demonstrated that complexity is a multicomponent concept for both populations. The authors also reported an effect of the expertise on their mental constructs. For example, novices' mental representation was related to subjective experience and was also influenced by extrinsic components such as brand and image. In the case of experts, technical knowledge contributed to their mental representation of complexity (Parr et al. 2011). The intrinsic components seemed more salient for the consumers. Lévy and Köster (1999) demonstrated that consumer preferences for many foods were related to the perceived complexity of the product.

However, focusing on the perceived complexity in Sauvignon Blanc wines, Schlich et al. (2015) suggested that experts were more consistent with the sensory concept of complexity than novices were. Interestingly, their data demonstrated that harmony and balance were positively correlated with the perception of the wine complexity. Moreover, the ease to identify different flavors in wine was not associated with perceived complexity by any of the participant groups.

These results could be explained by human difficulties to discriminate the odor of molecules presented as a mixture. Identification abilities in novices and also in experts are limited to three or four molecular components in a complex mixture (Laing and Francis 1989; Livermore and Laing 1996). Furthermore, the results obtained by Schlich et al. (2015) also argued in favor of a representative and holistic process involved in wine complexity perception called configural processing. In configural processing, the parts of odorant mixtures are integrated and perceived into unique wholes, in opposition to the analytical processing implying that the perception of mixtures is the sum of its parts (Thomas-Danguin et al. 2014).

As reported by Schlich et al. (2015), chemical complexity in wine is not representative of its sensory complexity. Indeed, Moskowitz and Barbe (1977) and Jellinek and Köster (1979) demonstrated no correlation between the number of components in an odorant mixture and its complexity perception. They suggested that complexity perception is not additive. Moreover, complexity evaluation of one single odorant component could be equal to or greater than a multicomponent mixture.

The best example in wine is the case of the off flavors. The key compounds of wine faults do not just add a more or less unpleasant aromatic note but participate to the standardization of the product. Actually, they indirectly decrease wine complexity. For example, a few decades ago, some studies have proposed that a mixture of 4-ethylphenol and 4-ethylguaiacol at low concentrations contributes to the complexity (Etievant et al. 1989; Mahaney et al. 1998). Nowadays, it is largely demonstrated that these compounds overwhelmed the fruity and varietal aroma in wine even at infra-threshold concentrations (Gerbaux and Vincent 2001; San-Juan et al. 2011; Tempere et al. 2016).

The complexity is generally associated with the presence of many aromatic elements, which are not easily recognizable (Jackson 2017). Notably, the flavor complexity can be increased by blending similar wines (Singleton and Ough

1962). However, Parr et al. (2011) highlighted that the mental representations of wine complexity for both consumers and experts were not only related to flavor but also to gustatory traits. Previously, Drewnowski (2001) reported that non-dominant bitter molecules could significantly affect complexity which relies on perceptive interactions between taste-active compounds. The wine perceived complexity results from multisensory integration and the concept of balance appears predominant, with high inter-individual variations in its evaluation (Smith 2012).

Wine complexity is thus a complex concept, which—contrary to the intuitive supposition—is not related to wine chemical complexity. Several factors were shown or suggested to be associated with the increase of wine complexity, like viticulture practices—grape varieties (Schreier and Jennings 1979), irrigation (Balint and Reynolds 2017), oenological practices—grape skin maceration (Pineau et al. 2011), micro-oxygenation (Day et al. 2015), or microorganisms. In the following part, we will focus on refining the impact of microorganisms on wine complexity and wine perception.

## The impact of microorganisms on wine complexity

While a large amount of publications deal with "wine complexity", and either "yeast" or "bacteria" occurrences (280 publications, Fig. 1b), only half of them (139/280) include word occurrences beginning with "sensor" (sensory, sensorial, etc.), and fewer still (55/280, 19%) actually include a sensorial analysis. Furthermore, only a small subset of those sensory evaluations (8) have a "complexity" descriptor, which may be related to the difficulties of defining and evaluating wine complexity (see above).

In fact, several authors evaluate the impact of microorganisms on wine chemical composition, and then mention in the discussion part that the evidenced changes in chemical composition should be assessed by further sensorial analysis in order to precise the actual impact on wine perception. Although it is legitimate to speculate that any change in wine composition could modify its perception, the actual relationship between a change in one compound (or a family compound) and wine perception remains elusive. Hence, we decided to consider for this review only the 55 publications including a sensory evaluation of the impact of microorganisms on wine.

### *Saccharomyces* species: the leaders

Unsurprisingly, the yeast *Saccharomyces cerevisiae* is the most studied microorganism whose impact on wine is evaluated at the sensorial level. The wordcloud (Fig. 2) produced from the 55 publications shows that *S. cerevisiae* and

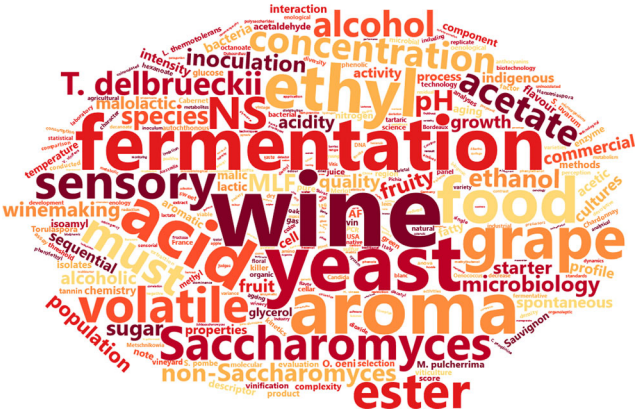


**Fig. 2** Wordcloud representation of the main occurrences in publications including a sensory evaluation of the impact of wine microorganisms. Only the 55 manuscripts including a sensory analysis (see Fig. 1.) were used to produce a wordcloud by means of R packages *tm*, *textreadr*, and *wordcloud2*. Only nouns were kept (verbs, adjectives, adverb, pronoun, preposition, etc. were excluded). Twenty most cited words are as follows (number of occurrences enclosed in parentheses): wine (7280), yeast (3649), fermentation (3176), acid (1973), *S. cerevisiae* (1661), aroma (1558), compound (1344), food (1247), grape (1207), ethyl (1204), sensory (981), volatile (972), NS (941), must (940), ester (932), *Saccharomyces* (902), acetate (714), alcohol (701), concentration (661), and pH (587)

*Saccharomyces* respectively are the 5th and 16th most cited words, with 1661 and 902 occurrences, respectively. In winemaking, *S. cerevisiae* is—in very most cases—the yeast responsible of the alcoholic fermentation (AF), and is usually employed as starter cultures (active dried yeast (ADY)). Thus, several papers considered the impact of various commercial strains of *S. cerevisiae* on wine perception: for example, Gammacurta et al. (2014) showed that *S. cerevisiae* strains impacted significantly the aromatic profile of red wines. At the chemical level, the measure of several families of esters was performed, whose abundances were strongly influenced by yeast strain also. However, despite their important role, neither specific ester nor specific ester family could alone explain the sensorial differences (Gammacurta et al. 2014). In another study, King et al. (2008) explored the possibility of mixing and co-inoculating commercial strains of *S. cerevisiae*. Those co-inoculations modify the sensory profile of Sauvignon Blanc wines and were associated with changes in volatile thiols. Interestingly, the authors pointed out that co-inoculation was not equal to blends of single-strain inoculation, suggesting the existence of interactions between yeast strains as a possible underlying mechanism explaining wine perception (King et al. 2008). Interaction mechanisms between microorganisms are of great interest and will be detailed below.

*S. cerevisiae* strain may also impacts the sensorial characteristics of wine through velum formation (Moreno et al. 2016) or during aging on lees: del Barrio-Galan et al. (2015) showed that strains having contrasted capabilities of polysaccharide liberation during AF also showed perceptive

differences. However, although aging on lees is commonly said to improve the complexity of wines (del Barrio-Galan et al. 2015; Loira et al. 2013), the precise compounds involved remains unclear: several families (polysaccharides, anthocyanins, proanthocyanidins, and volatile compounds) could be involved, yet their variation alone cannot account for the differences in wine perception.

The use of starter cultures has greatly helped to control the fermentation process and has improved the overall quality of the wines. This positive contribution is certainly due to the reduction of many off flavors (volatile acidity, undesirable sulfur compounds, phenolic off flavors) that have a negative masking effect on wine quality (Ribéreau-Gayon et al. 2006). However, in the past decennia, some authors have suggested that ADY use has led to an organoleptic standardization of the wines (Vigentini et al. 2016) and could be responsible for the loss of typicality of wines (Di Maro et al. 2007). Although those views are still debated (Ribéreau-Gayon et al. 2006), a new practice is gaining considerable momentum and consists in the selection of so-called indigenous, autochthonous, or native *S. cerevisiae* local strains. Indigenous strains are believed to be able to maintain the typical sensory properties and enhance the peculiarities of a wine and to show better acclimation to their original environment (Grieco et al. 2011; Nikolaou et al. 2006). This last statement remains to be demonstrated, yet several research programs report the isolation, selection, and analysis of indigenous *S. cerevisiae* strains associated with local wines, like Dealurile Olteniei wines from Romania (Grigorica et al. 2017), Apulian and Sicilian wines (Grieco et al. 2011; Sannino 2013), productions from Shanshan County in China (Liu et al. 2015), or Patagonian wines (Lopes et al. 2007). Specific grape varieties are also investigated, like Negroamaro, Xynomavro, Mencia, Godello, Albariño, or Treixadura grapes (Blanco et al. 2013a, b, 2014; Nikolaou et al. 2006; Tristezza et al. 2012). As a rule, different indigenous strains were shown to result in different aromatic profiles, and the selection of a few native *S. cerevisiae* strains having well-rated sensory perception was possible. In addition, the change in wine perception was usually associated by changes in a wide variety of aroma-related compounds, including volatile thiols, higher alcohols, ethyl esters, acetates, ethyl esters, or fatty acids (Blanco et al. 2013a, 2014; King et al. 2008). Although less studied, aldehydes, ketones, terpenes, lactones, and phenols were also considered by a few authors and their concentrations usually varied along with the strains (Callejon et al. 2010). Interestingly, in some cases, the ability of indigenous strains to produce extracellular enzymes during wine fermentation was evaluated, and several enzymatic activities of oenological interest were identified, such as β-glucosidase, glucanase, xylanase, protease, or pectinase (Tristezza et al. 2012). However, although a plethora of works co-investigate wine aromatic profiling and wine composition (particularly regarding volatile compounds), it was not possible to find one compound or one family that accounts alone for the differences in wine perception, let alone wine complexity.

Finally, a few papers investigated the use of non-*Saccharomyces cerevisiae* species for winemaking. The organoleptic impact of *S. paradoxus* and *Saccharomyces uvarum* (formerly called *Saccharomyces bayanus* or *S. bayanus* var. *uvarum*) was evaluated, as well as a wide range of volatile compounds (Budić-Leto et al. 2010; Domizio et al. 2007; Majdak et al. 2002; Ugliano et al. 2010). Interspecific hybrids emerged as another alternative to *S. cerevisiae* starters (da Silva et al. 2015; Dubourdieu et al. 2006; Perez-Torrado et al. 2017). However, once again, if significant differences in both sensory profiles and wine composition were revealed when testing different species of hybrids, their precise relationship remains unclear.

## Non-*Saccharomyces* yeasts: the rank outsiders

Non-*Saccharomyces* (NS) or non-conventional yeasts have been long considered undesired or spoilage microorganisms in wine, partly because NS are not able to complete wine alcoholic fermentation (i.e., they are not able to consume all the sugar contained in grape must) and usually result in stuck or sluggish fermentations when used alone. However, this limitation can be overcome through the use of mixed inoculations, including a NS partner and a *S. cerevisiae* partner to secure AF completion (Contreras et al. 2014). In the wake of the selection of indigenous *S. cerevisiae* strains to improve the organoleptic characteristics of wine, the use of mixed starters has gained significant interest. Mixed starters can be used either through co-inoculation (i.e., inoculation of different strains at the same moment) or sequential inoculation (the inoculation of one partner being delayed, usually *S. cerevisiae*, to allow the implantation of the NS partner). One of the most studied NS is *Torulaspora delbrueckii*, whose low production of volatile acidity and other undesirable volatile compounds contributed to its reputation of "high-purity" fermenter (Renault et al. 2009). The abundance of several wine compounds are modified along with the metabolic activity of *T. delbrueckii*, such as volatile thiols (Renault et al. 2016) and other volatile compounds (vinylphenols, esters, higher alcohols), pigments, or mannoprotein contents (Azzolini et al. 2015; Belda et al. 2015; Loira et al. 2014; Renault et al. 2015). Besides those characteristics, the killer activity of some strains of *T. delbrueckii* promotes the subsequent malolactic fermentation (Ramírez et al. 2016), pointing the existence of direct or indirect interactions between wine microorganisms. Many other NS species have been studied over the last 20 years, including *Lachancea thermotolerans*—formerly *Kluyveromyces thermotolerans* (Hranilovic et al. 2018; Takush and Osborne 2012), *Metschnikowia pulcherrima* (Hranilovic et al. 2018; Varela et al. 2017),

*Pichia kluyveri* (Benito et al. 2015), *Pichia kudriavzevii* (Del Mónaco et al. 2016), *Candida stellata* (García et al. 2017; Soden et al. 2000), *Starmerella bacillaris*—formerly *Candida zemplinina* (Tofalo et al. 2016), *Hanseniaspora* species (Medina et al. 2013; Mendoza et al. 2007; Tofalo et al. 2016; Varela et al. 2017), *Schizosaccharomyces pombe* (García et al. 2017), or *Issatchenkia terricola* (de Ovalle et al. 2018), and some of them are even commercialized for winemaking. Some rarer assays include the use of less-known species, like *Rhodotorula mucilaginosa* (Wang et al. 2017), *Zygosaccharomyces kombuchaensis*, or *Kazachstania gamospora* (Dashko et al. 2015).

In most cases, the contribution of these NS is discussed and shed light on their ability to excrete enzymes of oenological interest (de Ovalle et al. 2018; Hu et al. 2016), the production of secondary metabolites (including low glycerol and ethanol production), the release of mannoproteins, etc. (Henick-Kling et al. 1998; Padilla et al. 2016). The corresponding biochemical reactions and/or metabolic pathways are well described for some of those compounds, see for extensive mechanistic reviews Belda et al. (2017) or Swiegers and Pretorius (2005). All those features led to the general agreement that the use of certain NS could improve wine aroma complexity (Padilla et al. 2016). However, while the variation of many wine compounds were studied in relationship with changes in sensory profiles, it is still unclear which compounds account the best for the perceived changes, and their relative contribution to wine complexity. It is tempting to assume that increasing the number of compounds studied could help unraveling such relationship. Indeed, large-scale metabolomics allowing the quantification of hundreds of volatile compounds allowed the identification of unique features associated with different NS species (Beckner Whitener et al. 2015, 2016) but failed in clarifying the link between those metabolites and wine complexity. Another difficulty that has to be taken into account when dealing with NS species is that their use may increase the risk of stuck or sluggish fermentations (Hranilovic et al. 2018). Stuck or sluggish fermentations could depend on the yeast strains combination, with some mix performing better than other in identical conditions. The choice of the strains couple (NS, *S. cerevisiae*) to mix together is thus one key parameter to manage successfully mixed NS fermentations, one possible explanation would be the existence of yeast-yeast interactions (Chasseriaud 2015; Hranilovic et al. 2018; Kemsawasd et al. 2015; Nissen et al. 2003). Finally, the impact of spontaneous fermentations has recently been studied (Budić-Leto et al. 2010; Domizio et al. 2007; Patrignani et al. 2017). The sensory profiles of spontaneous fermentations are usually heterogeneous, and the multiplicity of biological and oenological factors involved tangles in their understanding. Use of local strains is not systematically the best way to reveal sensory complexity and typicality associated to a given *terroir*.

## Lactic acid bacteria, the malolactic drivers

Malolactic fermentation (MLF) is sometimes required as part of the winemaking process in order to reduce the acidity, which can be beneficial to the quality of certain types of wines. MLF is driven by lactic acid bacteria (LAB), the most famous one being *Oenococcus oeni* (Lonvaud-Funel 1999), followed by *Leuconostoc* species (Rodriguez et al. 1990). Besides their ability to produce lactic acid, LAB are able to produce or release several compounds impacting the sensorial properties of the wine, positively or negatively depending on their nature, their concentrations, the physical-chemical properties of the wine, etc. (Costello et al. 2012; Lonvaud-Funel 1999). The abundance of esters, volatile acids, and higher alcohols have been measured following MLF (Costello et al. 2012; López et al. 2011) and associated to changes in wine sensorial perception. The underlying mechanisms could involve the production of enzymatic activities, particularly esterases (Antalick et al. 2012). However, although significant, the impact of bacteria on wine aroma appears lower than the impact of yeast (Gammacurta et al. 2017). Until recently, the traditional MLF management adopted by most wineries was a sequential inoculation: the LAB were inoculated after AF completion. However, co-inoculation, which is the practice of inoculating bacteria shortly after yeast inoculation, is gaining in popularity because it could help securing and improving the MLF, and preventing the risk of *Brettanomyces* bruxellensis contamination by shortening the time between AF and MLF (Cañas et al. 2012; Versari et al. 2016). MLF co-inoculations were shown to be associated with the modification of several volatile compounds and sensory perception by comparison with sequential inoculations (Cañas et al. 2012; Versari et al. 2016). This suggests the existence of direct or indirect yeast-bacteria interactions (Versari et al. 2016) whose mechanisms remain poorly understood. In addition, it should be noted that MLF co-inoculations are associated with increased risks of stuck or sluggish alcoholic fermentation (Alexandre et al. 2004) when the latter is not well controlled. Another limit to the implementation of MLF co-inoculation is related to its incompatibility with post-maceration fermentation. Indeed, this practice—traditionally used to improve the organoleptic properties of red wines (Ribéreau-Gayon et al. 2006)—requires a higher temperature than the one recommended for MLF (30 vs. 20 °C). Finally, all those publications establish a significant impact of MLF drivers on wine composition and wine perception. However, the extent to which LAB impact wine complexity is still unclear.

The impact of microorganisms on wine perception and composition has been the object of considerable interests from the last 20 years. Nonetheless, most of the studies published are essentially descriptive rather than explicative. The involvement of some molecular mechanisms has been suggested, among which the regulation of the Ehrlich pathway

or the enzymes involved in ester production (Belda et al. 2017; Swiegers and Pretorius 2005). To date, the formal demonstration of the impact of such mechanisms on wine complexity still remains to be done. The discrepancy between the number of publications and our actual knowledge on the subject could be related to the multiplicity of factors involved in wine complexity (biological, chemical) and to the fact that those multiple factors, far from being additive, could interact together in a myriad of ways. Several layers of interaction mechanisms could be involved: interaction between wine compounds, interaction between microorganisms, and interaction between compounds and microorganisms. In addition, poorly investigated phenomenon could also explain the impact of microorganisms on wine complexity, like the intriguing case of the sweet taste of dry wines (i.e., sweetness perception in absence of sugar). Such phenomena emerge as promising future research trends that could improve our understanding of the impact of microorganisms on wine complexity and perception and are detailed in the following part.

## Wine complexity: challenges and future prospects

### Perceptive interactions

At least a thousand aromatic compounds have been described in wine, some of them at higher levels than their olfactory threshold, and others below (Darriet et al. 2013). However, wine aroma is not the simple additive results of all the molecules present, and describing the interactions between those compounds is one of the greatest challenges of wine chemistry. An important milestone was reached by Pineau et al. in 2009: they demonstrated that the fruity aroma of red wines resulted—at least in part—from particular perceptive interactions between various aromatic compounds (Pineau et al. 2009). The term "perceptive interactions" is used for odor mixtures and describes the olfactory impact of (the addition/subtraction of) one or several compounds on the perception of others, and particularly emphasizes the impact of compounds present at concentrations below their olfactory thresholds. Several other works identified molecules having enhancing and/or synergistic effects (Atanasova et al. 2004; Lytra et al. 2014; Segurel et al. 2004), for example, Lytra et al. (2013) showed that the perception of fruity aromas was increased in the presence of certain acetates and ethyl esters. Conversely, some compounds could have a masking effect on wine fruity aroma. As examples, Cameleyre et al. (2015) showed how higher alcohols produced during AF impacted more or less the fruitiness according to their concentrations, and Lytra et al. (2012) demonstrated the role of diactyl, acetoin, and γ-butyrolactone, particularly produced during MLF.

So far, most of the identified "interacting" compounds are metabolites produced during alcoholic and/or malolactic fermentations and the impact of these technological phases should also be considered as an indirect way to manage wine olfactive characteristics. In this context, subsequent yeast and lactic acid bacteria strain selection could be considered from a new perspective. However, assessing exhaustively the role of microorganisms does not seem to be an easy task. Recent advances showed that substituted esters, involved in fruity aroma, are produced by yeasts during AF. Yeast is also responsible for the production of the corresponding acids which are esterified during the first years of aging (Lytra et al. 2017). This demonstrates that yeast impacts not only the aroma of the young wine but also its evolution.

### Microbial interactions: the combined forces of wine microorganisms

During winemaking, numerous microorganisms co-exist and may interact together, directly, or indirectly by modifying their common environment. Understanding microbial interaction is thus one of the great challenges to understand the impact of microorganisms on wine perception. In wine, several mechanisms of yeast-yeast interactions have been suggested: nutriments competition, release of toxic products, synergism, antagonism, and cell-cell contact which is a "quorum-sensing"-like mechanism (Fleet 2003; Nissen et al. 2003; Renault et al. 2013). Although the precise molecular mechanisms are not fully described, the impact of such interactions has been partially investigated: Renault et al. (2015) showed that mixed inoculations of grape must include *S. cerevisiae*, and *T. delbrueckii* was associated with an increase in four esters (ethyl propanoate, ethyl isobutanoate, ethyl dihydrocinnamate, and isobutyl acetate). The ester concentration enhancement was due to yeast-yeast interactions: *S. cerevisiae* increased its production of four esters in response to the presence of *T. delbrueckii*. In addition, sensorial analysis showed that the mixed inoculations enhanced the complexity and fruity notes of the wine in comparison with pure culture. An elegant experiment was then realized: the concentration of these four esters in pure culture of *S. cerevisiae* was artificially adjusted up to their measured concentrations in mixed cultures. These additions restored the fruity and complexity perception of the wine, demonstrating formally the involvement of these four esters in the perception of wine complexity.

Other levels of microbial interaction has been demonstrated or suggested: interactions between strains of the same species like within *S. cerevisiae* (Howell et al. 2006), suggesting that the choice of complementary strains could be useful for wine aroma improvement (King et al. 2008). Cross-kingdom interactions, between bacteria and yeast, have also been described recently (Jarosz et al. 2014; Ramakrishnan et al. 2016). The underlying mechanisms are particularly interesting: bacteria heritably transform yeast metabolism through prion signaling,

and such modifications were significantly related to the completion of alcoholic fermentation in winemaking (Jarosz et al. 2014). The impact of those mechanisms on wine perception or complexity remains unknown, yet yeast-bacteria interactions emerge as a promising research field.

## Complexity and the taste of wine

The organoleptic perception of wine results from a combination of several phenomena occurring in the oral cavity, including aroma, taste, and tactile sensations (Sáenz-Navajas et al. 2012). Most of the literature focuses on the impact of microorganism on wine odorous compounds, ignoring gustatory ones. However, wine quality is strongly linked to the harmony of taste balance that plays a non-negligible role in wine complexity. The taste balance is mainly due to interactions between sweet, sour, and bitter tastes. Several molecules present in wine contribute to this balance, and microorganisms modulate the concentration of some of them. This is particularly the case of sourness that is partly due to the concentrations of lactic or malic acids, which are impacted by several wine microorganisms including LAB, *Saccharomyces*, and non-*Saccharomyces* species (Volschenk et al. 2006). Moreover, variations of sourness intensity can also modulate perception of other sensory attributes like astringency (Fontoin et al. 2008). Astringency is a tactile sensation that is mostly related to phenolic compounds that can enhance either dryness or mouth feel (Frost et al. 2015). In enology, the contact of wine with yeast lees is a current practice for modulating the fullness and mouth length of wines. During lees aging, yeast autolysis is correlated with a reduction of astringency (Fernández et al. 2011). The polysaccharides released during yeast autolysis interact with phenolic compounds (Carvalho et al. 2006; Vidal et al. 2004), suggesting their possible role in the mouth feel perception (Escot et al. 2001). However, the impact of polysaccharides on wine taste is still debated and remains unclear: it appears more likely to be an indirect relationship (Jones et al. 2008). The first direct role of microorganisms in gustatory perception in wine was given by Marchal et al. (2011). In this study, the authors demonstrated that increasing the quantities of yeast lees enhances sweetness perception. This effect is partially due to a peptidic molecule derived from the heat shock protein Hsp12p. The expression level of the related gene *HSP12* is strongly modulated by the fermentation conditions (sugar and temperature) but also by the yeast strain used. The expression level of such gene was partially correlated with the sweetness perception in red wine providing a first demonstration of a strain impact on this trait (Marchal et al. 2015). The quantity of the protein Hsp12p produced during the alcoholic fermentation shows a great variability (Blein-Nicolas et al. 2015; Léger et al. 2017). This variability offers promising perspectives for screening new strains conferring a more intense sweetness to dry wines. Interestingly, the *HSP12* gene is strongly overexpressed in mixed culture between *S. cerevisiae* and *T. delbrueckii* (Tronchoni et al. 2017). Moreover, the secretion of Hsp12p seems to be involved in inter-strain cross-talk in mixed *S. cerevisiae* cultures, as a signal to activate stress responses in surrounding cells (Rivero et al. 2015). The fact that mixed culture shows overexpression of this protein could explain the enhanced gustatory properties of mixed cultures that are often perceived as more complex. The effect of ethanol and glycerol, the main metabolites of alcoholic fermentation, on wine sweetness has been overestimated for a long time. While glycerol has been shown to have no impact on wine taste within the concentration range observed in dry wines (Gawel et al. 2013; Jones et al. 2008; Marchal et al. 2011), the sensory influence of ethanol on sweetness appears less important than suggested previously. However, the enhancing role of ethanol in bitterness and astringency perception has been well established (Cretin et al. 2018; Gawel et al. 2013). Beyond ethanol, the effect of microorganisms on wine bitterness has been poorly investigated. Further studies could improve the understanding of taste modifications consecutive to alcoholic and malolactic fermentations. In any case, those examples demonstrate the need for studies focusing on non-volatile wine compounds and their impact on wine perception and complexity.

## Yeast lag-phase duration: increasing wine complexity through the delay of alcoholic fermentation

Recently, Zimmer et al. investigated the genetic basis of lag-phase duration in winemaking conditions (Zimmer et al. 2014). The authors showed that a translocation involving the *SSU1* gene conferred an increased expression of the sulfite pump during the first hours of alcoholic fermentation, resulting in shorter lag-phase duration. Surprisingly, the use of long lag-phased *S. cerevisiae* during winemaking was associated with increased fruitiness and complexity in a Sauvignon Blanc wine (Albertin et al. 2017). In addition, the combination of a long lag-phased *S. cerevisiae* with grape must $CO_2$ saturation treatment allowed the stimulation of the growth of non-*Saccharomyces* species of interest (Chasseriaud et al. 2018). The relationship between lag-phase duration and wine perception is rather unclear and may involve physicochemical phenomena, metabolic reactions, or a combination of both. Further research programs are needed to confirm those results in a wider variety of wine and to elucidate the underlying molecular mechanisms.

## Conclusion

Wine complexity is a multicomponent concept whose perception may vary from one person to another. Besides interindividual variation that greatly complicates its evaluation,

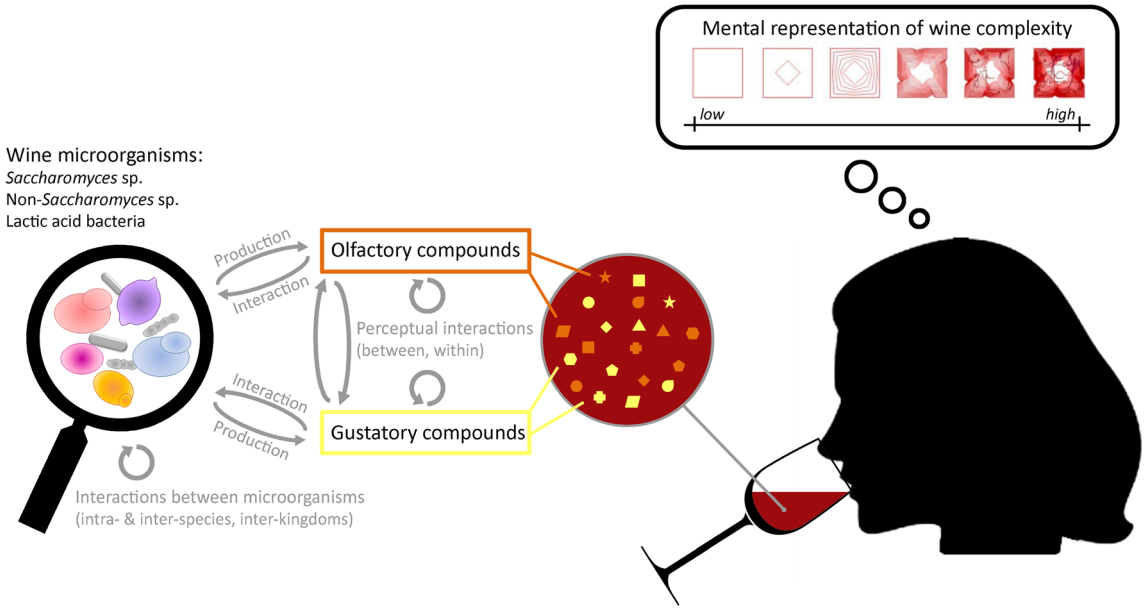


**Fig. 3** Schematic representation of direct or indirect microbiological factors shaping wine complexity. The mental representation of wine complexity was proposed by Schlich et al. (2015). Images included in the figure were either home-made or taken among the "free to use share or modify" images provided by Google

another difficulty when dealing with wine complexity lies in the existence of false assumptions and shortcuts. First, contrary to expectations, there is no clear relationship between the perception of wine complexity and wine chemical complexity. Adding one compound in wine, even an odorant or gustatory one, will not necessarily results in increased complexity. Adding multiple molecules in wine (even odorant or gustatory ones) will not necessarily results in increased complexity either. Moreover, such "additive" hypothesis ignores the importance of interactions between compounds, whose significance at the perception level was formally demonstrated. Those results point out the fact that, to deal with wine complexity, sensorial analyses are mandatory. This obvious statement is at odds with the scientific literature that proposes—in most cases—wine chemical analyses without sensorial evaluation. This issue is probably related to the difficulty to perform sensory analysis: in a collective experts' report published by OIV (2015), the general conditions to perform meaningful sensory analysis include, for example, a dedicated tasting room, temperature and humidity control, tasting glasses, specific organization (preferably morning sensory sessions), etc. The tasting panel is another key issue, since professionals with a good level of expertise seem to be the most apt to evaluate the sensory concept of complexity of wines. Large volumes of wine samples are required, conversely only a small number of wines per session should be presented to reduce effects of fatigue. Measuring the chemical composition can thus be used as a first step to evaluate the modalities that are more likely to have a sensory effect. However, sensory analysis remains mandatory in the end. In addition, most chemical analyses concentrate on specific compounds (esters, volatile thiols, etc.), and thus, disregard other/unknown compounds that could be involved in wine perception. In particular, the impact of taste-active molecules should be better investigated as they play a non-negligible role in wine perception.

The second common assumption that should be revised is that microbial diversity does not necessarily results in wine complexity and/or in multiple molecules addition in wine. While there is a definite trend to increase the number of microorganisms involved in winemaking (both at the intraspecific or interspecific levels), some combinations of microorganism (yeast-yeast and/or yeast-bacteria) can achieve a great wine complexity where other will fail. Indeed, as previously described, harmony and balance are of paramount importance for the perception of wine complexity, and microorganism interactions, directly or indirectly, could affect significantly wine perception.

Therefore, untangling wine complexity and the impact of microorganisms will not be a simple thing. Figure 3 shows a schematic representation of how microorganisms may impact wine complexity. Wine microorganisms can impact directly wine complexity through the production of olfactory compounds (volatile thiols, esters, higher alcohols, etc.) or gustatory molecules (polysaccharides, polyphenols, proteins, etc.). This research area is the most studied one to date, although gustatory compounds are less studied than olfactory ones. Understanding the impact of microorganism on wine taste is thus a key research field that should be thoroughly investigated. Perceptual interactions within olfactory of gustatory compounds, or between them, represent a very promising research

avenue to explore, particularly to refine the direct or indirect role played by microorganisms. Another line of research also lies with interaction, albeit between microorganisms themselves, at the intraspecific or interspecific levels, and also between kingdoms (yeast-bacteria). Microbial interactions could be mediated by cell-cell contact mechanisms or mediated by wine compounds. Thus, future research should focus on the multiple layers of biological and chemical interactions occurring during winemaking and should include joint approaches at the microbial, chemical, and sensorial levels. Altogether, these new avenues will help in improving our knowledge of wine complexity and will help in refining the impact of microorganisms on wine complexity.

**Author contributions statement** All authors wrote the paper.

## Compliance with ethical standards

**Conflict of interest** Sophie Tempère, Axel Marchal, Jean-Chistophe Barbe, Marina Bely, Isabelle Masneuf-Pomarede, and Warren Albertin declare that they have no conflict of interest. Philippe Marullo is affiliated with Biolaffort Company.

**Informed consent** This article does not contain any studies with human participants or animals performed by any of the authors.

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**Résumé**

Depuis 2006, mes travaux ont été centrés sur l'étude de la diversité génétique et phénotypique des levures du raisin et du vin, qu'il s'agisse d'espèces bénéfiques utilisées comme auxiliaires technologiques (*Saccharomyces* sp., non-*Saccharomyces* d'intérêt œnologique) ou qu'il s'agisse d'espèces d'altération (*Brettanomyces bruxellensis*). Nous avons développé des approches multi-échelles, combinant des outils classiques (microbiologie traditionnelle, génétique des populations), des approches spécifiques de l'œnologie (suivi des cinétiques fermentaires, analyse sensorielle), et des analyses –*omic* à haut-débit couplées à des outils bio-informatiques. Ces différents projets ont été financés sur fonds publiques (ANR, région, Bordeaux INP, etc.) ou privés (industriels de l'oenologie, interprofession, etc.), et ont nécessité l'engagement de nombreux collègues, collaborateurs et étudiants – nationaux ou internationaux. Sur le plan fondamental, nos travaux ont permis d'améliorer nos connaissances des levures d'oenologie à travers la publication de >40 articles scientifiques dans des revues avec comité de lecture. Quelques applications finalisées ont également vu le jour (sélection de souches de levure pour l'oenologie, développement de marqueurs moléculaires, etc.). Nous avons également contribué au transfert de connaissance vers la profession sous la forme d'articles ou de communications techniques.

Les projets que j'aimerai développer à l'avenir se déclinent en trois grands thèmes :
1- mieux comprendre l'adaptation des espèces et sous-populations de levure à des environnements variés, anthropisés ou non ;
2- améliorer nos connaissances des mécanismes d'interactions entre espèces microbiennes, notamment dans l'environnement vitivinicole ;
3- approfondir l'étude de certains phénotypes d'intérêt œnologique.
Comme pour nos travaux passés, les projets futurs comporteront lorsque cela est possible un volet fondamental et appliqué afin de mieux maitriser la qualité et l'identité des vins d'aujourd'hui et demain.