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1 *Brettanomyces bruxellensis*: Overview of the genetic and phenotypic  
2 diversity of an anthropized yeast

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## 19 Abstract

20 Human-associated microorganisms are ideal models to study the impact of environmental changes on  
21 species evolution and adaptation because of their small genome, short generation time, and their  
22 colonization of contrasting and ever-changing ecological niches. The yeast *Brettanomyces bruxellensis*  
23 is a good example of organism facing anthropogenic-driven selective pressures. It is associated with  
24 fermentation processes in which it can be considered either as a spoiler (*e.g.* winemaking, bioethanol  
25 production) or as a beneficial microorganism (*e.g.* production of specific beers, kombucha). Besides its  
26 industrial interests, noteworthy parallels and dichotomies with *Saccharomyces cerevisiae* propelled  
27 *B. bruxellensis* as a valuable complementary yeast model. In this review, we emphasize that the broad  
28 genetic and phenotypic diversity of this species is only beginning to be uncovered. Population genomic  
29 studies have revealed the co-existence of auto- and allotriploidization events with different  
30 evolutionary outcomes. The different diploid, autotriploid and allotriploid subpopulations are  
31 associated with specific fermented processes, suggesting independent adaptation events to  
32 anthropized environments. Phenotypically, *B. bruxellensis* is renowned for its ability to metabolize a  
33 wide variety of carbon and nitrogen sources, which may explain its ability to colonize already  
34 fermented environments showing low-nutrient contents. Several traits of interest could be related to  
35 adaptation to human activities (*e.g.* nitrate metabolization in bioethanol production, resistance to  
36 sulphite treatments in winemaking). However, phenotypic traits are insufficiently studied in view of  
37 the great genomic diversity of the species. Future work will have to take into account strains of varied  
38 substrates, geographical origins as well as displaying different ploidy levels to improve our  
39 understanding of an anthropized yeast's phenotypic landscape.

40

41 Keywords: polyploidy, hybridization, adaptation, fermentation, beer, wine

## 42 1. Introduction

43 Yeasts are eukaryotic species encountered in most, if not all, earth biomes (Starmer & Lachance, 2011).  
44 Yeasts are heterotrophic, they are described as primary decomposers of organic matter, and are  
45 particularly associated with the early colonization of nutrient-rich substrates. The ability of some yeast  
46 species to efficiently perform alcoholic fermentation, *i.e.* to convert sugar into ethanol, made it  
47 possible to forge close ties with human beings, leading to a tight co-evolution between yeast and  
48 human (Starmer & Lachance, 2011). Their simple life cycle, their small genomes (10 to 20Mbp) which  
49 are genetically diverse and their multifaceted metabolisms have propelled yeasts as valuable models  
50 to study evolutionary genetics and ecology (Gladieux et al., 2014; Hittinger et al., 2015). Besides the  
51 famous yeast model organism *Saccharomyces cerevisiae*, the genus *Brettanomyces* (of which *Dekkera*  
52 is a synonym) has received a lot of attention recently, due to its association with various food processes  
53 and to its unusual genomic composition. This genus belongs to the *Pichiaceae* family, which is a part  
54 of the *Saccharomycotina* subphylum (Kufferath & Van Laer, 1921; Riley et al., 2016). It is composed of  
55 six species (*B. nanus*, *B. naardenensis*, *B. bruxellensis*, *B. anomalus*, *B. custersianus* and  
56 *B. acidodurans*), with *Allodekkera sacchari* being the closest non-*Brettanomyces* species known to date  
57 (Jutakanoke et al., 2017; Péter et al., 2017) (Figure 1A). Recently, high quality genomes were obtained  
58 for five *Brettanomyces* species, revealing distant species separated in two clades with *B. nanus* and  
59 *B. naardenensis* on one hand, and *B. bruxellensis*, *B. anomalus* and *B. custersianus* on the other hand  
60 (Roach & Borneman, 2020). The calculation of average nucleotide identity along the genomes between  
61 each species pairs, ranging between 60.6% and 77.1%, revealed that *Brettanomyces* species are  
62 relatively distant of each other: as a comparison, the most distant species within *Saccharomyces* genus  
63 show 79.9% nucleotide identity (*S. cerevisiae* and *S. eubayanus*) and 75.2% within *Metschnikowia*  
64 genus (*M. hawaiiiana* and *M. orientalis*), two yeast genus frequently associated with fermented foods  
65 (Lachance, Lee, & Hsiang, 2020; Roach & Borneman, 2020). The five *Brettanomyces* species whose  
66 genome is fully sequenced exhibit gene family expansions related to fermentation, such as glucosidase  
67 enzymes involved in starch or galactose metabolism, as well as in nitrogen assimilation. In addition,

68 twelve horizontal gene transfer (HGT) events were detected within *Brettanomyces* genus, and may  
69 explain the ability of *B. bruxellensis* and *B. anomalus* to utilize sucrose (Roach & Borneman, 2020).  
70 Gene expansions and HGTs are well described markers of domesticated subpopulations of *S. cerevisiae*  
71 (Gallone et al., 2018; Giannakou, Cotterrell, & Delneri, 2020; Legras et al., 2018; Peter et al., 2018). The  
72 identification of interesting parallels with the evolution of *S. cerevisiae* supported *Brettanomyces* as a  
73 valuable yeast model to study adaptation to fermentative environments.

74 Due to its positive or negative role in different industrial applications, *B. bruxellensis* is the species that  
75 has received the most attention. It was first isolated by N. Hjelte Claussen in 1904 as being responsible  
76 for the special flavor of British beers, and therefore was the first patented microorganism (Claussen,  
77 1904; Steensels et al., 2015). In brewery, *B. bruxellensis* plays a major role for some special beer types  
78 such as the Lambics from Belgium, Flanders Red and Brown ales, or the 'coolship ales' from the USA  
79 (Bokulich, Bamforth, & Mills, 2012; Claussen, 1904; De Roos & De Vuyst, 2019). It contributes to the  
80 peculiar flavour of spontaneous fermented beers, bringing additional complexity to their aromatic  
81 bouquet. In addition, over the past decades, the growing popularity of craft beers and the rise of  
82 microbreweries led to an increased interest among brewers who use *B. bruxellensis* alone or in co-  
83 culture with other species. For example, *B. bruxellensis* has been described as a good candidate for  
84 beers with a spicy phenolic note (Holt, Mukherjee, Lievens, Verstrepen, & Thevelein, 2018).  
85 *Brettanomyces bruxellensis'* scientific literature with a focus on brewery is substantial (Capece,  
86 Romaniello, Siesto, & Romano, 2018; De Roos & De Vuyst, 2019; Lentz, 2018; Serra Colomer, Funch, &  
87 Forster, 2019; Steensels et al., 2015). *Brettanomyces bruxellensis* is also involved in the elaboration of  
88 other spontaneous fermented beverages such as kombucha, kefir, etc. in which its role is mostly  
89 considered as positive (Lynch, Wilkinson, Daenen, & Arendt, 2021; Tran et al., 2020).

90 Despite its positive contribution to particular beer flavours and traditional beverages, *B. bruxellensis* is  
91 also recognised as a major spoiler for top-selling ale and lager beers due to sensory or turbidity defects  
92 (Shimotsu et al., 2015). The same account for wine, where *B. bruxellensis* negative contribution is also

93 widely described, amplified by its resistance to sulphite, one of the common control method  
94 historically used (Monica Agnolucci, Tirelli, Cocolin, & Toffanin, 2017; Avramova et al., 2019; Blomqvist  
95 & Passoth, 2015; Malfeito-Ferreira, 2018; Schifferdecker, Dashko, Ishchuk, & Piškur, 2014; Suárez,  
96 Suárez-Lepe, Morata, & Calderón, 2007). Its presence in wine is associated with the production of  
97 volatile molecules (called volatile phenols), associated to unpleasant aromas described as barnyard,  
98 horse sweat or burnt plastic (Chatonnet, Dubourdie, Boidron, & Pons, 1992; Kheir, Salameh,  
99 Strehaiano, Brandam, & Lteif, 2013). The 'Brett' taint negatively affects up to 25-30% of red wines in  
100 the world (Alston, Arvik, Hart, & Lapsley, 2021; Gerbaux, Jeudy, & Monamy, 2000; A. Romano, Perello,  
101 Revel, & Lonvaud-Funel, 2008). *Brettanomyces bruxellensis* is able to penetrate the micropores of the  
102 barrels and therefore reused barrels – a frequent practice in oenology – are a recurrent cause of  
103 contamination (Cartwright, Glawe, & Edwards, 2018; Fabrizio et al., 2015). In bioethanol production,  
104 this species is considered as the main yeast spoiler as it can reduce the ethanol yield and cause large  
105 economic losses (Basílio et al., 2008; Bassi, Meneguello, Paraluppi, Sanches, & Ceccato-Antonini, 2018;  
106 Blomqvist & Passoth, 2015; A.T. de Souza Liberal et al., 2007; Radecka et al., 2015; Seo, Park, Jung, Ryu,  
107 & Kim, 2020). Besides its beneficial-spoiler duality, another oddity of *B. bruxellensis* is its ability to act  
108 as a first fermenter (even starter) yeast in some processes (*e.g.* craft beer, kombucha, bioethanol) or  
109 as a fermentation 'finisher' (*e.g.* wine, Lambic beers, etc.), the actual fermentation usually being  
110 carried out by *S. cerevisiae*. This starter-finisher versatility suggests multiple fermentative adaptations  
111 that may have arisen from the complex structure of *B. bruxellensis* genome that includes large  
112 chromosomal rearrangements, hybridization events, as well as ploidy level variation (Gounot et al.,  
113 2020). In particular, a remarkable characteristic of *B. bruxellensis* species resides in the rare  
114 coexistence of diploid and steady triploid isolates (Curtin, Kennedy, & Henschke, 2012; Piškur et al.,  
115 2012).

116 In this review, we first focus on the genetic and genomic composition of *B. bruxellensis* revealed by  
117 population genomic surveys. This genetic diversity is reflected at the phenotypic level, with contrasting  
118 behaviours regarding metabolic and life-history traits that have ecological significances. We describe

119 the impact of such diversity on central metabolism characteristics and technological traits, and we  
120 discuss how future researches including both genomic and phenotypic approaches may shed lights on  
121 the evolutionary history of a human-associated yeast.

## 122 2. Genetic and genomic diversity of *Brettanomyces bruxellensis*

### 123 2.1. Population structure of *Brettanomyces bruxellensis*

124 A wide variety of molecular tools (*e.g.* AFLP, RAPD) were developed to assess the genetic diversity of  
125 *B. bruxellensis* species (reviewed in (Renouf, Lonvaud-Funel, & Coulon, 2007)). Most of these studies  
126 focused on the intraspecific diversity of small cohorts: between 10 to 100 isolates mainly from the  
127 same process (Agnolucci et al., 2009; Conterno, Lucy Joseph, Arvik, Henick-Kling, & Bisson, 2006;  
128 Curtin, Bellon, Henschke, Godden, & de Barros Lopes, 2007; Miot-Sertier & Lonvaud-Funel, 2007).  
129 Thus, an important step has been taken with the genotyping of more than 1,000 worldwide strains  
130 using 12 microsatellite markers (Avramova, Cibrario, et al., 2018). This study provided deep insights  
131 into the genetic diversity and ploidy level in several subpopulations of different ecological origin. The  
132 population structure was mainly explained by the ploidy level (~47% of the variance), followed by the  
133 geographical origin (~5% of the variance) and the fermented product from which the strain had been  
134 isolated (~6% of the variance). However, when considering non-wine isolates, the geographical origin  
135 explains ~55% of the total variance, indicating that wine isolates are highly disseminated across the  
136 world compared to strains isolated from other substrates. Isolates from different origins (such as beer,  
137 kombucha, tequila and bioethanol isolates) were mostly clustered into one or two genetic groups,  
138 suggesting different adaptation processes to various anthropic environments (Avramova, Cibrario, et  
139 al., 2018). This large population study allowed the identification of different genetic subpopulations  
140 within *B. bruxellensis*, which were subsequently validated and refined by whole genome sequencing  
141 approaches (Eberlein, Abou Saada, Friedrich, Albertin, & Schacherer, 2021; Gounot et al., 2020; Serra  
142 Colomer, Chailyan, et al., 2020) (Supplemental Table 1). At least six subgroups can now be considered,  
143 either diploid (two groups) or triploid (four groups) (Figure 1B). Among diploid groups, one

144 subpopulation is composed mainly of strains isolated from wine, and is named the wine 3 group  
145 (known as G2N3, CBS2499-like or Wine 2n in various publications, Supplemental Table 1), the other is  
146 associated with kombucha, beer and wine isolates, and we named it the kombucha group (known as  
147 G2N1, L14165-like, Farmhouse). Regarding the triploid subpopulations, the beer group contains strains  
148 from beer and wine (G3N1, AWRI1608-like, Lambic), the two other groups are strongly associated with  
149 winemaking and are referred as wine 1 (or G3N2, AWRI1499-like, Wine 3n) and wine 2 groups (or  
150 G3N3, L0308-like). The last group, named teq/EtOH group, contains most isolates from bioethanol and  
151 tequila process (CBS5212-like, Tequila). Recent genomic data suggested that the teq/EtOH group was  
152 not monophyletic, yet more in-depth analyses are necessary to refine precisely the actual number of  
153 teq/EtOH groups. Recently still, genome sequencing revealed an autotetraploid strain (Figure 1B),  
154 genetically close to a small subpopulation already observed but described as diploid (G2N2, KOM1449-  
155 like). More isolates are needed to delineate this subpopulation and its ecological and genomic features.  
156 The vast majority (~80%) of wine strains are found in three groups (wine 1 and wine 2 triploid groups,  
157 wine 3 diploid group, Figure 1B), yet some wine isolates can be found occasionally in other groups,  
158 with variations depending on the wine producing region (Avramova, Cibrario, et al., 2018; Cibrario et  
159 al., 2019). Beer isolates are also distributed in different groups, although to a lesser extent (two main  
160 groups). Regarding the other isolation niches (such as kombucha, tequila, bioethanol), additional works  
161 and more strains are needed to determine whether they fall within one specific subpopulation, or if  
162 they are more disseminated as for winemaking. Dissemination via equipment could play an important  
163 role in exchanges within and between ecological niches: craft brewers frequently age beer in wood  
164 barrel that previously contained red or white wines (Sanna & Pretti, 2015) and similar practice is  
165 common for tequila's ageing (Aguilar-Méndez et al., 2017). *Brettanomyces bruxellensis*' population  
166 structure seems to be well-defined, but so far, only anthropic isolates of the species have been  
167 described, with no wild strains identified so far.

## 168 2.2. Genomic landscape of *Brettanomyces bruxellensis*



169 Within the *Saccharomycotina* subphylum, *B. bruxellensis* diverged from the model species *S. cerevisiae*  
170 between 200 and 300 MY ago, before the whole-genome duplication (WGD) event that appeared in  
171 the *Saccharomyces* lineage 100 MY ago (Fisher, Buskirk, Vignogna, Marad, & Lang, 2018; Guo et al.,  
172 2016; Wolfe & Shields, 1997). WGD outcomes (e.g. gene duplication, promoter rewiring) were  
173 suggested to be involved in the acquisition of the make-accumulate-consume (MAC) strategy in the  
174 *Saccharomyces* genus (Thomson et al., 2005). Thus, it seems that both species, *S. cerevisiae* and  
175 *B. bruxellensis*, have independently acquired the MAC ability (Rozpędowska et al., 2011). The first  
176 partial genome sequence of *B. bruxellensis* strain CBS 2499 showed around 50% of nucleotide identity  
177 with *S. cerevisiae* (Woolfit, Rozpędowska, Piškur, & Wolfe, 2007), and more recent studies identified  
178 that at least 3,300 orthologous gene families were conserved across both clades (out of 5000 for  
179 *B. bruxellensis*) (Cheng et al., 2017).

180 Over the past decade, several *de novo* assemblies have been published with increasing quality (Table  
181 1), in particular thanks to the combination of long-read sequencing (e.g. Oxford Nanopore sequencing)  
182 with short-read sequencing (Fournier et al., 2017). Such high quality assemblies were used as reference  
183 genomes for population genomic surveys (Eberlein et al., 2021; Gounot et al., 2020; Serra Colomer,  
184 Chailyan, et al., 2020). These population studies revealed that the pangenome is composed of 5,409  
185 ORFs (open reading frames) with 5,106 core and 303 accessory ORFs within the species. Although no  
186 significant functional enrichment was found for the set of accessory genes, some of them were shown  
187 to be involved in drug and sugar transports (Gounot et al., 2020). Many core genes involved in carbon  
188 and nitrogen uptake have been identified, consistent with the ability of *B. bruxellensis* to metabolise a  
189 wide range of complex nutrients (e.g. xylose, lactose, cellobiose and nitrate).

190 In *B. bruxellensis*, the nucleotide diversity estimated by the average number of pairwise nucleotide  
191 differences,  $\pi$ , is high ( $\pi = 1.2 \times 10^{-2}$ ) compared to *S. cerevisiae* ( $\pi = 3 \times 10^{-3}$ ) (Gounot et al., 2020). In  
192 addition, roughly 50% of the available strains of *B. bruxellensis* (exclusively isolated from anthropic  
193 niches) are triploids (Avramova, Cibrario, et al., 2018). Polyploidy in industrial yeasts is known to confer

194 robustness and environmental stress resistance (Albertin & Marullo, 2012; Querol & Bond, 2009;  
195 Steensels, Gallone, & Verstrepen, 2021). Interestingly, while triploidy appears to be predominant in  
196 the available strains, aneuploidy is rarer in *B. bruxellensis* than in *S. cerevisiae* with 5.6% and 19.1% of  
197 aneuploidy isolates, respectively (Gounot et al., 2020; Peter et al., 2018). Genomic studies have shown  
198 that the triploid genomes have different genomic architectures arising from intra- and interspecific  
199 hybridization events (Borneman, Zeppel, Chambers, & Curtin, 2014; Eberlein et al., 2021; Gounot et  
200 al., 2020). In a recent study, the polyploid genomes were reconstructed using different phasing  
201 strategies, revealing that each polyploid subpopulation had a unique history (Abou Saada, Tsouris,  
202 Eberlein, Friedrich, & Schacherer, 2021). The different triploid genomes (at least four) are composed  
203 of a core diploid *B. bruxellensis* genome and an additional haploid one. These additional genomes are  
204 either genetically closely related to the diploid one (with a genetic divergence lower than 1%) or  
205 genetically divergent (greater than 3%), indicating auto- as well as allopolyploidization events.  
206 Interestingly, the three allopolyploidization events have occurred independently with a specific and  
207 unique donor for each of the polyploid subpopulations (Table 1). The closest *Brettanomyces* sister  
208 species, *B. anomalus*, shows >23% of genetic dissimilarity with the diploid *B. bruxellensis*' genome,  
209 excluding the known *Brettanomyces* sister species as possible donors. Large-scale population genomic  
210 surveys with a long-read sequencing strategy will help refine precisely the evolutionary history of each  
211 subgenome, and any intertwined relationships.

212 In addition, *B. bruxellensis* genomes show different levels of Loss Of Heterozygosity (LOH). LOH events  
213 are a source of genomic rearrangements and can contribute to the rapid onset of phenotypic diversity  
214 (Dutta, Dutreux, & Schacherer, 2021; Sampaio et al., 2020; Smukowski Heil et al., 2017). LOH has  
215 gained attention for its frequent association with fitness, adaptation, polyploid stabilization, or even  
216 pathogenesis in yeasts but also in other organisms such as the oomycete *Phytophthora capsica*, hybrids  
217 of the cultivated rice *Oryza* or the *Cobitis* fish species (Beekman & Ene, 2020; Forche et al., 2011; Janko  
218 et al., 2021; Lamour et al., 2012; Morales & Dujon, 2012; Todd, Wikoff, Forche, & Selmecki, 2019; R.  
219 R.-C. Wang, Li, & Chatterton, 1999). Compared to *S. cerevisiae* where LOH represents 50% of the

220 genome, *B. bruxellensis* presents a low level of LOH, which is variable across subpopulations (Gounot  
221 et al., 2020; Peter et al., 2018). In the diploid isolates (wine 3 and kombucha groups), the LOH regions  
222 represent 13% of the entire genome. In the triploid subpopulations, different scenarios were observed.  
223 For subpopulations that have undergone an allopolyploidization event, a higher proportion of the  
224 genome is under LOH in the beer and wine 1 groups (Figure 1B) with 26.6% and 22.3%, respectively  
225 (Eberlein et al., 2021). However, this fraction mainly concerns the acquired haploid genome. By  
226 contrast, the genomes of the teq/EtOH group are less impacted by LOH events (10.5%), which mainly  
227 involve the core diploid genome from *B. bruxellensis*. For the autotriploid population, LOH was also  
228 highlighted and conserved across six sequenced strains, resulting in the presence of only two  
229 haplotypes while three were expected (Eberlein et al., 2021). The conservation of a given haplotype  
230 over the others may indicate specific selection pressures on the alleles present in such LOH regions.  
231 Future studies will have to focus on the gene content of these LOH regions to identify possible genetic  
232 signatures of adaptation to anthropized environments.

233 Regarding the Copy Number Variation (CNV), genes affected by CNV harbour functions related to drug  
234 transporters, nitrogen assimilation or ethanol production (Borneman et al., 2014; Sam Crauwels et al.,  
235 2014; C. D. Curtin, Borneman, Chambers, & Pretorius, 2012; Gounot et al., 2020). Ploidy level and CNV  
236 could play an important role in the adaptation to the ecological niche, where multiple copies of a  
237 particular gene or gene family can be beneficial in the new environment. For example, in *S. cerevisiae*  
238 yeast, beer isolates have more increased copies of genes involved in maltose uptake and breakdown  
239 are amplified (Gallone et al., 2016; Gladieux et al., 2014). In the pathogenic *C. albicans*, CNV are  
240 associated to a significant fitness benefit to antifungal drugs (Todd et al., 2019). Within allotriploid  
241 isolates, the diploid core genome was more prone to duplication events than the acquired haploid  
242 genome (Gounot et al., 2020). Further studies will be needed to determine if CNVs are actually  
243 associated with increased adaptation to specific ecological niche, as suggested for nitrate assimilation  
244 in bioethanol production process (Galafassi, Capusoni, Moktaduzzaman, & Compagno, 2013). Indeed,  
245 genes involved in the nitrogen pathway have been independently lost in several diploid isolates within

246 different subpopulations, indicating differential selective pressure (Gounot et al., 2020). Genomic  
247 rearrangements affecting genes associated with traits of ecological interests have also been reported.  
248 For example, genes coding beta-glucosidase activity are lost in beer isolates but conserved in wine  
249 isolates (Sam Crauwels et al., 2017). This enzymatic activity could be useful to consume efficiently  
250 peculiar carbon sources found in wines aged in oak barrels, due to the liberation of specific wood  
251 polysaccharides. These variations were related to different aroma production, indicating technological  
252 interest besides ecological significance (Serra Colomer, Funch, Solodovnikova, Hopley, & Förster,  
253 2020). Variations were also observed for maltose-related genes, although it was not possible to  
254 associate a phenotype to a specific genotype (S. Crauwels et al., 2015; Serra Colomer, Chailyan, et al.,  
255 2020). Concerning Horizontal Gene Transfer (HGT) in *B. bruxellensis*, three events from bacteria were  
256 recently highlighted, of which one encompassing an invertase-coding gene (from *Asaia* bacteria). This  
257 enzymatic activity is speculated to have conferred the ability to hydrolyse sucrose (Roach & Borneman,  
258 2020). Horizontal gene transfers are drivers of adaptive evolution in eukaryotes and may have  
259 contributed to adaptation to high-sugar environments (Gladieux et al., 2014; Schönknecht, Weber, &  
260 Lercher, 2014). The prevalence of these HGTs in the different subpopulations has yet to be described,  
261 and it will be interesting to assess their possible involvement in niches' adaptation.

262 Besides the nuclear genome, genetic diversity has also been described for *B. bruxellensis'*  
263 mitochondrial genome. The mitogenome is large and variable in size (between 75Kb to 100kb),  
264 compact with introns and intergenic sequences (Eberlein et al., 2021; Procházka, Poláková, Piškur, &  
265 Sulo, 2010). By contrast to *S. cerevisiae*, the mitochondrial genome contains NADH dehydrogenase  
266 subunit genes, which allow the recycling of NAD in presence of oxygen during the fermentation process  
267 (Procházka et al., 2010). Across the different genetic subpopulations, the synteny of the mitogenome  
268 is well conserved with the exception of the teq/EtOH group for which a large inversion event and  
269 increased size (up to 100 kb) due to high intron content in the *COB* and *COX1* genes were evidenced  
270 (Eberlein et al., 2021). The intron content is known to be variable within *Saccharomyces* genus,  
271 especially for *COX1* and *COB* genes and was shown to be a marker of hybridization events (especially

272 *COX1* introns) (De Chiara et al., 2020; Prasai, Robinson, Scott, Tatchell, & Harrison, 2017). In terms of  
273 nucleotide diversity, the genetic subgroups are highly homogeneous. The teq/EtOH group is more  
274 distant, with a genetic variation of 2% to 3% with the reference mitogenome. In *Ascomycota* species,  
275 the mitochondria inheritance is biparental, implying initial heteroplasmy in case of hybridization  
276 events. The outcome of the different mitotypes can be variable: (i) one or the other might be retained  
277 stochastically, (ii) one or the other might be retained due to selective pressures (*e.g.* to purge nucleo-  
278 cytoplasmic incompatibility or because of higher fitness of one mitotype), (iii) a chimeric mitotype can  
279 emerge from the two parental ones due to recombination events (Albertin et al., 2013; De Chiara et  
280 al., 2020; Lee et al., 2008). Here, the well-conserved mitotypes across the populations of diploid and  
281 polyploidy isolates argue in favour of the selection hypothesis without exchange. By contrast, the  
282 teq/EtOH group might have acquired a chimeric mitochondrial DNA, or the mitotype from the donor  
283 of the acquired haploid genome. Further studies will be needed to understand the possible impact of  
284 these mitochondrial genomes on the phenotypic diversity.

285 The exploration of the diversity of *B. bruxellensis* genomes has just started. Population genomic  
286 surveys unveil a complex genome architecture, with a strong involvement of polyploidy and  
287 hybridization events and mechanisms generating intra-specific variation (*e.g.* CNVs, LOH). LOH events  
288 appear to have radically shaped the genomic landscape of *B. bruxellensis* polyploids. This phenomenon  
289 is widely observed in polyploid yeasts and is an essential source of interindividual variation in  
290 predominantly asexual species (Peter et al., 2018; Steensels et al., 2021). Subsequent large-scale  
291 whole-genome sequencing will help to identify and understand the forces that shape the evolution of  
292 *B. bruxellensis* genomes, especially in the context of ecological divergence and industrial adaptation.  
293 However, genomic approaches alone will not be sufficient to formally demonstrate the relationship  
294 between the genomic and the phenotypic diversification of the species. Largescale phenotyping  
295 approaches are therefore necessary to clarify the link between genetic diversity and adaptation to  
296 anthropic fermentation environments.

### 297 3. Phenotypic diversity of *Brettanomyces bruxellensis*

298 Although molecular tools bring priceless information to understand the origin of the species, their  
299 formation and diversification in ever-changing environments, the physiological abilities and metabolic  
300 features of the organisms can also be used to seek a deeper comprehension of their niche space use.

#### 301 3.1. Intraspecific diversity of central metabolism and nutrient requirements

302 The nutritional requirements of *B. bruxellensis* are less described than those of model species such as  
303 *S. cerevisiae*. Nevertheless, *B. bruxellensis* exhibits atypical characteristics related to the metabolism  
304 of carbon, oxygen, nitrogen and other nutrients, that may explain its ability to colonise harsh  
305 environments described as ‘apocalyptic’ (Smith & Divol, 2016). The main phenotypic characteristics of  
306 *B. bruxellensis* have already been reviewed (Blomqvist & Passoth, 2015; de Barros Pita et al., 2019;  
307 Schifferdecker et al., 2014; Serra Colomer et al., 2019; Smith & Divol, 2016; Steensels et al., 2015).  
308 However, phenotypic traits have been little studied in the light of genetic diversification.  
309 *Brettanomyces bruxellensis* is characterized by growth variability which is partly related to ecological  
310 origin and/or to the genetic group (da Silva et al., 2019; Louw, du Toit, Alexandre, & Divol, 2016). As  
311 an example, bioethanol isolates grow faster compared to wine isolates on seven carbon sources  
312 (sucrose, cellobiose, maltose, lactose, glucose, fructose and galactose) (da Silva et al., 2019) and the  
313 variability of growth was highlighted for other genetic groups as well (Figure 2).

314 Generally, central regulatory mechanisms like Glucose Catabolite Repression GCR (da Silva et al., 2019;  
315 Leite et al., 2012) and Nitrogen Catabolite Repression NCR (Cajueiro, Parente, Leite, de Morais Junior,  
316 & de Barros Pita, 2017; de Barros Pita, Leite, de Souza Liberal, Simões, & de Morais, 2011; de Barros  
317 Pita & Tiukova, 2013; Galafassi, Capusoni, et al., 2013) seem to be less strict in *B. bruxellensis* compared  
318 to *S. cerevisiae* (*i.e.* non-glucose and non-ammonium sources can be metabolized even in presence of  
319 high glucose/ammonium concentration). Diversity was observed in GCR, with *B. bruxellensis*'  
320 bioethanol isolates being less susceptible to GCR than wine isolates (Blomqvist, Eberhard, Schnürer, &  
321 Passoth, 2010; da Silva et al., 2019). There is no data available to our knowledge for isolates from other

322 substrates, yet this feature needs more research as it may have significant ecological and technological  
323 implications: the simultaneous use of different carbon and nitrogen sources could increase the  
324 efficiency of absorption in low-nutrient media and be associated with higher fitness in specific  
325 environments.

326 The most striking characteristic of *B. bruxellensis* is its ability to use a wide range of carbon sources as  
327 shown by large-scale phenotypic analyses (Cibrario, Miot-Sertier, et al., 2020; Conterno et al., 2006;  
328 Crauwels et al., 2015; da Silva et al., 2019; Galafassi et al., 2011; Smith & Divol, 2018). However, the  
329 carbon utilisation varies among isolates: a survey on the assimilation of 190 different carbon sources  
330 on seven strains clearly showed a variability between isolates, that could be related to different  
331 processes (Crauwels et al., 2015). By contrast to wine isolates, most beer strains were not able to  
332 consume galactose, some  $\beta$ -disaccharides (cellobiose and gentiobiose) or some  $\beta$ -substituted  
333 monosaccharides (arbutin and  $\beta$ -methyl-D-glucoside) (Crauwels et al., 2015; Serra Colomer, Funch,  
334 Solodovnikova, Hoble, & Förster, 2020). This observation could be explained by a variation of beta-  
335 glucosidase and alpha-glucosidase activity between the genetic groups (Serra Colomer, Funch, et al.,  
336 2020). Different environmental selective pressures could be responsible for this feature (Serra  
337 Colomer, Funch, et al., 2020). However, further work involving more than seven isolates is needed,  
338 including strains representative of all described subpopulations to properly assess the relationship  
339 between carbon assimilation and process.

340 Like *S. cerevisiae*, *B. bruxellensis* is able to ferment sugars into ethanol even in the presence of oxygen,  
341 a phenomenon called the Crabtree effect, which supports the 'Make-Accumulate-Consume' (MAC)  
342 strategy (Rhind et al., 2011; Smith & Divol, 2016). The evolutionary and metabolic significances of the  
343 Crabtree effect remain highly discussed, although a simplistic viewpoint suggests that metabolic shifts  
344 (from respiration to fermentation and back again) are energetically costly and time-consuming  
345 (Pfeiffer & Morley, 2014). Furthermore, *B. bruxellensis* is an acetogenic yeast, which produces large  
346 amounts of acetic acid in addition to ethanol under aerobic conditions. This production varies a lot

347 between strains (up to 10-fold) (Freer, 2002; Freer, Dien, & Matsuda, 2003) and could have ecological  
348 implication since acetic acid release in the environments is described as a useful feature to outcompete  
349 other microorganisms (Rozpędowska et al., 2011) or to lure/deter flies involved in yeast dissemination  
350 (Dzialo, Park, Steensels, Lievens, & Verstrepen, 2017). Regarding oxygen impact, conflicting results  
351 have been highlighted, with some studies connecting oxygen input to increased or decreased growth  
352 (Aguilar Uscanga, Délia, & Strehaiano, 2003; da Silva et al., 2019; Smith & Divol, 2018). The  
353 conservation of the NADH dehydrogenase subunit genes in the mitogenome suggests specific oxygen's  
354 need during *B. bruxellensis*' fermentation (Procházka et al., 2010). The oxygen requirement is barely  
355 deciphered in *B. bruxellensis* and should be the subject of deeper studies in the near future, particularly  
356 to determine its possible impact on niche colonization.

357 In addition to peculiarities regarding carbon and oxygen metabolism, *B. bruxellensis* has atypical  
358 nitrogen and vitamins requirements, which may explain its ability to colonize certain niches. In general,  
359 *B. bruxellensis* seems to have higher nitrogen needs than *S. cerevisiae* (Leite et al., 2012), and  
360 *B. bruxellensis* is able to grow on nitrate as sole nitrogen sources (Borneman et al., 2014; Conterno et  
361 al., 2006). This could be advantageous in wine or in bioethanol environment where nitrate is present  
362 and not consumed by the first *S. cerevisiae* fermenter (Cajueiro et al., 2017; de Barros Pita et al., 2011).  
363 Pena-Moreno et al. even reported that the presence of nitrate boosted ethanol production and growth  
364 for some bioethanol strains (Morales-de la Peña, Welte-Chanes, & Martín-Belloso, 2019). Nitrate  
365 consumption capacity varies depending on the ecological origin of isolation (Borneman et al., 2014;  
366 Crauwels et al., 2015; da Silva et al., 2019). The nitrate assimilation cluster (NIT cluster) was found to  
367 be probably involved in the observed phenotypic variation: it encompasses genes encoding nitrate  
368 transporter, nitrate reductase, nitrite reductase and two related transcription factors. It has evolved  
369 differently between strains and ecological groups. While NIT cluster was duplicated in a wine diploid  
370 isolate capable of assimilating nitrate (belonging to the wine 3 subgroup) and it was partially deleted  
371 or subjected to gene conversion in beer isolates unable to assimilate nitrate (belonging to the beer  
372 subpopulation) (Borneman et al., 2014; Gounot et al., 2020). In a wine triploid isolate able to assimilate



373 nitrate (from the wine 1 group), three haplotypes are present. To sum up, selective pressures in  
374 different ecological niches may have allowed different evolutionary trajectories, although researches  
375 involving more representative strains are needed to have the full picture. Regarding vitamins  
376 requirements, *B. bruxellensis* was initially defined as an auxo-autotroph (Peynaud and Domercq, 1956)  
377 but recent results indicate that biotin is the only vitamin which strongly affects the biotic capacity of  
378 *B. bruxellensis* in the long term (von Cosmos & Edwards, 2016). Thiamine requirements appear to  
379 depend on the strain and on the presence of ethanol in the growth medium (von Cosmos & Edwards,  
380 2016).

### 381 3.2. Traits of technological interest

382 Another notable characteristic of *B. bruxellensis* species is its ability to convert hydroxycinnamic acids  
383 (HCAs) into volatile phenols. HCA metabolism may have an important impact from an ecological  
384 viewpoint: HCA have antimicrobial properties, and the ability to convert HAC into less toxic compounds  
385 could promote yeast growth (Richard, Viljanen, & Penttilä, 2015). Besides, HAC are important dietary  
386 antioxidants for flies. However, flies are not able to detect directly HCA and use ethyl phenols as an  
387 indirect indicator of their presence (Dweck, Ebrahim, Farhan, Hansson, & Stensmyr, 2015). Flies  
388 interaction with yeasts have consequences on their dissemination and the possibility to colonize new  
389 environments, and volatile phenols could be a key factor mediating yeast-insect interactions (Stefanini,  
390 2018). Besides their ecological importance, volatile phenols are considered as off-flavours in oenology  
391 (for review (Suárez et al., 2007; Wedral, Shewfelt, & Frank, 2010)). The term 'volatile phenols' generally  
392 includes 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG) and 4-ethylcatechol (4-EC) and their vinyl forms,  
393 4-vinylphenol (4-VP), 4-vinylguaiacol (4-VG), 4-vinylcatechol (4-VC). These are produced through the  
394 conversion of hydroxycinnamic acids (HCAs): p-coumaric acid, ferulic acid and caffeic acid respectively.  
395 The production of volatile phenols by *B. bruxellensis* strains is variable and was extensively described  
396 in oenological conditions (M. Agnolucci et al., 2009; Monica Agnolucci et al., 2010; Conterno et al.,  
397 2006; Di Toro et al., 2015; Hixson et al., 2012; Madsen et al., 2017; D. Romano et al., 2017; Vigentini

398 et al., 2008; Zepeda-Mendoza et al., 2018). Most studies involved end-point analyses, which makes it  
399 difficult to distinguish between the intrinsic capacities of the strains and their modulation by external  
400 parameters. Furthermore, the respective importance of the different factors governing such variability  
401 is poorly described: a few authors showed that volatile phenol production was both strain-dependent  
402 and wine matrix-dependent (Chandra, Madeira, Coutinho, Albergaria, & Malfeito-Ferreira, 2016;  
403 Cibrario, Miot-Sertier, et al., 2020; Dias, Pereira-da-Silva, Tavares, Malfeito-Ferreira, & Loureiro, 2003;  
404 Zhu, Zhang, & Lu, 2012). The niche of isolation could also play a role and brewing isolates have a more  
405 efficient metabolism toward ferulic acid (leading to 4-EG) than p-coumaric acid compared to wine  
406 isolates (Lentz & Harris, 2015). Some wine and soft drink isolates possess a duplication of the Vinyl  
407 Phenol Reductase gene, which is absent in beer isolates (Crauwels et al., 2017). However, while all  
408 these studies confirmed the existence of intraspecific variation, their impact on the production of  
409 volatile phenols and their possible ecological significance remains to be properly assessed.

410 *Brettanomyces bruxellensis* show important intra-specific variability regarding the production of other  
411 aroma-active molecules such as esters, fatty acids, terpenes, phenolic compounds, N-heterocycle, (see  
412 *Brettanomyces* aroma wheel, (Lucy Joseph, Albino, & Bisson, 2017; Serra Colomer, Funch, et al., 2020;  
413 Tyrawa, Preiss, Armstrong, & van der Merwe, 2019)). Although some environmental factors may  
414 impact ester production (*e.g.* ethanol and p-coumaric acid concentrations (Conterno, Aprea,  
415 Franceschi, Viola, & Vrhovsek, 2013; Lucy Joseph, Kumar, Su, & Bisson, 2007)), esterase activity is also  
416 strain-dependent (Holt et al., 2018; Spaepen & Verachtert, 1982; Steensels et al., 2015; Verstrepen et  
417 al., 2003). The intraspecific variation in the production of esters is mirrored at the aromatic level and  
418 impacts fruitiness, which is particularly important for Lambic beer style (Van Oevelen, Spaepen,  
419 Timmermans, & Verachtert, 1977). The activity of some alpha-glucosidase and beta-glucosidase  
420 enzymes may be involved in the release of aromatic compounds like terpenes (Crauwels et al., 2014;  
421 Daenen et al., 2007; Serra Colomer, Funch, et al., 2020; Vervoort et al., 2016). N-heterocycles can be  
422 produced by *B. bruxellensis* in beverages (mostly in wine or beer) (for review see (Snowdon, Bowyer,  
423 Grbin, & Bowyer, 2006)). These molecules are responsible for the 'mousy' off-flavor, which causes

424 rejection by the consumers. The metabolic pathway is not fully understood, but the amino-acid  
425 content and the oxygen availability in fermented beverages are key parameters for their production  
426 (Grbin & Henschke, 2000; Grbin, Herderich, Markides, Lee, & Henschke, 2007). The state of art is  
427 somewhat inconsistent, which may be explained by the difficulty to properly measure such compounds  
428 and their seemingly random occurrence in fermented beverages. From an ecological aspect, all these  
429 aromatic compounds could play key roles in natural environments, including signalling and  
430 communication with other organisms, and/or attractants for animals, particularly insects (Dzialo et al.,  
431 2017).

432 *Brettanomyces bruxellensis* shows an incredible ability to persist over long time in some industrial  
433 environments (Cibrario, Miot-Sertier, et al., 2020). Such capacity of persistence could be linked to the  
434 various physiological states of the cells, beside the classical free-living (planktonic) state. Indeed,  
435 *B. bruxellensis* is able to form biofilm, pseudo-mycelium, chlamydospore-like structure and VBNC  
436 (Viable But Non Culturable) cells. While many aspects remains unknown or contradictory, a  
437 physiological change between these forms could be induced by stress conditions related to the  
438 presence of some chemical compounds and/or to nutrient limitation (Uscanga, Delia, & Strehaiano,  
439 2000). Intraspecific diversity was observed in the ability to enter or exit the VBNC state (Capozzi et al.,  
440 2016; Longin et al., 2016) as well as to form pseudo-hyphae (Martyniak, Bolton, Kuksin, Shahin, & Chan,  
441 2017) or biofilm (Lucy Joseph et al., 2007). Recently, two concomitant studies (Dimopoulou, Renault,  
442 et al., 2019; Lebleux et al., 2020) have suggested that the ability to produce biofilms depends on the  
443 genetic groups. Although only a limited number of strains were tested, the beer group showed  
444 robustness regarding biofilm formation in glucose-rich or glucose-limited media. For other genetic  
445 groups, biofilm's production was more dependent on environmental characteristics, suggesting  
446 different regulatory mechanisms (Dimopoulou, Renault, et al., 2019). These differences were also  
447 partly mirrored by the biochemical and physicochemical properties of the surface of the strains  
448 (Dimopoulou, Renault, et al., 2019). Finally, whether *B. bruxellensis* is actually able to sporulate and to  
449 undergo a sexual cycle remains a mystery. Early description of ascospore-like forms by van der Walt

450 (Van der Walt, 1964) prompted the definition of a teleomorphic stage (ie the sexual reproductive stage  
451 of a fungal species in mycology). The teleomorph was named *Dekkera bruxellensis* (*B. bruxellensis*  
452 being the anamorph counterpart, that reproduces asexually). Since then, ascospores formation was  
453 scarcely described (A.T. de Souza Liberal et al., 2007), and most authors consider *B. bruxellensis* as  
454 lacking an effective sexual cycle (Hellborg & Piškur, 2009). In particular, the existence of a meiosis  
455 should be associated with triploid instability, which is not evidenced at the genomic level. The impact  
456 of polyploidy and hybridization on the ability to switch from one form to another (planktonic, sessile,  
457 biofilm, pseudo-mycelium) remains to be elucidated. From an ecological viewpoint, the possibility to  
458 adopt various lifestyles and switch from one to another cellular morphologies may facilitate niches  
459 adaptation and drastic environment changes, as it the case for dimorphic fungi whose yeast-hyphae  
460 switch is recognized as an essential adaptation for host colonization and pathogenicity (Boyce &  
461 Andrianopoulos, 2015).

462 As one of the main worldwide wine spoilers, winemakers try to prevent and/or control *Brettanomyces*  
463 contamination through several methods such as the use of filtration, application of ozone, high  
464 pressure, ultrasound, ultraviolet irradiation, pulsed electric fields, chitosan or the addition of sulphite  
465 (Supplemental Table 2). So far, most studies evaluating the efficiency of these different applications  
466 have included a very small subset of strains (Supplemental Table 2) with questionable  
467 representativeness, especially for wine isolates that are found into different genetic groups. As a result,  
468 conflicting conclusions have been recorded: for example, chitosan treatments have sometimes been  
469 described as very effective (Bağder Elmacı et al., 2015) or moderately effective against *B. bruxellensis*  
470 (Petrova, Cartwright, & Edwards, 2016). This incongruity has been partly resolved, a strain-dependent  
471 sensitivity to chitosan was demonstrated (Paulin et al., 2020). In future work, it could be interesting to  
472 define a standardized method to evaluate the efficiency of anti-microbial treatments against  
473 *B. bruxellensis* including a panel of isolates representative of the genetic diversity of the species.

474 In winemaking, the most common treatment is the addition of sulphur dioxide (Barata et al., 2008).  
475 High intraspecific diversity in sulphite tolerance was repeatedly demonstrated in several studies  
476 (Avramova, Cibrario, et al., 2018; Dimopoulou, Hatzikamari, Masneuf-Pomarede, & Albertin, 2019;  
477 Galafassi, Toscano, Vigentini, Piškur, & Compagno, 2013). On a cohort of 100 isolates from  
478 winemaking, three phenotypic groups were defined: sensitive (slowed growth), tolerant (delayed  
479 growth) and resistant (no impact on growth) (Vigentini, Lucy Joseph, Picozzi, Foschino, & Bisson, 2013).  
480 A recent study was able to connect sulphur sensitivity to the genetic groups, with two triploid groups  
481 (wine 1 and wine 2) containing most tolerant/resistant isolates (Avramova, Vallet-Courbin, Maupeu,  
482 Masneuf-Pomarède, & Albertin, 2018). Competition experiments between sensitive/tolerant isolates  
483 under increasing concentration of sulphite showed specific adaptation of isolates from wine 1  
484 allotriploid group to high SO<sub>2</sub> environments (Avramova et al., 2019). Different *SSU1* haplotypes (*SSU1*  
485 encoding sulphite efflux pump) related to variable SO<sub>2</sub> tolerance were characterized (Valdetara et al.,  
486 2020; C Varela, Bartel, Roach, Borneman, & Curtin, 2019; Cristian Varela, Bartel, Onetto, & Borneman,  
487 2020). It revealed that gene dosage effect (the number of *SSU1* haplotype) as well as the *SSU1*  
488 regulation could be at least partially involved in SO<sub>2</sub> tolerance (Varela et al., 2019). In a recent  
489 experiment, Bartel et al conducted a laboratory experimental evolution in presence of sulfur dioxide  
490 concentrations, and evidenced adaptive evolution in different genetic backgrounds targeting partly  
491 *SSU1* (Bartel et al., 2021). A genotyping study showed that the two genetic groups (wine 1 and wine 2)  
492 containing the most tolerant/resistant isolates of *B. bruxellensis* were scarcely isolated before 1990  
493 and that their proportion had increased steadily since then, possibly with the increase of sulphur use  
494 in oenology (Cibrario et al., 2019). Sulphur tolerance/resistance in *B. bruxellensis* could thus be the  
495 result of anthropization and adaptation to winemaking environments as for wine strains of  
496 *S. cerevisiae* (Gallone et al., 2016; García-Ríos & Guillamón, 2019; Kaewkod, Bovonsombut, &  
497 Tragoolpua, 2019). The fact that sulphite tolerant/resistant phenotypes are present in two different  
498 genetic cluster (one autotriploids, one allotriploid) may indicate independent acquisition and possibly

499 different adaptive mechanisms. How much and how the change in ploidy contributes to the  
500 resistant/tolerant phenotype has to be resolved in future studies.

501 In conclusion, although a high number of surveys investigated various *B. bruxellensis* traits with  
502 fundamental and applied interest, many of these published studies used a small subset of strains  
503 usually not representative of the genetic diversity of the species (Figure 3, Supplemental Table 3).  
504 *Brettanomyces bruxellensis* literature is scattered with incongruences that could be directly related to  
505 intraspecific diversity and the lack of representability of the isolates tested. It highlights the necessity,  
506 for future research, to properly take into consideration strains from different ploidy levels, with varied  
507 hybridization backgrounds (auto- and allotriploids), isolated from various substrates and geographical  
508 origins and distributed within the different genetic populations described.

## 509 4. Discussion and perspectives

### 510 4.1. *Brettanomyces bruxellensis*, the fermentation finisher

511 *Brettanomyces bruxellensis* has attracted increasing attention recently due to its involvement in  
512 industrial processes and its unusual genomic composition. *Saccharomyces cerevisiae* is one of the most  
513 intensively studied eukaryotic models, at the fundamental level in molecular, cellular and ecology  
514 biology, but also from an applied point of view as it is widely used as a fermentation starter for several  
515 human processes (Boone, 2014; Chambers & Pretorius, 2010; Goddard & Greig, 2015; Peter &  
516 Schacherer, 2016). Interestingly, while *B. bruxellensis* species is associated with similar anthropic  
517 processes (*e.g.* oenology, brewery), it is particularly recognized for its competence to colonize already  
518 fermented environments (such as wine or beer rather than grape must or wort) (Schifferdecker et al.,  
519 2014). *Brettanomyces bruxellensis* is thus a perfect model of fermentation finisher, a complementary  
520 counterpoint to *S. cerevisiae* model of fermentation starter, sharing the same industrial niches, but not  
521 with the same temporality. Interestingly, no isolates of *B. bruxellensis* were identified outside human  
522 related processes so far. Several reasons may explain the non-detection of wild isolates yet. First,  
523 *B. bruxellensis* is a slow-growing yeast species, so it may be easily outcompeted by other yeast species

524 whenever culturable methods are used (Agnolucci et al., 2017). Another possibility is that  
525 *B. bruxellensis* could be part of biofilm communities in natural environments – albeit at low-abundance  
526 and/or in Viable But Not Cultivable (VBNC) state preventing its isolation. Indeed, 40-80% of cells on  
527 Earth live and persist in multispecies communities (bacteria, archaea, eukaryotes, etc.) forming  
528 biofilms, *e.g.* aggregated structures frequently enclosed into matrixes of extracellular polymeric  
529 substances (Flemming et al., 2016; Flemming & Wuertz, 2019). Growth in polymicrobial biofilms is  
530 relatively protected from environmental variations, and could improve survival in hostile environments  
531 (Flemming & Wuertz, 2019). The identification of low-abundance or VBNC species within complex  
532 communities remains a challenge, prompting the use of metagenomics, culturomics or reverse  
533 genomics approaches (Martellacci et al., 2019; Ryu et al., 2021). *Brettanomyces bruxellensis* has  
534 abilities to form VBNC or to be involved in microbial consortia like kombucha’s SCOBY (Symbiotic  
535 Community of Bacteria and Yeast) (Savary et al., 2021). These characteristics make it plausible the  
536 hypothesis of natural biofilms as one of their wild environments, although no definite evidences are  
537 described so far. Besides biofilms, natural fermentations are another putative example of ecosystems  
538 that could foster the growth of wild *B. bruxellensis*. Many insects, mammals or birds store foods (fruits,  
539 grains, meat, etc.) for times of less-plentiful sustenance. Food storage is prone to microbial growth or  
540 spoilage, and natural fermentations occurred long before humans, and long before human-directed  
541 fermentations (Carrigan et al., 2015; Post & Urban, 1993; Ruxton, Wilkinson, Schaefer, & Sherratt,  
542 2014; Wiens et al., 2008). These naturally-occurring fermentations are poorly described, but they  
543 probably display similar characteristics to *B. bruxellensis*’ anthropic environments: presence of ethanol  
544 and organic acids, wide variety of ever-evolving sugar, nitrogen and other nutrients contents, oxygen  
545 availability, succession of complex microbial communities, etc. In such natural fermentations,  
546 *B. bruxellensis* could play second fiddle and colonize the environments after more efficiently-growing  
547 and fermenting microorganisms. In natural environments also, the role of wild-fermentation finisher  
548 would be congruent with *B. bruxellensis*’ scavenging abilities (Smith & Divol, 2016). However, this  
549 hypothesis remains purely speculative: the literature is scarce regarding the microbial communities

550 associated with food caching or hoarding , and so are the available isolates from these niches (Herrera,  
551 Kramer, & Reichman, 1997; Post & Urban, 1993). Obtaining and studying natural isolates would be an  
552 ideal option, but even in the absence of data on their wild counterparts, future works both at the  
553 genomic and phenotypic levels will help outlining the characteristics of *B. bruxellensis'* natural  
554 reservoirs. In particular, study of the central metabolism and the molecular mechanisms (HGT, LOH,  
555 CNV, etc) driving their evolution will help to determine which metabolic functions are conserved, lost  
556 or gained and their possible involvement into adaptations to anthropic environments. For example,  
557 determining whether maltose metabolization or beta-glucosidase activity were acquired before  
558 *B. bruxellensis'* anthropic associations may give clues concerning its possible natural niches (*i.e.* crops-  
559 or wood-related). Future works should explore more thoroughly all these aspects (central metabolism,  
560 VBNC and biofilm abilities, etc.) to gain more insights into a fermentation-related microorganism and  
561 to improve our possibilities of controlling the species in various processes.

562 4.2. Exploring the relationships between polyploidy/hybridization and adaptation to anthropized  
563 environments

564 Genomic analyses unveiled a genetic diversity of *B. bruxellensis* related to specific substrates. Diploid  
565 isolates coexist with triploid ones of hybrid origins, and several subpopulations of diploid and triploid  
566 individuals are described. Some of these groups are related to specific substrate origins (wine, beer,  
567 kombucha, tequila) and display adaptive traits related to their ecological niche (*e.g.* sulphite  
568 tolerance/resistance in winemaking, ability to metabolize nitrate in bioethanol production, maltose  
569 utilization in beer process). In industrial processes, where stress is omnipresent, polyploidy and  
570 aneuploidy are recurrent events in domesticated populations of *S. cerevisiae* (Peter et al., 2018; Querol  
571 & Bond, 2009; Steensels et al., 2021). In *B. bruxellensis*, the level of aneuploidy (roughly 5%) is  
572 surprisingly low considering: 1- the proportion of aneuploidy in other yeast species (19% in  
573 *S. cerevisiae*, 15% and 33% in the pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans*,  
574 respectively (Gounot et al., 2020; Peter et al., 2018; Rhodes et al., 2017; Scopel, Hose, Bensasson, &  
575 Gasch, 2021; Selmecki, Forche, & Berman, 2006)); and 2- the proportion of triploids in *B. bruxellensis*



576 (>50%) which are known to evolve quickly toward aneuploidy and diploidy in *Saccharomyces* species  
577 (Avramova, Cibrario, et al., 2018; Gerstein, McBride, & Otto, 2008; Todd, Forche, & Selmecki, 2017).  
578 The phylogeny and evolutionary history of *B. bruxellensis* species are particularly complex to  
579 reconstruct because of the intertwined events of polyploidization and hybridization (Linder, Moret, &  
580 Nakhleh, 2003). New approaches will be needed to unravel its complex genetic architecture and to  
581 elucidate the precise relationship between genomic evolution and the actual adaptation to  
582 anthropized environments. The evolution of many species (plant, animal, microorganism) is strongly  
583 shaped by human activities. When anthropogenic-driven transformation is purposefully associated  
584 with improved attributes, the species are labelled as domesticated, as in *S. cerevisiae* for which several  
585 independent domestication events were evidenced (Gallone et al., 2018; Giannakou et al., 2020;  
586 Steensels, Gallone, Voordeckers, & Verstrepen, 2019). In the case of *B. bruxellensis*, the situation  
587 seems to be more complicated: the fact that some beer isolates are used by brewers for their improved  
588 ability to metabolize specific sugars may correspond to domestication. Conversely, adaptation to  
589 winemaking environments through acquisition of sulphite resistance is probably an unintentional  
590 consequence of anthropogenic influence. Thus, for wine groups, the term 'domestication' does not  
591 apply to what seems to be an adaptive evolution at the expense of humans. A few works already  
592 provided evidence of various beneficial or detrimental traits associated with the different anthropized  
593 environments colonized by *B. bruxellensis*. The underlying molecular mechanisms remain to be  
594 explored more thoroughly, in particular the role played by LOH, HGT, CNV and other mechanisms in  
595 the evolution and adaptation of the species. How polyploidization and hybridization actually impacted  
596 the evolutionary routes of *B. bruxellensis* is still an open question. Future directions should take  
597 advantage of this model yeast to examine closely the influence of anthropogenic activities on the  
598 species and their genomic, phenotypic and adaptive consequences, whether positive or negative for  
599 humans.

600 The description of both auto- and allotriploid groups raises the question of their origin. The recent  
601 genomic approaches, and particularly phasing methods, allowed the precise description of the

602 genomic content of these triploids that harbour a core diploid genome added with an additional  
603 haploid one of various intra- and inter-specific origins (Abou Saada et al., 2021). Triploid formation in  
604 *B. bruxellensis* happened at least four times, suggesting the successive occurrence of a not-so-rare  
605 event of polyploid formation, followed by a not-so-rare establishment mechanism (selection or  
606 random genetic drift). Regarding the possible mechanisms of polyploid formation, accidental mis-  
607 repartition of chromosomes during mitosis can lead to unbalanced number of chromosomes in  
608 daughter cells (Todd et al., 2017; Wertheimer, Stone, & Berman, 2016). However, this pathway would  
609 account only for the formation of autotriploids (not allotriploids), furthermore without heterozygosity  
610 increase, which is described in *B. bruxellensis* wine 2 autotriploid group. In addition, the presence of a  
611 complete haploid set of chromosomes tends to invalidate the hypothesis of mitosis mishaps, which  
612 would more frequently lead to aneuploids rather than polyploids. Autopolyploidy can also occur via  
613 endoreduplication – replication of the whole nuclear genome in absence of mitosis – a phenomenon  
614 frequently described in plants (Harari, Ram, Rappoport, Hadany, & Kupiec, 2018; Sugimoto-Shirasu &  
615 Roberts, 2003). However, endoreduplication accounts only for even ploidy levels, not for odd (triploid)  
616 ones, and is not associated with increased in heterozygosity levels. The production of unreduced  
617 gametes, followed by intra- or interspecific hybridization, is another route of polyploid formation  
618 frequently described in plants and animals (Otto & Whitton, 2000). The absence of evidence of sexual  
619 cycle in *B. bruxellensis* makes this mechanism less likely. Protoplast (spheroplast) fusion could be an  
620 interesting hypothesis: protoplast formation could occur after cell wall digestion in insects' guts and  
621 subsequent protoplasts fusion could allow intra- and inter-specific hybridization of non-sexual species  
622 (Steensels et al., 2014). To date, protoplast fusion is the most likely hypothesis of auto- and allotriploid  
623 formation, yet the literature lacks of formal evidence and future genomic and ecological analyses may  
624 shed lights on the possible routes of polyploid formation in *B. bruxellensis*. In addition to  
625 polyploidization/hybridization mechanism, the prominence of *B. bruxellensis*' triploids indicates that  
626 triploid formation was followed by the successful establishment of these lineages, through either  
627 neutral genetic drift or natural/artificial selection. It seems highly unlikely that random genetic drift

628 could account alone for the presence of triploid lineages in *B. bruxellensis*: firstly, polyploidy is related  
629 to energetic and resource costs that, far from being neutral, should lead to its counter-selection  
630 (Comai, 2005; Neiman, Kay, & Krist, 2013). Secondly, at least four auto- and allotriploid events have  
631 led to the independent establishment of triploid lineages, a high number for a purportedly stochastic  
632 phenomenon. Thirdly, some of these triploid lineages show (or are suspected to show) higher fitness  
633 in their environments of predilection, such as sulphite resistance for the wine 1 and wine 2 groups, the  
634 possible metabolization of maltose and other complex sugars for the beer group, or increased growth  
635 and the ability to metabolize nitrate for teq/EtOH group (Avramova, Vallet-Courbin, et al., 2018; S.  
636 Crauwels et al., 2015; Galafassi, Capusoni, et al., 2013; Serra Colomer, Chailyan, et al., 2020). All these  
637 elements suggest that polyploidization in *B. bruxellensis* is not neutral from an evolutionary viewpoint,  
638 although more genomic and phenotypic studies are needed to deepen our understanding of the  
639 evolutionary fates of these polyploids compared to their diploid counterparts.

640 Finally, the large genetic differences (up to 3%) recorded between subpopulations raise the question  
641 of the actual number of species within this clade (Eberlein et al., 2021). No sexual cycle was formally  
642 recorded to date and no gene flow between *B. bruxellensis* subpopulations was ever described,  
643 suggesting independent evolution within each clade, and speciation in progress. Future large-scale  
644 genomic and phenotypic analyses will help determine whether we should still consider *B. bruxellensis*  
645 as a single but complex species with diverse subpopulations, or whether we should redefine  
646 *B. bruxellensis* as a complex of single species.

#### 647 4.3 *Brettanomyces bruxellensis*, a yeast model to study the relationship between polyploidy, 648 hybridization and adaptation to human-related environments

649 Polyploidy and hybridization, two frequently associated events, have long been described as key  
650 evolutionary mechanisms underlying radiation and adaptation in many clades of plants, animals or  
651 fungi (Gregory & Mable, 2005; Otto & Whitton, 2000; Todd et al., 2017; Van De Peer, Mizrachi, &  
652 Marchal, 2017). In flowering plants, where the impact of polyploidy is the most studied, polyploidy and  
653 hybridization are frequently associated with phenotypic diversification, trait innovation, increased

654 fitness, invasiveness abilities, adaptation to harsh environments and domestication (Ainouche &  
655 Wendel, 2014; Soltis & Soltis, 2009). Historically less studied in microorganisms, polyploidy and  
656 hybridization are now well established as driving evolutionary forces in yeast, Chromalveolata, but also  
657 in prokaryotes, including Bacteria and Archaea (Albertin & Marullo, 2012; Marcet-Houben & Gabaldón,  
658 2015; Soppa, 2022). Yeast usually enables the development of systems biology approaches, and  
659 *B. bruxellensis* triploids may allow to investigate the fates of genes duplicated by whole genome  
660 duplication and their impact on phenotypic traits. In addition, the close association between various  
661 human processes and different ploidy/hybridization status makes of *B. bruxellensis* a valuable model  
662 to decipher the evolutionary mechanisms involved in the adaptation to anthropized niches. The study  
663 of *B. bruxellensis*' diploid and triploid populations may provide valuable insights into the ecological and  
664 evolutionary significance of natural polyploidy.

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668 **References**

- 669 Abbott, D. A., Hynes, S. H., & Ingledew, W. M. (2005). Growth rates of *Dekkera/Brettanomyces* yeasts  
 670 hinder their ability to compete with *Saccharomyces cerevisiae* in batch corn mash  
 671 fermentations. *Applied Microbiology and Biotechnology*, 66(6), 641–647.  
 672 <https://doi.org/10.1007/s00253-004-1769-1>
- 673 Abou Saada, O., Tsouris, A., Eberlein, C., Friedrich, A., & Schacherer, J. (2021). nPhase: an accurate  
 674 and contiguous phasing method for polyploids. *Genome Biology*, 22(1), 126.  
 675 <https://doi.org/10.1186/s13059-021-02342-x>
- 676 Agnolucci, M., Vigentini, I., Capurso, G., Merico, A., Tirelli, A., Compagno, C., ... Nuti, M. (2009).  
 677 Genetic diversity and physiological traits of *Brettanomyces bruxellensis* strains isolated from  
 678 Tuscan Sangiovese wines. *International Journal of Food Microbiology*, 130(3), 238–244.  
 679 <https://doi.org/10.1016/j.ijfoodmicro.2009.01.025>
- 680 Agnolucci, Monica, Rea, F., Sbrana, C., Cristani, C., Fracassetti, D., Tirelli, A., & Nuti, M. (2010).  
 681 Sulphur dioxide affects culturability and volatile phenol production by *Brettanomyces/Dekkera*  
 682 *bruxellensis*. *International Journal of Food Microbiology*, 143(1–2), 76–80.  
 683 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.022>
- 684 Agnolucci, Monica, Scarano, S., Rea, F., Toffanin, A., & Nuti, M. (2007). Detection of  
 685 *Dekkera/Brettanomyces bruxellensis* in pressed Sangiovese grapes by real time PCR. In *Italian*  
 686 *Journal of Food Science* (Vol. 19).
- 687 Agnolucci, Monica, Tirelli, A., Cocolin, L., & Toffanin, A. (2017). *Brettanomyces bruxellensis* yeasts:  
 688 impact on wine and winemaking. *World Journal of Microbiology and Biotechnology*, 33(10), 180.  
 689 <https://doi.org/10.1007/s11274-017-2345-z>
- 690 Aguilar-Méndez, O., López-Álvarez, J. A., Díaz-Pérez, A. L., Altamirano, J., Reyes De la Cruz, H.,  
 691 Rutiaga-Quiñones, J. G., & Campos-García, J. (2017). Volatile compound profile conferred to  
 692 tequila beverage by maturation in recycled and regenerated white oak barrels from *Quercus*  
 693 *alba*. *European Food Research and Technology*, 243(12), 2073–2082.  
 694 <https://doi.org/10.1007/s00217-017-2901-7>
- 695 Aguilar Uscanga, M. G., Délia, M.-L., & Strehaiano, P. (2003). *Brettanomyces bruxellensis*: effect of  
 696 oxygen on growth and acetic acid production. *Applied Microbiology and Biotechnology*, 61(2),  
 697 157–162. <https://doi.org/10.1007/s00253-002-1197-z>
- 698 Ainouche, M. L., & Wendel, J. F. (2014). Polyploid Speciation and Genome Evolution: Lessons from  
 699 Recent Allopolyploids. In P. Pontarotti (Ed.), *Evolutionary Biology: Genome Evolution,*  
 700 *Speciation, Coevolution and Origin of Life* (pp. 87–113). [https://doi.org/10.1007/978-3-319-](https://doi.org/10.1007/978-3-319-07623-2_5)  
 701 [07623-2\\_5](https://doi.org/10.1007/978-3-319-07623-2_5)
- 702 Albertin, W., da Silva, T., Rigoulet, M., Salin, B., Masneuf-Pomarede, I., de Vienne, D., ... Marullo, P.  
 703 (2013). The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific  
 704 *Saccharomyces* Hybrids. *PLoS ONE*, 8(9), e75121.  
 705 <https://doi.org/10.1371/journal.pone.0075121>
- 706 Albertin, W., & Marullo, P. (2012). Polyploidy in fungi: Evolution after whole-genome duplication.  
 707 *Proceedings of the Royal Society B: Biological Sciences*, 279(1738), 2497–2509.  
 708 <https://doi.org/10.1098/rspb.2012.0434>
- 709 Albertin, W., Masneuf-Pomarede, I., & Peltier, E. (2018). Molecular diagnosis of *Brettanomyces*  
 710 *bruxellensis*' sulfur dioxide sensitivity through genotype specific method. *Patent Application*

- 711 *Publication, 1.*
- 712 Alston, J. M., Arvik, T., Hart, J., & Lapsley, J. T. (2021). Brettanomics I : The Cost of Brettanomyces in  
713 California Wine Production. *Journal of Wine Economics*, 16(1), 4–31.  
714 <https://doi.org/10.1017/jwe.2020.20>
- 715 Angela, C., Young, J., Kordayanti, S., Virginia Partha Devanthi, P., & . K. (2020). Isolation and Screening  
716 of Microbial Isolates from Kombucha Culture for Bacterial Cellulose Production in Sugarcane  
717 Molasses Medium. *KnE Life Sciences*, 2020, 111–127. <https://doi.org/10.18502/cls.v5i2.6444>
- 718 Arroyo-López, F. N., Durán-Quintana, M. C., Ruiz-Barba, J. L., Querol, A., & Garrido-Fernández, A.  
719 (2006). Use of molecular methods for the identification of yeast associated with table olives.  
720 *Food Microbiology*, 23(8), 791–796. <https://doi.org/10.1016/j.fm.2006.02.008>
- 721 Avramova, M., Cibrario, A., Peltier, E., Coton, M., Coton, E., Schacherer, J., ... Masneuf-Pomarede, I.  
722 (2018). Brettanomyces bruxellensis population survey reveals a diploid-triploid complex  
723 structured according to substrate of isolation and geographical distribution. *Scientific Reports*,  
724 8(1), 4136. <https://doi.org/10.1038/s41598-018-22580-7>
- 725 Avramova, M., Grbin, P., Borneman, A., Albertin, W., Masneuf-Pomarede, I., & Varela, C. (2019).  
726 Competition experiments between Brettanomyces bruxellensis strains reveal specific  
727 adaptation to sulfur dioxide and complex interactions at intraspecies level. *FEMS Yeast*  
728 *Research*, 19(3), 1–9. <https://doi.org/10.1093/femsyr/foz010>
- 729 Avramova, M., Vallet-Courbin, A., Maupeu, J., Masneuf-Pomarede, I., & Albertin, W. (2018).  
730 Molecular Diagnosis of Brettanomyces bruxellensis' Sulfur Dioxide Sensitivity Through  
731 Genotype Specific Method. *Frontiers in Microbiology*, 9(JUN), 1–9.  
732 <https://doi.org/10.3389/fmicb.2018.01260>
- 733 Bağder Elmacı, S., Gülgör, G., Tokatlı, M., Erten, H., İşci, A., & Özçelik, F. (2015). Effectiveness of  
734 chitosan against wine-related microorganisms. *Antonie van Leeuwenhoek*, 107(3), 675–686.  
735 <https://doi.org/10.1007/s10482-014-0362-6>
- 736 Barata, A., Caldeira, J., Botelho, R., Pagliara, D., Malfeito-Ferreira, M., & Loureiro, V. (2008).  
737 Survival patterns of Dekkera bruxellensis in wines and inhibitory effect of sulphur dioxide.  
738 *International Journal of Food Microbiology*, 121(2), 201–207.  
739 <https://doi.org/10.1016/j.ijfoodmicro.2007.11.020>
- 740 Bartel, C., Roach, M., Onetto, C., Curtin, C., Varela, C., & Borneman, A. (2021). Adaptive evolution of  
741 sulfite tolerance in Brettanomyces bruxellensis. *FEMS Yeast Research*, 21(5), foab036.  
742 <https://doi.org/10.1093/femsyr/foab036>
- 743 Basílio, A. C. M., De Araújo, P. R. L., De Moraes, J. O. F., Da Silva Filho, E. A., De Moraes, M. A., &  
744 Simões, D. A. (2008). Detection and identification of wild yeast contaminants of the industrial  
745 fuel ethanol fermentation process. *Current Microbiology*, 56(4), 322–326.  
746 <https://doi.org/10.1007/s00284-007-9085-5>
- 747 Bassi, A. P. G., Meneguello, L., Paraluppi, A. L., Sanches, B. C. P., & Ceccato-Antonini, S. R. (2018).  
748 Interaction of Saccharomyces cerevisiae–Lactobacillus fermentum–Dekkera bruxellensis and  
749 feedstock on fuel ethanol fermentation. *Antonie van Leeuwenhoek*, 111(9), 1661–1672.  
750 <https://doi.org/10.1007/s10482-018-1056-2>
- 751 Beekman, C. N., & Ene, I. V. (2020). Short-Term evolution strategies for host adaptation and drug  
752 escape in human fungal pathogens. *PLoS Pathogens*, 16(5), 1–9.  
753 <https://doi.org/10.1371/journal.ppat.1008519>
- 754 Benito, S., Palomero, F., Morata, A., Calderón, F., & Suárez-Lepe, J. A. (2009). Factors affecting the

- 755 hydroxycinnamate decarboxylase/vinylphenol reductase activity of dekkera/brettanomyces:  
756 Application for dekkera/brettanomyces control in red wine making. *Journal of Food Science*,  
757 74(1), 15–22. <https://doi.org/10.1111/j.1750-3841.2008.00977.x>
- 758 Berbegal, C., Garofalo, C., Russo, P., Pati, S., Capozzi, V., & Spano, G. (2017). Use of Autochthonous  
759 Yeasts and Bacteria in Order to Control Brettanomyces bruxellensis in Wine. *Fermentation*, 3(4),  
760 65. <https://doi.org/10.3390/fermentation3040065>
- 761 Blomqvist, J., Eberhard, T., Schnürer, J., & Passoth, V. (2010). Fermentation characteristics of Dekkera  
762 bruxellensis strains. *Applied Microbiology and Biotechnology*, 87(4), 1487–1497.  
763 <https://doi.org/10.1007/s00253-010-2619-y>
- 764 Blomqvist, J., Nogué, V. S., Gorwa-Grauslund, M., & Passoth, V. (2012). Physiological requirements  
765 for growth and competitiveness of Dekkera bruxellensis under oxygen-limited or anaerobic  
766 conditions. *Yeast*, 29(7), 265–274. <https://doi.org/10.1002/yea.2904>
- 767 Blomqvist, J., & Passoth, V. (2015). Dekkera bruxellensis—spoilage yeast with biotechnological  
768 potential, and a model for yeast evolution, physiology and competitiveness. *FEMS Yeast  
769 Research*, 15(4), 1–9. <https://doi.org/10.1093/femsyr/fov021>
- 770 Bokulich, N. A., Bamforth, C. W., & Mills, D. A. (2012). Brewhouse-Resident Microbiota Are  
771 Responsible for Multi-Stage Fermentation of American Coolship Ale. *PLoS ONE*, 7(4), e35507.  
772 <https://doi.org/10.1371/journal.pone.0035507>
- 773 Boone, C. (2014). Yeast Systems Biology: Our Best Shot at Modeling a Cell. *Genetics*, 198(2), 435–437.  
774 <https://doi.org/10.1534/genetics.114.169128>
- 775 Borneman, A. R., Zeppel, R., Chambers, P. J., & Curtin, C. D. (2014). Insights into the Dekkera  
776 bruxellensis Genomic Landscape: Comparative Genomics Reveals Variations in Ploidy and  
777 Nutrient Utilisation Potential amongst Wine Isolates. *PLoS Genetics*, 10(2), e1004161.  
778 <https://doi.org/10.1371/journal.pgen.1004161>
- 779 Boyce, K. J., & Andrianopoulos, A. (2015). Fungal dimorphism: the switch from hyphae to yeast is a  
780 specialized morphogenetic adaptation allowing colonization of a host. *FEMS Microbiology  
781 Reviews*, 39(6), 797–811. <https://doi.org/10.1093/femsre/fuv035>
- 782 Branco, P., Sabir, F., Diniz, M., Carvalho, L., Albergaria, H., & Prista, C. (2019). Biocontrol of  
783 Brettanomyces/Dekkera bruxellensis in alcoholic fermentations using saccharomycin-  
784 overproducing Saccharomyces cerevisiae strains. *Applied Microbiology and Biotechnology*,  
785 103(7), 3073–3083. <https://doi.org/10.1007/s00253-019-09657-7>
- 786 Breniaux, M., Renault, P., Meunier, F., & Ghidossi, R. (2019). Study of High Power Ultrasound for Oak  
787 Wood Barrel Regeneration: Impact on Wood Properties and Sanitation Effect. *Beverages*, 5(1),  
788 10. <https://doi.org/10.3390/beverages5010010>
- 789 Cajueiro, D. B. B., Parente, D. C., Leite, F. C. B., de Moraes Junior, M. A., & de Barros Pita, W. (2017).  
790 Glutamine: a major player in nitrogen catabolite repression in the yeast Dekkera bruxellensis.  
791 *Antonie van Leeuwenhoek*, 110(9), 1157–1168. <https://doi.org/10.1007/s10482-017-0888-5>
- 792 Capece, A., Romaniello, R., Siesto, G., & Romano, P. (2018). Conventional and Non-Conventional  
793 Yeasts in Beer Production. *Fermentation*, 4(2), 38.  
794 <https://doi.org/10.3390/fermentation4020038>
- 795 Capozzi, V., Di Toro, M. R., Grieco, F., Michelotti, V., Salma, M., Lamontanara, A., ... Spano, G. (2016).  
796 Viable But Not Culturable (VBNC) state of Brettanomyces bruxellensis in wine: New insights on  
797 molecular basis of VBNC behaviour using a transcriptomic approach. *Food Microbiology*, 59,  
798 196–204. <https://doi.org/10.1016/j.fm.2016.06.007>

- 799 Carrigan, M. A., Uryasev, O., Frye, C. B., Eckman, B. L., Myers, C. R., Hurley, T. D., & Benner, S. A.  
800 (2015). Hominids adapted to metabolize ethanol long before human-directed fermentation.  
801 *Proceedings of the National Academy of Sciences*, *112*(2), 458–463.  
802 <https://doi.org/10.1073/pnas.1404167111>
- 803 Cartwright, Z. M., Glawe, D. A., & Edwards, C. G. (2018). Reduction of *Brettanomyces bruxellensis*  
804 Populations from Oak Barrel Staves Using Steam. *American Journal of Enology and Viticulture*,  
805 *69*(4), 400–409. <https://doi.org/10.5344/ajev.2018.18024>
- 806 Chambers, P. J., & Pretorius, I. S. (2010). Fermenting knowledge: the history of winemaking, science  
807 and yeast research. *EMBO Reports*, *11*(12), 914–920. <https://doi.org/10.1038/embor.2010.179>
- 808 Chandra, M., Madeira, I., Coutinho, A.-R., Albergaria, H., & Malfeito-Ferreira, M. (2016). Growth and  
809 volatile phenol production by *Brettanomyces bruxellensis* in different grapevine varieties during  
810 fermentation and in finished wine. *European Food Research and Technology*, *242*(4), 487–494.  
811 <https://doi.org/10.1007/s00217-015-2559-y>
- 812 Chatonnet, P., Dubourdie, D., Boidron, J., & Pons, M. (1992). The origin of ethylphenols in wines.  
813 *Journal of the Science of Food and Agriculture*, *60*(2), 165–178.  
814 <https://doi.org/10.1002/jsfa.2740600205>
- 815 Cheng, J., Guo, X., Cai, P., Cheng, X., Piškur, J., Ma, Y., ... Gu, Z. (2017). Parallel Evolution of Chromatin  
816 Structure Underlying Metabolic Adaptation. *Molecular Biology and Evolution*, *34*(11), 2870–  
817 2878. <https://doi.org/10.1093/molbev/msx220>
- 818 Chescheir, S., Philbin, D., & Osborne, J. P. (2015). Impact of *Oenococcus oeni* on Wine  
819 Hydroxycinnamic Acids and Volatile Phenol Production by *Brettanomyces bruxellensis*.  
820 *American Journal of Enology and Viticulture*, *66*(3), 357–362.  
821 <https://doi.org/10.5344/ajev.2015.14108>
- 822 Cibrario, A., Avramova, M., Dimopoulou, M., Magani, M., Miot-Sertier, C., Mas, A., ... Dols-Lafargue,  
823 M. (2019). *Brettanomyces bruxellensis* wine isolates show high geographical dispersal and long  
824 persistence in cellars. *PLOS ONE*, *14*(12), e0222749.  
825 <https://doi.org/10.1371/journal.pone.0222749>
- 826 Cibrario, A., Miot-Sertier, C., Paulin, M., Bullier, B., Riquier, L., Perello, M.-C., ... Dols-Lafargue, M.  
827 (2020). *Brettanomyces bruxellensis* phenotypic diversity, tolerance to wine stress and wine  
828 spoilage ability. *Food Microbiology*, *87*(September 2018), 103379.  
829 <https://doi.org/10.1016/j.fm.2019.103379>
- 830 Cibrario, A., Perello, M. C., Miot-Sertier, C., Riquier, L., de Revel, G., Ballestra, P., & Dols-Lafargue, M.  
831 (2020). Carbohydrate composition of red wines during early aging and incidence on spoilage by  
832 *Brettanomyces bruxellensis*. *Food Microbiology*, *92*(January), 1–8.  
833 <https://doi.org/10.1016/j.fm.2020.103577>
- 834 Claussen, N. H. (1904). On a Method for the Application of Hansen's Pure Yeast System in the  
835 Manufacturing of Well-Conditioned English Stock Beers. *Journal of the Institute of Brewing*,  
836 *10*(4), 308–331. <https://doi.org/10.1002/j.2050-0416.1904.tb04656.x>
- 837 Codato, C. B., Martini, C., Ceccato-Antonini, S. R., & Bastos, R. G. (2018). Ethanol production from  
838 *Dekkera bruxellensis* in synthetic media with pentose. *Brazilian Journal of Chemical Engineering*,  
839 *35*(1), 11–17. <https://doi.org/10.1590/0104-6632.20180351s20160475>
- 840 Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*,  
841 *6*(11), 836–846. <https://doi.org/10.1038/nrg1711>
- 842 Conterno, L., Aprea, E., Franceschi, P., Viola, R., & Vrhovsek, U. (2013). Overview of *Dekkera*



- 843 bruxellensis behaviour in an ethanol-rich environment using untargeted and targeted  
844 metabolomic approaches. *Food Research International*, 51(2), 670–678.  
845 <https://doi.org/10.1016/j.foodres.2013.01.049>
- 846 Conterno, L., Lucy Joseph, C. M. L., Arvik, T. J., Henick-Kling, T., & Bisson, L. F. (2006). Genetic and  
847 physiological characterization of *Brettanomyces bruxellensis* strains isolated from wines.  
848 *American Journal of Enology and Viticulture*, 57(2), 139–147.
- 849 Coton, M., Pawtowski, A., Taminiou, B., Burgaud, G., Deniel, F., Coulloume-Labarthe, L., ... Coton, E.  
850 (2017). Unraveling microbial ecology of industrial-scale Kombucha fermentations by  
851 metabarcoding and culture-based methods. *FEMS Microbiology Ecology*, 93(5), 1–16.  
852 <https://doi.org/10.1093/femsec/fix048>
- 853 Coulon, J., Perello, M. C., Lonvaud-Funel, A., De Revel, G., & Renouf, V. (2010). *Brettanomyces*  
854 *bruxellensis* evolution and volatile phenols production in red wines during storage in bottles.  
855 *Journal of Applied Microbiology*, 108(4), 1450–1458. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.2009.04561.x)  
856 [2672.2009.04561.x](https://doi.org/10.1111/j.1365-2672.2009.04561.x)
- 857 Crauwels, S., Van Assche, A., de Jonge, R., Borneman, A. R., Verreth, C., Troels, P., ... Lievens, B.  
858 (2015). Comparative phenomics and targeted use of genomics reveals variation in carbon and  
859 nitrogen assimilation among different *Brettanomyces bruxellensis* strains. *Applied Microbiology*  
860 *and Biotechnology*, 99(21), 9123–9134. <https://doi.org/10.1007/s00253-015-6769-9>
- 861 Crauwels, Sam, Van Opstaele, F., Jaskula-Goiris, B., Steensels, J., Verreth, C., Bosmans, L., ... Lievens,  
862 B. (2017). Fermentation assays reveal differences in sugar and (off-) flavor metabolism across  
863 different *Brettanomyces bruxellensis* strains. *FEMS Yeast Research*, 17(1), 1–10.  
864 <https://doi.org/10.1093/femsyr/fow105>
- 865 Crauwels, Sam, Zhu, B., Steensels, J., Busschaert, P., De Samblanx, G., Marchal, K., ... Lievens, B.  
866 (2014). Assessing Genetic Diversity among *Brettanomyces* Yeasts by DNA Fingerprinting and  
867 Whole-Genome Sequencing. *Applied and Environmental Microbiology*, 80(14), 4398–4413.  
868 <https://doi.org/10.1128/AEM.00601-14>
- 869 Cravero, F., Englezos, V., Rantsiou, K., Torchio, F., Giacosa, S., Río Segade, S., ... Cocolin, L. (2018).  
870 Control of *Brettanomyces bruxellensis* on wine grapes by post-harvest treatments with  
871 electrolyzed water, ozonated water and gaseous ozone. *Innovative Food Science & Emerging*  
872 *Technologies*, 47(August 2017), 309–316. <https://doi.org/10.1016/j.ifset.2018.03.017>
- 873 Curtin, C. D., Bellon, J. R., Henschke, P. A., Godden, P. W., & de Barros Lopes, M. A. (2007). Genetic  
874 diversity of *Dekkera bruxellensis* yeasts isolated from Australian wineries. *FEMS Yeast Research*,  
875 7(3), 471–481. <https://doi.org/10.1111/j.1567-1364.2006.00183.x>
- 876 Curtin, C. D., Borneman, A. R., Chambers, P. J., & Pretorius, I. S. (2012). De-Novo Assembly and  
877 Analysis of the Heterozygous Triploid Genome of the Wine Spoilage Yeast *Dekkera bruxellensis*  
878 AWRI1499. *PLoS ONE*, 7(3), e33840. <https://doi.org/10.1371/journal.pone.0033840>
- 879 Curtin, C., Kennedy, E., & Henschke, P. A. (2012). Genotype-dependent sulphite tolerance of  
880 Australian *Dekkera* (*Brettanomyces*) *bruxellensis* wine isolates. *Letters in Applied Microbiology*,  
881 55(1), 56–61. <https://doi.org/10.1111/j.1472-765X.2012.03257.x>
- 882 da Silva, J. M., da Silva, G. H. T. G., Parente, D. C., Leite, F. C. B., Silva, C. S., Valente, P., ... de Morais  
883 Jr, M. A. (2019). Biological diversity of carbon assimilation among isolates of the yeast *Dekkera*  
884 *bruxellensis* from wine and fuel-ethanol industrial processes. *FEMS Yeast Research*, 19(3),  
885 1689–1699. <https://doi.org/10.1093/femsyr/foz022>
- 886 Da Silva, T. C. D., Leite, F. C. B., & De Morais, M. A. (2016). Distribution of *Dekkera bruxellensis* in a

- 887 sugarcane-based fuel ethanol fermentation plant. *Letters in Applied Microbiology*, 62(4), 354–  
888 358. <https://doi.org/10.1111/lam.12558>
- 889 Daenen, L., Saison, D., Sterckx, F., Delvaux, F. R., Verachtert, H., & Derdelinckx, G. (2007). Screening  
890 and evaluation of the glucoside hydrolase activity in *Saccharomyces* and *Brettanomyces*  
891 brewing yeasts. *Journal of Applied Microbiology*, 104(2), 071010063119005-???  
892 <https://doi.org/10.1111/j.1365-2672.2007.03566.x>
- 893 de Barros Pita, W., Leite, F. C. B., de Souza Liberal, A. T., Simões, D. A., & de Morais, M. A. (2011). The  
894 ability to use nitrate confers advantage to *Dekkera bruxellensis* over *S. cerevisiae* and can  
895 explain its adaptation to industrial fermentation processes. *Antonie van Leeuwenhoek*, 100(1),  
896 99–107. <https://doi.org/10.1007/s10482-011-9568-z>
- 897 de Barros Pita, W., Teles, G. H., Peña-Moreno, I. C., da Silva, J. M., Ribeiro, K. C., & de Morais Junior,  
898 M. A. (2019). The biotechnological potential of the yeast *Dekkera bruxellensis*. *World Journal of*  
899 *Microbiology and Biotechnology*, 35(7), 103. <https://doi.org/10.1007/s11274-019-2678-x>
- 900 de Barros Pita, W., & Tiukova, I. (2013). The influence of nitrate on the physiology of the yeast  
901 *Dekkera bruxellensis* grown under oxygen limitation. *Yeast (Chichester, England)*, 26(10), 545–  
902 551. <https://doi.org/10.1002/yea>
- 903 De Chiara, M., Friedrich, A., Barré, B., Breitenbach, M., Schacherer, J., & Liti, G. (2020). Discordant  
904 evolution of mitochondrial and nuclear yeast genomes at population level. *BMC Biology*, 18(1),  
905 1–15. <https://doi.org/10.1186/s12915-020-00786-4>
- 906 De Roos, J., & De Vuyst, L. (2019). Microbial acidification, alcoholization, and aroma production  
907 during spontaneous lambic beer production. *Journal of the Science of Food and Agriculture*,  
908 99(1), 25–38. <https://doi.org/10.1002/jsfa.9291>
- 909 de Souza Liberal, A.T., Basílio, A. C. M., do Monte Resende, A., Brasileiro, B. T. V., da Silva-Filho, E. A.,  
910 de Morais, J. O. F., ... de Morais, M. A. (2007). Identification of *Dekkera bruxellensis* as a major  
911 contaminant yeast in continuous fuel ethanol fermentation. *Journal of Applied Microbiology*,  
912 102(2), 538–547. <https://doi.org/10.1111/j.1365-2672.2006.03082.x>
- 913 de Souza Liberal, Anna Theresa, Carazzolle, M. F., Pereira, G. A., Simões, D. A., & de Morais, M. A.  
914 (2012). The yeast *Dekkera bruxellensis* genome contains two orthologs of the ARO10 gene  
915 encoding for phenylpyruvate decarboxylase. *World Journal of Microbiology and Biotechnology*,  
916 28(7), 2473–2478. <https://doi.org/10.1007/s11274-012-1054-x>
- 917 Di Toro, M. R., Capozzi, V., Beneduce, L., Alexandre, H., Tristezza, M., Durante, M., ... Spano, G.  
918 (2015). Intraspecific biodiversity and ‘spoilage potential’ of *Brettanomyces bruxellensis* in  
919 Apulian wines. *LWT - Food Science and Technology*, 60(1), 102–108.  
920 <https://doi.org/10.1016/j.lwt.2014.06.059>
- 921 Dias, L., Dias, S., Sancho, T., Stender, H., Querol, A., Malfeito-Ferreira, M., & Loureiro, V. (2003).  
922 Identification of yeasts isolated from wine-related environments and capable of producing 4-  
923 ethylphenol. *Food Microbiology*, 20(5), 567–574. [https://doi.org/10.1016/S0740-](https://doi.org/10.1016/S0740-0020(02)00152-1)  
924 [0020\(02\)00152-1](https://doi.org/10.1016/S0740-0020(02)00152-1)
- 925 Dias, L., Pereira-da-Silva, S., Tavares, M., Malfeito-Ferreira, M., & Loureiro, V. (2003). Factors  
926 affecting the production of 4-ethylphenol by the yeast *Dekkera bruxellensis* in enological  
927 conditions. *Food Microbiology*, 20(4), 377–384. [https://doi.org/10.1016/S0740-0020\(03\)00023-](https://doi.org/10.1016/S0740-0020(03)00023-6)  
928 [6](https://doi.org/10.1016/S0740-0020(03)00023-6)
- 929 Dimopoulou, M., Hatzikamari, M., Masneuf-Pomarede, I., & Albertin, W. (2019). Sulfur dioxide  
930 response of *Brettanomyces bruxellensis* strains isolated from Greek wine. *Food Microbiology*,

- 931 78, 155–163. <https://doi.org/10.1016/j.fm.2018.10.013>
- 932 Dimopoulou, M., Renault, M., Dols-Lafargue, M., Albertin, W., Herry, J.-M., Bellon-Fontaine, M.-N., &  
933 Masneuf-Pomarede, I. (2019). Microbiological, biochemical, physicochemical surface properties  
934 and biofilm forming ability of *Brettanomyces bruxellensis*. *Annals of Microbiology*.  
935 <https://doi.org/10.1007/s13213-019-01503-5>
- 936 Duarte, F. L., Coimbra, L., & Baleiras-Couto, M. (2017). Filter Media Comparison for the Removal of  
937 *Brettanomyces bruxellensis* from Wine. *American Journal of Enology and Viticulture*, *68*(4),  
938 504–508. <https://doi.org/10.5344/ajev.2017.17003>
- 939 DuBois, A. (2017). Interactions between *Oenococcus oeni* and *Brettanomyces bruxellensis* during  
940 Winemaking and Consequences for Wine Quality. *Young*, *c*(1), 105–106.
- 941 Dutta, A., Dutreux, F., & Schacherer, J. (2021). Loss of heterozygosity results in rapid but variable  
942 genome homogenization across yeast genetic backgrounds. *ELife*, *10*, 1–19.  
943 <https://doi.org/10.7554/eLife.70339>
- 944 Dweck, H. K. M., Ebrahim, S. A. M., Farhan, A., Hansson, B. S., & Stensmyr, M. C. (2015). Olfactory  
945 proxy detection of dietary antioxidants in drosophila. *Current Biology*, *25*(4), 455–466.  
946 <https://doi.org/10.1016/j.cub.2014.11.062>
- 947 Dzialo, M. C., Park, R., Steensels, J., Lievens, B., & Verstrepen, K. J. (2017). Physiology, ecology and  
948 industrial applications of aroma formation in yeast. *FEMS Microbiology Reviews*, *41*(Supp\_1),  
949 S95–S128. <https://doi.org/10.1093/femsre/fux031>
- 950 Eberlein, C., Abou Saada, O., Friedrich, A., Albertin, W., & Schacherer, J. (2021). Different trajectories  
951 of polyploidization shape the genomic landscape of the *Brettanomyces bruxellensis* yeast  
952 species. *Genome Research*, *31*(12), 2316–2326. <https://doi.org/10.1101/gr.275380.121>
- 953 Englezos, V., Rantsiou, K., Torchio, F., Pollon, M., Giacosa, S., Río Segade, S., ... Cocolin, L. (2019).  
954 Efficacy of Ozone against Different Strains of *Brettanomyces bruxellensis* on Winegrapes  
955 Postharvest and Impact on Wine Composition. *American Journal of Enology and Viticulture*,  
956 *70*(3), 249–258. <https://doi.org/10.5344/ajev.2019.18058>
- 957 Fabrizio, V., Vigentini, I., Parisi, N., Picozzi, C., Compagno, C., & Foschino, R. (2015). Heat inactivation  
958 of wine spoilage yeast *Dekkera bruxellensis* by hot water treatment. *Letters in Applied*  
959 *Microbiology*, *61*(2), 186–191. <https://doi.org/10.1111/lam.12444>
- 960 Fadda, M. ., Cosentino, S., Deplano, M., & Palmas, F. (2001). Yeast populations in Sardinian feta  
961 cheese. *International Journal of Food Microbiology*, *69*(1–2), 153–156.  
962 [https://doi.org/10.1016/S0168-1605\(01\)00586-4](https://doi.org/10.1016/S0168-1605(01)00586-4)
- 963 Fisher, K. J., Buskirk, S. W., Vignogna, R. C., Marad, D. A., & Lang, G. I. (2018). Adaptive genome  
964 duplication affects patterns of molecular evolution in *Saccharomyces cerevisiae*. *PLOS Genetics*,  
965 *14*(5), e1007396. <https://doi.org/10.1371/journal.pgen.1007396>
- 966 Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms:  
967 an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), 563–575.  
968 <https://doi.org/10.1038/nrmicro.2016.94>
- 969 Flemming, H.-C., & Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms.  
970 *Nature Reviews Microbiology*, *17*(4), 247–260. <https://doi.org/10.1038/s41579-019-0158-9>
- 971 Forche, A., Abbey, D., Pisithkul, T., Weinzierl, M. A., Ringstrom, T., Bruck, D., ... Berman, J. (2011).  
972 Stress alters rates and types of loss of heterozygosity in *Candida albicans*. *MBio*, *2*(4), 1–9.  
973 <https://doi.org/10.1128/mBio.00129-11>

- 974 Fournier, T., Gounot, J.-S., Freel, K., Cruaud, C., Lemainque, A., Aury, J.-M., ... Friedrich, A. (2017).  
 975 High-Quality de Novo Genome Assembly of the *Dekkera bruxellensis* Yeast Using Nanopore  
 976 MinION Sequencing. *G3; Genes/Genomes/Genetics*, *7*(10), 3243–3250.  
 977 <https://doi.org/10.1534/g3.117.300128>
- 978 Fredericks, I. N., du Toit, M., & Krügel, M. (2011). Efficacy of ultraviolet radiation as an alternative  
 979 technology to inactivate microorganisms in grape juices and wines. *Food Microbiology*, *28*(3),  
 980 510–517. <https://doi.org/10.1016/j.fm.2010.10.018>
- 981 Freer, S. N. (2002). Acetic acid production by *Dekkera/Brettanomyces* yeasts. *World Journal of*  
 982 *Microbiology and Biotechnology*, *18*(3), 271–275.  
 983 <https://doi.org/doi.org/10.1023/A:1014927129259>
- 984 Freer, S. N., Dien, B., & Matsuda, S. (2003). Production of acetic acid by *Dekkera/Brettanomyces*  
 985 yeasts under conditions of constant pH. *World Journal of Microbiology and Biotechnology*,  
 986 *19*(1), 101–105. <https://doi.org/10.1023/A:1022592810405>
- 987 Galafassi, S., Capusoni, C., Muktaduzzaman, M., & Compagno, C. (2013). Utilization of nitrate  
 988 abolishes the “Custers effect” in *Dekkera bruxellensis* and determines a different pattern of  
 989 fermentation products. *Journal of Industrial Microbiology and Biotechnology*, *40*(3–4), 297–303.  
 990 <https://doi.org/10.1007/s10295-012-1229-3>
- 991 Galafassi, S., Merico, A., Pizza, F., Hellborg, L., Molinari, F., Piškur, J., & Compagno, C. (2011).  
 992 *Dekkera/Brettanomyces* yeasts for ethanol production from renewable sources under oxygen-  
 993 limited and low-pH conditions. *Journal of Industrial Microbiology & Biotechnology*, *38*(8), 1079–  
 994 1088. <https://doi.org/10.1007/s10295-010-0885-4>
- 995 Galafassi, S., Toscano, M., Vigentini, I., Piškur, J., & Compagno, C. (2013). Osmotic stress response in  
 996 the wine yeast *Dekkera bruxellensis*. *Food Microbiology*, *36*(2), 316–319.  
 997 <https://doi.org/10.1016/j.fm.2013.06.011>
- 998 Gallone, B., Mertens, S., Gordon, J. L., Maere, S., Verstrepen, K. J., & Steensels, J. (2018). Origins,  
 999 evolution, domestication and diversity of *Saccharomyces* beer yeasts. *Current Opinion in*  
 1000 *Biotechnology*, *49*, 148–155. <https://doi.org/10.1016/j.copbio.2017.08.005>
- 1001 Gallone, B., Steensels, J., Prah, T., Soriaga, L., Saels, V., Herrera-Malaver, B., ... Verstrepen, K. J.  
 1002 (2016). Domestication and Divergence of *Saccharomyces cerevisiae* Beer Yeasts. *Cell*, *166*(6),  
 1003 1397–1410.e16. <https://doi.org/10.1016/j.cell.2016.08.020>
- 1004 García-Ríos, E., & Guillamón, J. M. (2019). Sulfur dioxide resistance in *Saccharomyces cerevisiae*:  
 1005 beyond SSU1. *Microbial Cell*, *6*(12), 527–530. <https://doi.org/10.15698/mic2019.12.699>
- 1006 García-Ríos, E., Ruiz-Rico, M., Guillamón, J. M., Pérez-Esteve, É., & Barat, J. M. (2018). Improved  
 1007 antimicrobial activity of immobilised essential oil components against representative spoilage  
 1008 wine microorganisms. *Food Control*, *94*(July), 177–186.  
 1009 <https://doi.org/10.1016/j.foodcont.2018.07.005>
- 1010 Gerbaux, V., Jeudy, S., & Monamy, C. (2000). Étude des phénols volatils dans les vins de Pinot noir en  
 1011 Bourgogne. *Bulletin de l'OIV*, *73*(835), 581–599.
- 1012 Gerstein, A. C., McBride, R. M., & Otto, S. P. (2008). Ploidy reduction in *Saccharomyces cerevisiae*.  
 1013 *Biology Letters*, *4*(1), 91–94. <https://doi.org/10.1098/rsbl.2007.0476>
- 1014 Giannakou, K., Cotterrell, M., & Delneri, D. (2020). Genomic Adaptation of *Saccharomyces* Species to  
 1015 Industrial Environments. *Frontiers in Genetics*, *11*(August), 1–10.  
 1016 <https://doi.org/10.3389/fgene.2020.00916>

- 1017 Gil-Sánchez, I., Monge, M., Miralles, B., Armentia, G., Cueva, C., Crespo, J., ... Moreno-Arribas, M. V.  
 1018 (2019). Some new findings on the potential use of biocompatible silver nanoparticles in  
 1019 winemaking. *Innovative Food Science & Emerging Technologies*, 51(April 2018), 64–72.  
 1020 <https://doi.org/10.1016/j.ifset.2018.04.017>
- 1021 Gladieux, P., Ropars, J., Badouin, H., Branca, A., Aguilera, G., Vienne, D. M., ... Giraud, T. (2014).  
 1022 Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in  
 1023 eukaryotes. *Molecular Ecology*, 23(4), 753–773. <https://doi.org/10.1111/mec.12631>
- 1024 Goddard, M. R., & Greig, D. (2015). *Saccharomyces cerevisiae*: A nomadic yeast with no niche? *FEMS*  
 1025 *Yeast Research*, 15(3), 1–6. <https://doi.org/10.1093/femsyr/fov009>
- 1026 Godoy, L., Martínez, C., Carrasco, N., & Ganga, M. (2008). Purification and characterization of a p-  
 1027 coumarate decarboxylase and a vinylphenol reductase from *Brettanomyces bruxellensis*.  
 1028 *International Journal of Food Microbiology*, 127(1–2), 6–11.  
 1029 <https://doi.org/10.1016/j.ijfoodmicro.2008.05.011>
- 1030 Godoy, Liliana, García, V., Peña, R., Martínez, C., & Ganga, M. A. (2014). Identification of the *Dekkera*  
 1031 *bruxellensis* phenolic acid decarboxylase (PAD) gene responsible for wine spoilage. *Food*  
 1032 *Control*, 45, 81–86. <https://doi.org/10.1016/j.foodcont.2014.03.041>
- 1033 Godoy, Liliana, Silva-Moreno, E., Mardones, W., Guzman, D., Cubillos, F. A., & Ganga, A. (2017).  
 1034 Genomics Perspectives on Metabolism, Survival Strategies, and Biotechnological Applications of  
 1035 ***Brettanomyces bruxellensis*** LAMAP2480. *Journal of Molecular Microbiology and Biotechnology*,  
 1036 27(3), 147–158. <https://doi.org/10.1159/000471924>
- 1037 Godoy, Liliana, Vera-Wolf, P., Martínez, C., Ugalde, J. A., & Ganga, M. A. (2016). Comparative  
 1038 transcriptome assembly and genome-guided profiling for *Brettanomyces bruxellensis*  
 1039 LAMAP2480 during p-coumaric acid stress. *Scientific Reports*, 6(1), 34304.  
 1040 <https://doi.org/10.1038/srep34304>
- 1041 González-Arenzana, L., López-Alfaro, I., Gutiérrez, A. R., López, N., Santamaría, P., & López, R. (2019).  
 1042 Continuous pulsed electric field treatments' impact on the microbiota of red Tempranillo wines  
 1043 aged in oak barrels. *Food Bioscience*, 27(October 2018), 54–59.  
 1044 <https://doi.org/10.1016/j.fbio.2018.10.012>
- 1045 González, C., Godoy, L., & Ganga, M. A. (2017). Identification of a second PAD1 in *Brettanomyces*  
 1046 *bruxellensis* LAMAP2480. *Antonie van Leeuwenhoek*, 110(2), 291–296.  
 1047 <https://doi.org/10.1007/s10482-016-0793-3>
- 1048 Gounot, J.-S., Neuvéglise, C., Freel, K. C., Devillers, H., Piškur, J., Friedrich, A., & Schacherer, J. (2020).  
 1049 High complexity and degree of genetic variation in *Brettanomyces bruxellensis* population.  
 1050 *Genome Biology and Evolution*. <https://doi.org/10.1093/gbe/evaa077>
- 1051 Gracin, L., Jambrak, A. R., Juretić, H., Dobrović, S., Barukčić, I., Grozdanović, M., & Smoljanić, G.  
 1052 (2016). Influence of high power ultrasound on *Brettanomyces* and lactic acid bacteria in wine in  
 1053 continuous flow treatment. *Applied Acoustics*, 103(March 2016), 143–147.  
 1054 <https://doi.org/10.1016/j.apacoust.2015.05.005>
- 1055 Granato, T. M., Romano, D., Vigentini, I., Foschino, R. C., Monti, D., Mamone, G., ... Molinari, F.  
 1056 (2015). New insights on the features of the vinyl phenol reductase from the wine-spoilage yeast  
 1057 *Dekkera/Brettanomyces bruxellensis*. *Annals of Microbiology*, 65(1), 321–329.  
 1058 <https://doi.org/10.1007/s13213-014-0864-5>
- 1059 GRBIN, P. R., & HENSCHKE, P. A. (2000). Mousy off-flavour production in grape juice and wine by  
 1060 *Dekkera* and *Brettanomyces* yeasts. *Australian Journal of Grape and Wine Research*, 6(3), 255–

- 1061 262. <https://doi.org/10.1111/j.1755-0238.2000.tb00186.x>
- 1062 Grbin, P. R., Herderich, M., Markides, A., Lee, T. H., & Henschke, P. A. (2007). The Role of Lysine  
1063 Amino Nitrogen in the Biosynthesis of Mousy Off-Flavor Compounds by *Dekkera anomala*.  
1064 *Journal of Agricultural and Food Chemistry*, 55(26), 10872–10879.  
1065 <https://doi.org/10.1021/jf071243e>
- 1066 Gregory, T. R., & Mable, B. K. (2005). *Polyploidy in Animals*. In : *The evolution of the genome*.
- 1067 Guo, Y. C., Zhang, L., Dai, S. X., Li, W. X., Zheng, J. J., Li, G. H., & Huang, J. F. (2016). Independent  
1068 evolution of winner traits without whole genome duplication in *Dekkera* yeasts. *PLoS ONE*,  
1069 11(5), 1–13. <https://doi.org/10.1371/journal.pone.0155140>
- 1070 Harari, Y., Ram, Y., Rappoport, N., Hadany, L., & Kupiec, M. (2018). Spontaneous Changes in Ploidy  
1071 Are Common in Yeast. *Current Biology*, 28(6), 825-835.e4.  
1072 <https://doi.org/10.1016/j.cub.2018.01.062>
- 1073 Hellborg, L., & Piškur, J. (2009). Complex Nature of the Genome in a Wine Spoilage Yeast, *Dekkera*  
1074 *bruxellensis*. *Eukaryotic Cell*, 8(11), 1739–1749. <https://doi.org/10.1128/EC.00115-09>
- 1075 Heresztyn, T. (1986). Metabolism of volatile phenolic compounds from hydroxycinnamic acids  
1076 by *Brettanomyces* yeast. *Archives of Microbiology*, 146(1), 96–98.  
1077 <https://doi.org/10.1007/BF00690165>
- 1078 Herrera, J., Kramer, C. L., & Reichman, O. J. (1997). Patterns of fungal communities that inhabit  
1079 rodent food stores: effect of substrate and infection time. *Mycologia*, 89(6), 846–857.  
1080 <https://doi.org/10.1080/00275514.1997.12026855>
- 1081 Hittinger, C. T., Rokas, A., Bai, F. Y., Boekhout, T., Gonçalves, P., Jeffries, T. W., ... Kurtzman, C. P.  
1082 (2015). Genomics and the making of yeast biodiversity. *Current Opinion in Genetics and*  
1083 *Development*, 35, 100–109. <https://doi.org/10.1016/j.gde.2015.10.008>
- 1084 Hixson, J. L., Sleep, N. R., Capone, D. L., Elsey, G. M., Curtin, C. D., Sefton, M. A., & Taylor, D. K.  
1085 (2012). Hydroxycinnamic Acid Ethyl Esters as Precursors to Ethylphenols in Wine. *Journal of*  
1086 *Agricultural and Food Chemistry*, 60(9), 2293–2298. <https://doi.org/10.1021/jf204908s>
- 1087 Holt, S., Mukherjee, V., Lievens, B., Verstrepen, K. J., & Thevelein, J. M. (2018). Bioflavoring by non-  
1088 conventional yeasts in sequential beer fermentations. *Food Microbiology*, 72, 55–66.  
1089 <https://doi.org/10.1016/j.fm.2017.11.008>
- 1090 Janko, K., Bartoš, O., Kočí, J., Roslein, J., Drdová, E. J., Kotusz, J., ... Štefková-Kašparová, E. (2021).  
1091 Genome Fractionation and Loss of Heterozygosity in Hybrids and Polyploids: Mechanisms,  
1092 Consequences for Selection, and Link to Gene Function. *Molecular Biology and Evolution*,  
1093 38(12), 5255–5274. <https://doi.org/10.1093/molbev/msab249>
- 1094 Joseph, C. M. L., Gorton, L. W., Ebeler, S. E., & Bisson, L. F. (2013). Production of Volatile Compounds  
1095 by Wine Strains of *Brettanomyces bruxellensis* Grown in the Presence of Different Precursor  
1096 Substrates. *American Journal of Enology and Viticulture*, 64(2), 231–240.  
1097 <https://doi.org/10.5344/ajev.2013.12095>
- 1098 Joseph, C. M. Lucy, Kumar, G., Su, E., & Bisson, L. F. (2007). Adhesion and biofilm production by wine  
1099 isolates of *Brettanomyces bruxellensis*. *American Journal of Enology and Viticulture*, 58(3), 373–  
1100 378.
- 1101 Joseph, C.M. Lucy, Albino, E., & Bisson, L. F. (2017). Creation and Use of a *Brettanomyces* Aroma  
1102 Wheel. *Catalyst: Discovery into Practice*, 1(1), 12–20.  
1103 <https://doi.org/10.5344/catalyst.2016.16003>

- 1104 Jutakanoke, R., Endoh, R., Takashima, M., Ohkuma, M., Tanasupawat, S., & Akaracharanya, A. (2017).  
 1105 Allodekkera sacchari gen. nov., sp. nov., a yeast species in the Saccharomycetales isolated from  
 1106 a sugar factory. *International Journal of Systematic and Evolutionary Microbiology*, 67(2), 250–  
 1107 255. <https://doi.org/10.1099/ijsem.0.001607>
- 1108 Kaewkod, T., Bovonsombut, S., & Tragoolpua, Y. (2019). Efficacy of kombucha obtained from green,  
 1109 oolong and black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on  
 1110 colorectal cancer cell line. *Microorganisms*, 7(12), 1–18.  
 1111 <https://doi.org/10.3390/microorganisms7120700>
- 1112 Kheir, J., Salameh, D., Strehaiano, P., Brandam, C., & Lteif, R. (2013). Impact of volatile phenols and  
 1113 their precursors on wine quality and control measures of Brettanomyces/Dekkera yeasts.  
 1114 *European Food Research and Technology*, 237(5), 655–671. <https://doi.org/10.1007/s00217-013-2036-4>  
 1115
- 1116 Kregiel, D., James, S. A., Rygala, A., Berlowska, J., Antolak, H., & Pawlikowska, E. (2018). Consortia  
 1117 formed by yeasts and acetic acid bacteria Asaia spp. in soft drinks. *Antonie van Leeuwenhoek*,  
 1118 111(3), 373–383. <https://doi.org/10.1007/s10482-017-0959-7>
- 1119 Križanović, S., Tomašević, M., Režek Jambrak, A., Čurko, N., Gracin, L., Lukić, K., & Kovačević Ganić, K.  
 1120 (2020). Effect of Thermosonication and Physicochemical Properties of Wine on Culturability,  
 1121 Viability, and Metabolic Activity of Brettanomyces bruxellensis Yeast in Red Wines. *Journal of*  
 1122 *Agricultural and Food Chemistry*, 68(11), 3302–3311. <https://doi.org/10.1021/acs.jafc.9b03661>
- 1123 Kufferath, H., & Van Laer, M. (1921). Études sur les levures du Lambic. *Bull Soc Chim Belgique*, 30,  
 1124 270–276.
- 1125 Kumara, H. M. C. S., De Cort, S., & Verachtert, H. (1993). Localization and characterization of  $\alpha$ -  
 1126 glucosidase activity in Brettanomyces lambicus. *Applied and Environmental Microbiology*, 59(8),  
 1127 2352–2358.
- 1128 Kuo, H. P., Wang, R., Huang, C. Y., Lai, J. T., Lo, Y. C., & Huang, S. T. (2018). Characterization of an  
 1129 extracellular  $\beta$ -glucosidase from Dekkera bruxellensis for resveratrol production. *Journal of*  
 1130 *Food and Drug Analysis*, 26(1), 163–171. <https://doi.org/10.1016/j.jfda.2016.12.016>
- 1131 Lachance, M.-A., Lee, D. K., & Hsiang, T. (2020). Delineating yeast species with genome average  
 1132 nucleotide identity: a calibration of ANI with haplontic, heterothallic Metschnikowia species.  
 1133 *Antonie van Leeuwenhoek*, 113(12), 2097–2106. <https://doi.org/10.1007/s10482-020-01480-9>
- 1134 Lamour, K. H., Mudge, J., Gobena, D., Hurtado-Gonzales, O. P., Schmutz, J., Kuo, A., ... Kingsmore, S. F.  
 1135 (2012). Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for  
 1136 rapid adaptation in the vegetable pathogen Phytophthora capsici. *Molecular Plant-Microbe*  
 1137 *Interactions*, 25(10), 1350–1360. <https://doi.org/10.1094/MPMI-02-12-0028-R>
- 1138 Lebleux, M., Abdo, H., Coelho, C., Basmacıyan, L., Albertin, W., Maupeu, J., ... Rousseaux, S. (2020).  
 1139 New advances on the Brettanomyces bruxellensis biofilm mode of life. *International Journal of*  
 1140 *Food Microbiology*, 318(June), 108464. <https://doi.org/10.1016/j.ijfoodmicro.2019.108464>
- 1141 Lee, H.-Y., Chou, J.-Y., Cheong, L., Chang, N.-H., Yang, S.-Y., & Leu, J.-Y. (2008). Incompatibility of  
 1142 Nuclear and Mitochondrial Genomes Causes Hybrid Sterility between Two Yeast Species. *Cell*,  
 1143 135(6), 1065–1073. <https://doi.org/10.1016/j.cell.2008.10.047>
- 1144 Legras, J. L., Galeote, V., Bigey, F., Camarasa, C., Marsit, S., Nidelet, T., ... Dequin, S. (2018).  
 1145 Adaptation of s. Cerevisiae to fermented food environments reveals remarkable genome  
 1146 plasticity and the footprints of domestication. *Molecular Biology and Evolution*, 35(7), 1712–  
 1147 1727. <https://doi.org/10.1093/molbev/msy066>

- 1148 Leite, F. C. B., Basso, T. O., Pita, W. B., Gombert, A. K., Simões, D. A., & de Morais Júnior, M. A. (2012).  
 1149 Quantitative aerobic physiology of the yeast *Dekkera bruxellensis*, a major contaminant in  
 1150 bioethanol production plants. *FEMS Yeast Research*, *13*, n/a-n/a.  
 1151 <https://doi.org/10.1111/j.1567-1364.2012.12007.x>
- 1152 Lentz, M. (2018). The Impact of Simple Phenolic Compounds on Beer Aroma and Flavor.  
 1153 *Fermentation*, *4*(1), 20. <https://doi.org/10.3390/fermentation4010020>
- 1154 Lentz, M., & Harris, C. (2015). Analysis of Growth Inhibition and Metabolism of Hydroxycinnamic  
 1155 Acids by Brewing and Spoilage Strains of *Brettanomyces* Yeast. *Foods*, *4*(4), 581–593.  
 1156 <https://doi.org/10.3390/foods4040581>
- 1157 Linder, C. R., Moret, B. M. E., & Nakhleh, L. (2003). Network (reticulate) evolution: biology, models,  
 1158 and algorithms. *The Pacific Symposium ...*, 41. Retrieved from  
 1159 [http://www.cs.utexas.edu/~phylo/resources/pdf/papers/psb04.pdf%5Cnpapers2://publication/](http://www.cs.utexas.edu/~phylo/resources/pdf/papers/psb04.pdf%5Cnpapers2://publication/uuid/A12A09EE-0B73-4B6E-8A57-64DAE806F49A%5Cnhttps://www.cs.rice.edu/~nakhleh/Papers/psb04.pdf)  
 1160 [uuid/A12A09EE-0B73-4B6E-8A57-](http://www.cs.utexas.edu/~phylo/resources/pdf/papers/psb04.pdf%5Cnpapers2://publication/uuid/A12A09EE-0B73-4B6E-8A57-64DAE806F49A%5Cnhttps://www.cs.rice.edu/~nakhleh/Papers/psb04.pdf)  
 1161 [64DAE806F49A%5Cnhttps://www.cs.rice.edu/~nakhleh/Papers/psb04.pdf](http://www.cs.utexas.edu/~phylo/resources/pdf/papers/psb04.pdf%5Cnpapers2://publication/uuid/A12A09EE-0B73-4B6E-8A57-64DAE806F49A%5Cnhttps://www.cs.rice.edu/~nakhleh/Papers/psb04.pdf)
- 1162 Longin, C., Degueurce, C., Julliat, F., Guilloux-Benatier, M., Rousseaux, S., & Alexandre, H. (2016).  
 1163 Efficiency of population-dependent sulfite against *Brettanomyces bruxellensis* in red wine. *Food*  
 1164 *Research International*, *89*, 620–630. <https://doi.org/10.1016/j.foodres.2016.09.019>
- 1165 Louw, M., du Toit, M., Alexandre, H., & Divol, B. (2016). Comparative morphological characteristics of  
 1166 three *Brettanomyces bruxellensis* wine strains in the presence/absence of sulfur dioxide.  
 1167 *International Journal of Food Microbiology*, *238*, 79–88.  
 1168 <https://doi.org/10.1016/j.ijfoodmicro.2016.08.040>
- 1169 Lynch, K. M., Wilkinson, S., Daenen, L., & Arendt, E. K. (2021). An update on water kefir:  
 1170 Microbiology, composition and production. *International Journal of Food Microbiology*, *345*,  
 1171 109128. <https://doi.org/10.1016/j.ijfoodmicro.2021.109128>
- 1172 Madsen, M. G., Edwards, N. K., Petersen, M. A., Mokwena, L., Swiegers, J. H., & Arneborg, N. (2017).  
 1173 Influence of *Oenococcus oeni* and *Brettanomyces bruxellensis* on hydroxycinnamic acids and  
 1174 volatile phenols of aged wine. *American Journal of Enology and Viticulture*, *68*(1), 23–29.  
 1175 <https://doi.org/10.5344/ajev.2016.16015>
- 1176 Malfeito-Ferreira, M. (2018). Two Decades of “Horse Sweat” Taint and *Brettanomyces* Yeasts in  
 1177 Wine: Where do We Stand Now? *Beverages*, *4*(2), 32.  
 1178 <https://doi.org/10.3390/beverages4020032>
- 1179 Marcet-Houben, M., & Gabaldón, T. (2015). Beyond the whole-genome duplication: Phylogenetic  
 1180 evidence for an ancient interspecies hybridization in the baker’s yeast lineage. *PLoS Biology*,  
 1181 *13*(8), 1–26. <https://doi.org/10.1371/journal.pbio.1002220>
- 1182 Martellacci, L., Quaranta, G., Patini, R., Isola, G., Gallenzi, P., & Masucci, L. (2019). A Literature  
 1183 Review of Metagenomics and Culturomics of the Peri-implant Microbiome: Current Evidence  
 1184 and Future Perspectives. *Materials*, *12*(18), 3010. <https://doi.org/10.3390/ma12183010>
- 1185 Martyniak, B., Bolton, J., Kuksin, D., Shahin, S. M., & Chan, L. L. Y. (2017). A novel concentration and  
 1186 viability detection method for *Brettanomyces* using the Cellometer image cytometry. *Journal of*  
 1187 *Industrial Microbiology and Biotechnology*, *44*(1), 119–128. [https://doi.org/10.1007/s10295-](https://doi.org/10.1007/s10295-016-1861-4)  
 1188 [016-1861-4](https://doi.org/10.1007/s10295-016-1861-4)
- 1189 Mazzucco, M. B., Ganga, M. A., & Sangorrín, M. P. (2019). Production of a novel killer toxin from  
 1190 *Saccharomyces eubayanus* using agro-industrial waste and its application against wine spoilage  
 1191 yeasts. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*,



- 1192 112(7), 965–973. <https://doi.org/10.1007/s10482-019-01231-5>
- 1193 Meroth, C. B., Hammes, W. P., & Hertel, C. (2003). Identification and Population Dynamics of Yeasts  
1194 in Sourdough Fermentation Processes by PCR-Denaturing Gradient Gel Electrophoresis. *Applied*  
1195 *and Environmental Microbiology*, 69(12), 7453–7461. [https://doi.org/10.1128/AEM.69.12.7453-](https://doi.org/10.1128/AEM.69.12.7453-7461.2003)  
1196 7461.2003
- 1197 Millet, V., & Lonvaud-Funel, A. (2000). The viable but non-culturable state of wine micro-organisms  
1198 during storage. *Letters in Applied Microbiology*, 30(2), 136–141. [https://doi.org/10.1046/j.1472-](https://doi.org/10.1046/j.1472-765x.2000.00684.x)  
1199 765x.2000.00684.x
- 1200 Miot-Sertier, C., & Lonvaud-Funel, A. (2007). Development of a molecular method for the typing of  
1201 *Brettanomyces bruxellensis* (*Dekkera bruxellensis*) at the strain level. *Journal of Applied*  
1202 *Microbiology*, 102(2), 555–562. <https://doi.org/10.1111/j.1365-2672.2006.03069.x>
- 1203 Mokatduzzaman, M., Galafassi, S., Capusoni, C., Vigentini, I., Ling, Z., Piškur, J., & Compagno, C.  
1204 (2015). Galactose utilization sheds new light on sugar metabolism in the sequenced strain  
1205 *Dekkera bruxellensis* CBS 2499. *FEMS Yeast Research*, 15(2), 1–9.  
1206 <https://doi.org/10.1093/femsyr/fou009>
- 1207 Mokatduzzaman, M., Galafassi, S., Vigentini, I., Foschino, R., Corte, L., Cardinali, G., ... Compagno, C.  
1208 (2016). Strain-dependent tolerance to acetic acid in *Dekkera bruxellensis*. *Annals of*  
1209 *Microbiology*, 66(1), 351–359. <https://doi.org/10.1007/s13213-015-1115-0>
- 1210 Morales-de la Peña, M., Welti-Chanes, J., & Martín-Belloso, O. (2019). Novel technologies to improve  
1211 food safety and quality. *Current Opinion in Food Science*, 30, 1–7.  
1212 <https://doi.org/10.1016/j.cofs.2018.10.009>
- 1213 Morales, L., & Dujon, B. (2012). Evolutionary Role of Interspecies Hybridization and Genetic  
1214 Exchanges in Yeasts. *Microbiology and Molecular Biology Reviews*, 76(4), 721–739.  
1215 <https://doi.org/10.1128/MMBR.00022-12>
- 1216 Morata, A., Vejarano, R., Ridolfi, G., Benito, S., Palomero, F., Uthurry, C., ... Suárez-Lepe, J. A. (2013).  
1217 Reduction of 4-ethylphenol production in red wines using HCDC+ yeasts and cinnamyl  
1218 esterases. *Enzyme and Microbial Technology*, 52(2), 99–104.  
1219 <https://doi.org/10.1016/j.enzmictec.2012.11.001>
- 1220 Morrissey, W. F., Davenport, B., Querol, A., & Dobson, A. D. W. (2004). The role of indigenous yeasts  
1221 in traditional Irish cider fermentations. *Journal of Applied Microbiology*, 97(3), 647–655.  
1222 <https://doi.org/10.1111/j.1365-2672.2004.02354.x>
- 1223 Nardi, T., Remize, F., & Alexandre, H. (2010). Adaptation of yeasts *Saccharomyces cerevisiae* and  
1224 *Brettanomyces bruxellensis* to winemaking conditions: A comparative study of stress genes  
1225 expression. *Applied Microbiology and Biotechnology*, 88(4), 925–937.  
1226 <https://doi.org/10.1007/s00253-010-2786-x>
- 1227 Neiman, M., Kay, A. D., & Krist, A. C. (2013). Can resource costs of polyploidy provide an advantage to  
1228 sex? *Heredity*, 110(2), 152–159. <https://doi.org/10.1038/hdy.2012.78>
- 1229 Neto, A. G. B., Pestana-Calsa, M. C., de Morais, M. A., & Calsa, T. (2014). Proteome responses to  
1230 nitrate in bioethanol production contaminant *Dekkera bruxellensis*. *Journal of Proteomics*, 104,  
1231 104–111. <https://doi.org/10.1016/j.jprot.2014.03.014>
- 1232 Olsen, R.-A., Bunikis, I., Tiukova, I., Holmberg, K., Lötstedt, B., Pettersson, O. V., ... Vezzi, F. (2015). De  
1233 novo assembly of *Dekkera bruxellensis*: a multi technology approach using short and long-read  
1234 sequencing and optical mapping. *GigaScience*, 4(1), 56. [https://doi.org/10.1186/s13742-015-](https://doi.org/10.1186/s13742-015-0094-1)  
1235 0094-1

- 1236 Oro, L., Canonico, L., Marinelli, V., Ciani, M., & Comitini, F. (2019). Occurrence of *Brettanomyces*  
1237 *bruxellensis* on Grape Berries and in Related Winemaking Cellar. *Frontiers in Microbiology*,  
1238 *10*(MAR), 1–10. <https://doi.org/10.3389/fmicb.2019.00415>
- 1239 Otto, S. P., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, *34*(1),  
1240 401–437.
- 1241 Parente, D. C., Cajueiro, D. B. B., Moreno, I. C. P., Leite, F. C. B., De Barros Pita, W., & De Morais, M.  
1242 A. (2018). On the catabolism of amino acids in the yeast *Dekkera bruxellensis* and the  
1243 implications for industrial fermentation processes. *Yeast*, *35*(3), 299–309.  
1244 <https://doi.org/10.1002/yea.3290>
- 1245 Parente, D. C., Vidal, E. E., Leite, F. C. B., de Barros Pita, W., & de Morais, M. A. (2014). Production of  
1246 sensory compounds by means of the yeast *Dekkera bruxellensis* in different nitrogen sources  
1247 with the prospect of producing cachaça. *Yeast*, (November 2013), n/a-n/a.  
1248 <https://doi.org/10.1002/yea.3051>
- 1249 Passoth, V., Blomqvist, J., & Schnürer, J. (2007). *Dekkera bruxellensis* and *Lactobacillus vini* Form a  
1250 Stable Ethanol-Producing Consortium in a Commercial Alcohol Production Process. *Applied and*  
1251 *Environmental Microbiology*, *73*(13), 4354–4356. <https://doi.org/10.1128/AEM.00437-07>
- 1252 Paulin, M., Miot-Sertier, C., Dutilh, L., Brasselet, C., Delattre, C., Pierre, G., ... Dols-Lafargue, M.  
1253 (2020). *Brettanomyces bruxellensis* Displays Variable Susceptibility to Chitosan Treatment in  
1254 Wine. *Frontiers in Microbiology*, *11*(September), 1–13.  
1255 <https://doi.org/10.3389/fmicb.2020.571067>
- 1256 Peña-Moreno, I. C., Castro Parente, D., da Silva, J. M., Andrade Mendonça, A., Rojas, L. A. V., de  
1257 Morais Junior, M. A., & de Barros Pita, W. (2019). Nitrate boosts anaerobic ethanol production  
1258 in an acetate-dependent manner in the yeast *Dekkera bruxellensis*. *Journal of Industrial*  
1259 *Microbiology & Biotechnology*, *46*(2), 209–220. <https://doi.org/10.1007/s10295-018-2118-1>
- 1260 Pereira, L. F., Bassi, A. P. G., Avansini, S. H., Neto, A. G. B., Brasileiro, B. T. R. V., Ceccato-Antonini, S.  
1261 R., & de Morais, M. A. (2012). The physiological characteristics of the yeast *Dekkera bruxellensis*  
1262 in fully fermentative conditions with cell recycling and in mixed cultures with *Saccharomyces*  
1263 *cerevisiae*. *Antonie van Leeuwenhoek*, *101*(3), 529–539. [https://doi.org/10.1007/s10482-011-](https://doi.org/10.1007/s10482-011-9662-2)  
1264 [9662-2](https://doi.org/10.1007/s10482-011-9662-2)
- 1265 Péter, G., Dlauchy, D., Tóbiás, A., Fülöp, L., Podgoršek, M., & Čadež, N. (2017). *Brettanomyces*  
1266 *acidodurans* sp. nov., a new acetic acid producing yeast species from olive oil. *Antonie van*  
1267 *Leeuwenhoek, International Journal of General and Molecular Microbiology*, *110*(5), 657–664.  
1268 <https://doi.org/10.1007/s10482-017-0832-8>
- 1269 Peter, J., De Chiara, M., Friedrich, A., Yue, J.-X., Pflieger, D., Bergström, A., ... Schacherer, J. (2018).  
1270 Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates. *Nature*, *556*(7701), 339–  
1271 344. <https://doi.org/10.1038/s41586-018-0030-5>
- 1272 Peter, J., & Schacherer, J. (2016). Population genomics of yeasts: towards a comprehensive view  
1273 across a broad evolutionary scale. *Yeast*, *33*(3), 73–81. <https://doi.org/10.1002/yea.3142>
- 1274 Petrova, B., Cartwright, Z. M., & Edwards, C. G. (2016). Effectiveness of chitosan preparations against  
1275 *Brettanomyces bruxellensis* grown in culture media and red wines. *OENO One*,  
1276 *50*(1), 49. <https://doi.org/10.20870/oeno-one.2016.50.1.54>
- 1277 Peynaud, E., & Domercq, S. (1956). Sur les *brettanomyces* isolés de raisins et de vins. *Archiv for*  
1278 *Mikrobiologie*, *24*(3), 266–280. <https://doi.org/10.1007/BF00419012>
- 1279 Pfeiffer, T., & Morley, A. (2014). An evolutionary perspective on the Crabtree effect. *Frontiers in*

- 1280 *Molecular Biosciences*, 1(OCT), 1–6. <https://doi.org/10.3389/fmolb.2014.00017>
- 1281 Phowchinda, O., Dalia-Dupuy, M. L., & Strehaiano, P. (1995). Effects of acetic acid on growth and  
1282 fermentative activity of *Saccharomyces cerevisiae*. *Biotechnology Letters*, 17(2), 237–242.  
1283 <https://doi.org/10.1007/BF00127996>
- 1284 Pilard, E., Harrouard, J., Miot-Sertier, C., Marullo, P., Albertin, W., & Ghidossi, R. (2021). Wine yeast  
1285 species show strong inter- and intra-specific variability in their sensitivity to ultraviolet  
1286 radiation. *Food Microbiology*, 100(June), 103864. <https://doi.org/10.1016/j.fm.2021.103864>
- 1287 Pinto, L., Baruzzi, F., Cocolin, L., & Malfeito-Ferreira, M. (2020). Emerging technologies to control  
1288 *Brettanomyces* spp. in wine: Recent advances and future trends. *Trends in Food Science &*  
1289 *Technology*, 99(March), 88–100. <https://doi.org/10.1016/j.tifs.2020.02.013>
- 1290 Piškur, J., Ling, Z., Marcet-Houben, M., Ishchuk, O. P., Aerts, A., LaButti, K., ... Phister, T. (2012). The  
1291 genome of wine yeast *Dekkera bruxellensis* provides a tool to explore its food-related  
1292 properties. *International Journal of Food Microbiology*, 157(2), 202–209.  
1293 <https://doi.org/10.1016/j.ijfoodmicro.2012.05.008>
- 1294 Post, D. M., & Urban, J. E. (1993). Change in Microbial Populations from Eastern Woodrat Caches. *The*  
1295 *Southwestern Naturalist*, 38(1), 30. <https://doi.org/10.2307/3671641>
- 1296 Prasai, K., Robinson, L. C., Scott, R. S., Tatchell, K., & Harrison, L. (2017). Evidence for double-strand  
1297 break mediated mitochondrial DNA replication in *Saccharomyces cerevisiae*. *Nucleic Acids*  
1298 *Research*, 45(13), 7760–7773. <https://doi.org/10.1093/nar/gkx443>
- 1299 Procházka, E., Poláková, S., Piškur, J., & Sulo, P. (2010). Mitochondrial genome from the facultative  
1300 anaerobe and petite-positive yeast *Dekkera bruxellensis* contains the NADH dehydrogenase  
1301 subunit genes. *FEMS Yeast Research*, 10(5), no-no. [https://doi.org/10.1111/j.1567-](https://doi.org/10.1111/j.1567-1364.2010.00644.x)  
1302 [1364.2010.00644.x](https://doi.org/10.1111/j.1567-1364.2010.00644.x)
- 1303 Querol, A., & Bond, U. (2009). The complex and dynamic genomes of industrial yeasts: MINIREVIEW.  
1304 *FEMS Microbiology Letters*, 293(1), 1–10. <https://doi.org/10.1111/j.1574-6968.2008.01480.x>
- 1305 Radecka, D., Mukherjee, V., Mateo, R. Q., Stojiljkovic, M., Foulquié-Moreno, M. R., & Thevelein, J. M.  
1306 (2015). Looking beyond *Saccharomyces*: The potential of non-conventional yeast species for  
1307 desirable traits in bioethanol fermentation. *FEMS Yeast Research*, 15(6), 1–13.  
1308 <https://doi.org/10.1093/femsyr/fov053>
- 1309 Reis, A. L. S., de Fátima Rodrigues de Souza, R., Baptista Torres, R. R. N., Leite, F. C. B., Paiva, P. M. G.,  
1310 Vidal, E. E., & de Moraes, M. A. (2014). Oxygen-limited cellobiose fermentation and the  
1311 characterization of the cellobiase of an industrial *Dekkera/Brettanomyces bruxellensis* strain.  
1312 *SpringerPlus*, 3(1), 38. <https://doi.org/10.1186/2193-1801-3-38>
- 1313 Renouf, V., Lonvaud-Funel, A., & Coulon, J. (2007). The origin of *Brettanomyces bruxellensis* in wines:  
1314 A review. *Journal International Des Sciences de La Vigne et Du Vin*, 41(3), 161–173.
- 1315 Renouf, Vincent, & Lonvaud-Funel, A. (2007). Development of an enrichment medium to detect  
1316 *Dekkera/Brettanomyces bruxellensis*, a spoilage wine yeast, on the surface of grape berries.  
1317 *Microbiological Research*, 162(2), 154–167. <https://doi.org/10.1016/j.micres.2006.02.006>
- 1318 Renouf, Vincent, Strehaiano, P., & Lonvaud-Funel, A. (2008). Effectiveness of dimethyldicarbonate to  
1319 prevent *Brettanomyces bruxellensis* growth in wine. *Food Control*, 19(2), 208–216.  
1320 <https://doi.org/10.1016/j.foodcont.2007.03.012>
- 1321 Rhind, N., Chen, Z., Yassour, M., Thompson, D. A., Haas, B. J., Habib, N., ... Nusbaum, C. (2011).  
1322 Comparative Functional Genomics of the Fission Yeasts. *Science*, 332(6032), 930–936.

- 1323 <https://doi.org/10.1126/science.1203357>
- 1324 Rhodes, J., Desjardins, C. A., Sykes, S. M., Beale, M. A., Vanhove, M., Sakthikumar, S., ... Cuomo, C. A.  
1325 (2017). Tracing Genetic Exchange and Biogeography of *Cryptococcus neoformans* var. *grubii* at  
1326 the Global Population Level. *Genetics*, *207*(1), 327–346.  
1327 <https://doi.org/10.1534/genetics.117.203836>
- 1328 Richard, P., Viljanen, K., & Penttilä, M. (2015). Overexpression of PAD1 and FDC1 results in significant  
1329 cinnamic acid decarboxylase activity in *Saccharomyces cerevisiae*. *AMB Express*, *5*(1), 12.  
1330 <https://doi.org/10.1186/s13568-015-0103-x>
- 1331 Riley, R., Haridas, S., Wolfe, K. H., Lopes, M. R., Hittinger, C. T., Göker, M., ... Jeffries, T. W. (2016).  
1332 Comparative genomics of biotechnologically important yeasts. *Proceedings of the National  
1333 Academy of Sciences*, *113*(35), 9882–9887. <https://doi.org/10.1073/pnas.1603941113>
- 1334 Roach, M. J., & Borneman, A. R. (2020). New genome assemblies reveal patterns of domestication  
1335 and adaptation across *Brettanomyces* (*Dekkera*) species. *BMC Genomics*, *21*(1), 194.  
1336 <https://doi.org/10.1186/s12864-020-6595-z>
- 1337 Romano, A., Perello, M. C., Revel, G. De, & Lonvaud-Funel, A. (2008). Growth and volatile compound  
1338 production by *Brettanomyces/Dekkera bruxellensis* in red wine. *Journal of Applied  
1339 Microbiology*, *104*(6), 1577–1585. <https://doi.org/10.1111/j.1365-2672.2007.03693.x>
- 1340 Romano, D., Valdetara, F., Zambelli, P., Galafassi, S., De Vitis, V., Molinari, F., ... Vigentini, I. (2017).  
1341 Cloning the putative gene of vinyl phenol reductase of *Dekkera bruxellensis* in *Saccharomyces  
1342 cerevisiae*. *Food Microbiology*, *63*, 92–100. <https://doi.org/10.1016/j.fm.2016.11.003>
- 1343 Rozpędowska, E., Hellborg, L., Ishchuk, O. P., Orhan, F., Galafassi, S., Merico, A., ... Piškur, J. (2011).  
1344 Parallel evolution of the make–accumulate–consume strategy in *Saccharomyces* and *Dekkera*  
1345 yeasts. *Nature Communications*, *2*(1), 302. <https://doi.org/10.1038/ncomms1305>
- 1346 Ruxton, G. D., Wilkinson, D. M., Schaefer, H. M., & Sherratt, T. N. (2014). Why fruit rots: theoretical  
1347 support for Janzen’s theory of microbe–macrobe competition. *Proceedings of the Royal Society  
1348 B: Biological Sciences*, *281*(1782), 20133320. <https://doi.org/10.1098/rspb.2013.3320>
- 1349 Ryu, S., Park, W. S., Yun, B., Shin, M., Go, G., Kim, J. N., ... Kim, Y. (2021). Diversity and characteristics  
1350 of raw milk microbiota from Korean dairy farms using metagenomic and culturomic analysis.  
1351 *Food Control*, *127*(March), 108160. <https://doi.org/10.1016/j.foodcont.2021.108160>
- 1352 Sampaio, N. M. V., Ajith, V. P., Watson, R. A., Heasley, L. R., Chakraborty, P., Rodrigues-Prause, A., ...  
1353 Argueso, J. L. (2020). Characterization of systemic genomic instability in budding yeast.  
1354 *Proceedings of the National Academy of Sciences*, *117*(45), 28221–28231.  
1355 <https://doi.org/10.1073/pnas.2010303117>
- 1356 Sanna, V., & Pretti, L. (2015). Effect of wine barrel ageing or sapa addition on total polyphenol  
1357 content and antioxidant activities of some Italian craft beers. *International Journal of Food  
1358 Science and Technology*, *50*(3), 700–707. <https://doi.org/10.1111/ijfs.12666>
- 1359 Santos, A., Navascués, E., Bravo, E., & Marquina, D. (2011). *Ustilago maydis* killer toxin as a new tool  
1360 for the biocontrol of the wine spoilage yeast *Brettanomyces bruxellensis*. *International Journal  
1361 of Food Microbiology*, *145*(1), 147–154. <https://doi.org/10.1016/j.ijfoodmicro.2010.12.005>
- 1362 Savary, O., Mounier, J., Thierry, A., Poirier, E., Jourden, J., Maillard, M.-B., ... Coton, M. (2021). Tailor-  
1363 made microbial consortium for Kombucha fermentation: Microbiota-induced biochemical  
1364 changes and biofilm formation. *Food Research International*, *147*(March), 110549.  
1365 <https://doi.org/10.1016/j.foodres.2021.110549>

- 1366 Schifferdecker, A. J., Dashko, S., Ishchuk, O. P., & Piškur, J. (2014). The wine and beer yeast *Dekkera*  
1367 *bruxellensis*. *Yeast*, *31*(9), 323–332. <https://doi.org/10.1002/yea.3023>
- 1368 Schifferdecker, A. J., Siurkus, J., Andersen, M. R., Joerck-Ramberg, D., Ling, Z., Zhou, N., ... Ishchuk, O.  
1369 P. (2016). Alcohol dehydrogenase gene ADH3 activates glucose alcoholic fermentation in  
1370 genetically engineered *Dekkera bruxellensis* yeast. *Applied Microbiology and Biotechnology*,  
1371 *100*(7), 3219–3231. <https://doi.org/10.1007/s00253-015-7266-x>
- 1372 Schönknecht, G., Weber, A. P. M., & Lercher, M. J. (2014). Horizontal gene acquisitions by eukaryotes  
1373 as drivers of adaptive evolution. *BioEssays*, *36*(1), 9–20.  
1374 <https://doi.org/10.1002/bies.201300095>
- 1375 Schopp, L. M., Lee, J., Osborne, J. P., Chescheir, S. C., & Edwards, C. G. (2013). Metabolism of  
1376 Nonesterified and Esterified Hydroxycinnamic Acids in Red Wines by *Brettanomyces*  
1377 *bruxellensis*. *Journal of Agricultural and Food Chemistry*, *61*(47), 11610–11617.  
1378 <https://doi.org/10.1021/jf403440k>
- 1379 Scopel, E. F. C., Hose, J., Bensasson, D., & Gasch, A. P. (2021). Genetic variation in aneuploidy  
1380 prevalence and tolerance across *Saccharomyces cerevisiae* lineages. *Genetics*, *217*(4).  
1381 <https://doi.org/10.1093/genetics/iyab015>
- 1382 Selmecki, A., Forche, A., & Berman, J. (2006). Aneuploidy and Isochromosome Formation in Drug-  
1383 Resistant *Candida albicans*. *Science*, *313*(5785), 367–370.  
1384 <https://doi.org/10.1126/science.1128242>
- 1385 Seo, S. O., Park, S. K., Jung, S. C., Ryu, C. M., & Kim, J. S. (2020). Anti-contamination strategies for  
1386 yeast fermentations. *Microorganisms*, Vol. 8, pp. 1–13.  
1387 <https://doi.org/10.3390/microorganisms8020274>
- 1388 Serpaggi, V., Remize, F., Recorbet, G., Gaudot-Dumas, E., Sequeira-Le Grand, A., & Alexandre, H.  
1389 (2012). Characterization of the “viable but nonculturable” (VBNC) state in the wine spoilage  
1390 yeast *Brettanomyces*. *Food Microbiology*, *30*(2), 438–447.  
1391 <https://doi.org/10.1016/j.fm.2011.12.020>
- 1392 Serra Colomer, M., Chailyan, A., Fennessy, R. T., Olsson, K. F., Johnsen, L., Solodovnikova, N., &  
1393 Forster, J. (2020). Assessing Population Diversity of *Brettanomyces* Yeast Species and  
1394 Identification of Strains for Brewing Applications. *Frontiers in Microbiology*, *11*(April), 1–21.  
1395 <https://doi.org/10.3389/fmicb.2020.00637>
- 1396 Serra Colomer, M., Funch, B., & Forster, J. (2019). The raise of *Brettanomyces* yeast species for beer  
1397 production. *Current Opinion in Biotechnology*, *56*, 30–35.  
1398 <https://doi.org/10.1016/j.copbio.2018.07.009>
- 1399 Serra Colomer, M., Funch, B., Solodovnikova, N., Hobbey, T. J., & Förster, J. (2020). Biotransformation  
1400 of hop derived compounds by *Brettanomyces* yeast strains. *Journal of the Institute of Brewing*,  
1401 (March). <https://doi.org/10.1002/jib.610>
- 1402 Shimotsu, S., Asano, S., Iijima, K., Suzuki, K., Yamagishi, H., & Aizawa, M. (2015). Investigation of beer-  
1403 spoilage ability of *Dekkera* / *Brettanomyces* yeasts and development of multiplex PCR method  
1404 for beer-spoilage yeasts. *Journal of the Institute of Brewing*, *121*(2), 177–180.  
1405 <https://doi.org/10.1002/jib.209>
- 1406 Smith, B. D., & Divol, B. (2016). *Brettanomyces bruxellensis*, a survivalist prepared for the wine  
1407 apocalypse and other beverages. *Food Microbiology*, *59*, 161–175.  
1408 <https://doi.org/10.1016/j.fm.2016.06.008>
- 1409 Smith, B. D., & Divol, B. (2018). The carbon consumption pattern of the spoilage yeast *Brettanomyces*

- 1410           bruxellensis in synthetic wine-like medium. *Food Microbiology*, 73, 39–48.  
1411           <https://doi.org/10.1016/j.fm.2017.12.011>
- 1412           Smukowski Heil, C. S., DeSevo, C. G., Pai, D. A., Tucker, C. M., Hoang, M. L., & Dunham, M. J. (2017).  
1413           Loss of Heterozygosity Drives Adaptation in Hybrid Yeast. *Molecular Biology and Evolution*,  
1414           34(7), 1596–1612. <https://doi.org/10.1093/molbev/msx098>
- 1415           Snowdon, E. M., Bowyer, M. C., Grbin, P. R., & Bowyer, P. K. (2006). Mousy Off-Flavor: A Review.  
1416           *Journal of Agricultural and Food Chemistry*, 54(18), 6465–6474.  
1417           <https://doi.org/10.1021/jf0528613>
- 1418           Soltis, P. S., & Soltis, D. E. (2009). The Role of Hybridization in Plant Speciation. *Annual Review of*  
1419           *Plant Biology*, 60(1), 561–588. <https://doi.org/10.1146/annurev.arplant.043008.092039>
- 1420           Soppa, J. (2022). Non-equivalent genomes in polyploid prokaryotes. *Nature Microbiology*, 7(2), 186–  
1421           188. <https://doi.org/10.1038/s41564-021-01034-3>
- 1422           Spaepen, M., & Verachtert, H. (1982). Esterase activity in the genus *Brettanomyces*. *Journal of the*  
1423           *Institute of Brewing*, 88(1), 11–17. <https://doi.org/10.1002/j.2050-0416.1982.tb04061.x>
- 1424           Spitaels, F., Wieme, A. D., Janssens, M., Aerts, M., Daniel, H.-M., Van Landschoot, A., ... Vandamme,  
1425           P. (2014). The Microbial Diversity of Traditional Spontaneously Fermented Lambic Beer. *PLoS*  
1426           *ONE*, 9(4), e95384. <https://doi.org/10.1371/journal.pone.0095384>
- 1427           Starmer, W. T., & Lachance, M. A. (2011). Yeast ecology. *The Yeasts*, 1(1987), 65–83.  
1428           <https://doi.org/10.1016/B978-0-444-52149-1.00006-9>
- 1429           Steensels, J., Daenen, L., Malcorps, P., Derdelinckx, G., Verachtert, H., & Verstrepen, K. J. (2015).  
1430           *Brettanomyces* yeasts — From spoilage organisms to valuable contributors to industrial  
1431           fermentations. *International Journal of Food Microbiology*, 206, 24–38.  
1432           <https://doi.org/10.1016/j.ijfoodmicro.2015.04.005>
- 1433           Steensels, J., Gallone, B., & Verstrepen, K. J. (2021). Interspecific hybridization as a driver of fungal  
1434           evolution and adaptation. *Nature Reviews Microbiology*, 0123456789.  
1435           <https://doi.org/10.1038/s41579-021-00537-4>
- 1436           Steensels, J., Gallone, B., Voordeckers, K., & Verstrepen, K. J. (2019). Domestication of Industrial  
1437           Microbes. *Current Biology*, 29(10), R381–R393. <https://doi.org/10.1016/j.cub.2019.04.025>
- 1438           Steensels, J., Snoek, T., Meersman, E., Nicolino, M. P., Voordeckers, K., & Verstrepen, K. J. (2014).  
1439           Improving industrial yeast strains: Exploiting natural and artificial diversity. *FEMS Microbiology*  
1440           *Reviews*, 38(5), 947–995. <https://doi.org/10.1111/1574-6976.12073>
- 1441           Stefanini, I. (2018). Yeast-insect associations: It takes guts. *Yeast*, 35(4), 315–330.  
1442           <https://doi.org/10.1002/yea.3309>
- 1443           Suárez, R., Suárez-Lepe, J. A., Morata, A., & Calderón, F. (2007). The production of ethylphenols in  
1444           wine by yeasts of the genera *Brettanomyces* and *Dekkera*: A review. *Food Chemistry*, 102(1),  
1445           10–21. <https://doi.org/10.1016/j.foodchem.2006.03.030>
- 1446           Sugimoto-Shirasu, K., & Roberts, K. (2003). “Big it up”: Endoreduplication and cell-size control in  
1447           plants. *Current Opinion in Plant Biology*, 6(6), 544–553.  
1448           <https://doi.org/10.1016/j.pbi.2003.09.009>
- 1449           Tchobanov, I., Gal, L., Guilloux-Benatier, M., Remize, F., Nardi, T., Guzzo, J., ... Alexandre, H. (2008).  
1450           Partial vinylphenol reductase purification and characterization from *Brettanomyces*  
1451           bruxellensis. *FEMS Microbiology Letters*, 284(2), 213–217. <https://doi.org/10.1111/j.1574-6968.2008.01192.x>  
1452

- 1453 Teles, G. H., da Silva, J. M., Mendonça, A. A., de Moraes Junior, M. A., & de Barros Pita, W. (2018).  
 1454 First aspects on acetate metabolism in the yeast *Dekkera bruxellensis* : a few keys for improving  
 1455 ethanol fermentation. *Yeast*, *35*(10), 577–584. <https://doi.org/10.1002/yea.3348>
- 1456 Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. *International*  
 1457 *Journal of Food Microbiology*, *95*(2), 119–126.  
 1458 <https://doi.org/10.1016/j.ijfoodmicro.2003.12.020>
- 1459 Thomson, J. M., Gaucher, E. A., Burgan, M. F., De Kee, D. W., Li, T., Aris, J. P., & Benner, S. A. (2005).  
 1460 Resurrecting ancestral alcohol dehydrogenases from yeast. *Nature Genetics*, *37*(6), 630–635.  
 1461 <https://doi.org/10.1038/ng1553>
- 1462 Tiukova, I. A., de Barros Pita, W., Sundell, D., Haddad Momeni, M., Horn, S. J., Ståhlberg, J., ...  
 1463 Passoth, V. (2014). Adaptation of *Dekkera bruxellensis* to lignocellulose-based substrate.  
 1464 *Biotechnology and Applied Biochemistry*, *61*(1), 51–57. <https://doi.org/10.1002/bab.1145>
- 1465 Tiukova, I. A., Jiang, H., Dainat, J., Hoepfner, M. P., Lantz, H., Piskur, J., ... Passoth, V. (2019).  
 1466 Assembly and Analysis of the Genome Sequence of the Yeast *Brettanomyces naardenensis* CBS  
 1467 7540. *Microorganisms*, *7*(11), 489. <https://doi.org/10.3390/microorganisms7110489>
- 1468 Tiukova, I. A., Petterson, M. E., Tellgren-Roth, C., Bunikis, I., Eberhard, T., Pettersson, O. V., &  
 1469 Passoth, V. (2013). Transcriptome of the Alternative Ethanol Production Strain *Dekkera*  
 1470 *bruxellensis* CBS 11270 in Sugar Limited, Low Oxygen Cultivation. *PLoS ONE*, *8*(3), e58455.  
 1471 <https://doi.org/10.1371/journal.pone.0058455>
- 1472 Tiukova, I. A., Pettersson, M. E., Hoepfner, M. P., Olsen, R.-A., Käller, M., Nielsen, J., ... Passoth, V.  
 1473 (2019). Chromosomal genome assembly of the ethanol production strain CBS 11270 indicates a  
 1474 highly dynamic genome structure in the yeast species *Brettanomyces bruxellensis*. *PLOS ONE*,  
 1475 *14*(5), e0215077. <https://doi.org/10.1371/journal.pone.0215077>
- 1476 Todd, R. T., Forche, A., & Selmecki, A. (2017). Ploidy Variation in Fungi: Polyploidy, Aneuploidy, and  
 1477 Genome Evolution. In *The Fungal Kingdom* (pp. 599–618).  
 1478 <https://doi.org/10.1128/9781555819583.ch28>
- 1479 Todd, R. T., Wikoff, T. D., Forche, A., & Selmecki, A. (2019). Genome plasticity in *Candida albicans* is  
 1480 driven by long repeat sequences. *ELife*, *8*, 1–33. <https://doi.org/10.7554/eLife.45954>
- 1481 Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H., & Tourdot-Maréchal, R. (2020).  
 1482 Microbial Dynamics between Yeasts and Acetic Acid Bacteria in Kombucha: Impacts on the  
 1483 Chemical Composition of the Beverage. *Foods*, *9*(7), 963.  
 1484 <https://doi.org/10.3390/foods9070963>
- 1485 Tyrawa, C., Preiss, R., Armstrong, M., & van der Merwe, G. (2019). The temperature dependent  
 1486 functionality of *Brettanomyces bruxellensis* strains in wort fermentations. *Journal of the*  
 1487 *Institute of Brewing*, *125*(3), 315–325. <https://doi.org/10.1002/jib.565>
- 1488 Umiker, N. L., Descenzo, R. A., Lee, J., & Edwards, C. G. (2013). Removal of *Brettanomyces bruxellensis*  
 1489 from red wine using membrane filtration. *Journal of Food Processing and Preservation*, *37*(5),  
 1490 799–805. <https://doi.org/10.1111/j.1745-4549.2012.00702.x>
- 1491 Uscanga, M. G. A., Delia, M.-L., & Strehaiano, P. (2000). Nutritional requirements of *Brettanomyces*  
 1492 *bruxellensis* : Growth and physiology in batch and chemostat cultures. *Canadian Journal of*  
 1493 *Microbiology*, *46*(11), 1046–1050. <https://doi.org/10.1139/w00-089>
- 1494 Valdes, J., Tapia, P., Cepeda, V., Varela, J., Godoy, L., Cubillos, F. A., ... Ganga, M. A. (2014). Draft  
 1495 genome sequence and transcriptome analysis of the wine spoilage yeast *Dekkera bruxellensis*  
 1496 LAMAP2480 provides insights into genetic diversity, metabolism and survival. *FEMS*

- 1497 *Microbiology Letters*, 361(2), 104–106. <https://doi.org/10.1111/1574-6968.12630>
- 1498 Valdetara, F., Škalič, M., Fracassetti, D., Louw, M., Compagno, C., du Toit, M., ... Vigentini, I. (2020).  
 1499 Transcriptomics unravels the adaptive molecular mechanisms of *Brettanomyces bruxellensis*  
 1500 under SO<sub>2</sub> stress in wine condition. *Food Microbiology*, 90, 103483.  
 1501 <https://doi.org/10.1016/j.fm.2020.103483>
- 1502 Van De Peer, Y., Mizrahi, E., & Marchal, K. (2017). The evolutionary significance of polyploidy.  
 1503 *Nature Reviews Genetics*, 18(7), 411–424. <https://doi.org/10.1038/nrg.2017.26>
- 1504 Van der Walt, J. P. (1964). Dekkera, a new genus of the Saccharomycetaceae. *Antonie Van*  
 1505 *Leeuwenhoek*, 30(1), 273–280.
- 1506 Van Oevelen, D., Spaepen, M., Timmermans, P., & Verachtert, H. (1977). Microbiological aspects of  
 1507 spontaneous wort fermentation in the production of Lambic and Gueuse. *Journal of the*  
 1508 *Institute of Brewing*, 83(6), 356–360. <https://doi.org/10.1002/j.2050-0416.1977.tb03825.x>
- 1509 van Wyk, S., & Silva, F. V. M. (2017). High pressure inactivation of *Brettanomyces bruxellensis* in red  
 1510 wine. *Food Microbiology*, 63, 199–204. <https://doi.org/10.1016/j.fm.2016.11.020>
- 1511 van Wyk, S., Silva, F. V. M., & Farid, M. M. (2019). Pulsed electric field treatment of red wine:  
 1512 Inactivation of *Brettanomyces* and potential hazard caused by metal ion dissolution. *Innovative*  
 1513 *Food Science & Emerging Technologies*, 52(June 2018), 57–65.  
 1514 <https://doi.org/10.1016/j.ifset.2018.11.001>
- 1515 Vanbeneden, N., Gils, F., Delvaux, F., & Delvaux, F. R. (2008). Formation of 4-vinyl and 4-ethyl  
 1516 derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in  
 1517 beer and distribution of Pad1-activity among brewing yeasts. *Food Chemistry*, 107(1), 221–230.  
 1518 <https://doi.org/10.1016/j.foodchem.2007.08.008>
- 1519 Varela, C, Bartel, C., Roach, M., Borneman, A., & Curtin, C. (2019). *Brettanomyces bruxellensis* SSU1  
 1520 Haplotypes Confer Different Levels of Sulfite Tolerance When Expressed in a *Saccharomyces*  
 1521 *cerevisiae* SSU1 Null Mutant. *Applied and Environmental Microbiology*, 85(4), 1–16.  
 1522 <https://doi.org/10.1128/AEM.02429-18>
- 1523 Varela, Cristian, Bartel, C., Onetto, C., & Borneman, A. (2020). Targeted gene deletion in  
 1524 *Brettanomyces bruxellensis* with an expression-free CRISPR-Cas9 system. *Applied Microbiology*  
 1525 *and Biotechnology*. <https://doi.org/10.1007/s00253-020-10750-5>
- 1526 Verstreppe, K. J., Derdelinckx, G., Dufour, J.-P., Winderickx, J., Thevelein, J. M., Pretorius, I. S., &  
 1527 Delvaux, F. R. (2003). Flavor-active esters: Adding fruitiness to beer. *Journal of Bioscience and*  
 1528 *Bioengineering*, 96(2), 110–118. [https://doi.org/10.1016/S1389-1723\(03\)90112-5](https://doi.org/10.1016/S1389-1723(03)90112-5)
- 1529 Vervoort, Y., Herrera-Malaver, B., Mertens, S., Guadalupe Medina, V., Duitama, J., Michiels, L., ...  
 1530 Verstreppe, K. J. (2016). Characterization of the recombinant *Brettanomyces anomalus*  $\beta$ -  
 1531 glucosidase and its potential for bioflavouring. *Journal of Applied Microbiology*, 121(3), 721–  
 1532 733. <https://doi.org/10.1111/jam.13200>
- 1533 Vigentini, I., Lucy Joseph, C. M., Picozzi, C., Foschino, R., & Bisson, L. F. (2013). Assessment of the  
 1534 *Brettanomyces bruxellensis* metabolome during sulphur dioxide exposure. *FEMS Yeast*  
 1535 *Research*, 13(7), 597–608. <https://doi.org/10.1111/1567-1364.12060>
- 1536 Vigentini, I., Romano, A., Compagno, C., Merico, A., Molinari, F., Tirelli, A., ... Volonterio, G. (2008).  
 1537 Physiological and oenological traits of different Dekkera/*Brettanomyces bruxellensis* strains  
 1538 under wine-model conditions. *FEMS Yeast Research*, 8(7), 1087–1096.  
 1539 <https://doi.org/10.1111/j.1567-1364.2008.00395.x>



- 1540 von Cosmos, N., & Edwards, C. (2016). Use of Nutritional Requirements for *Brettanomyces*  
 1541 *bruxellensis* to Limit Infections in Wine. *Fermentation*, 2(4), 17.  
 1542 <https://doi.org/10.3390/fermentation2030017>
- 1543 Wang, R., Chen, Y.-C., Lai, Y.-J., Lu, T.-J., Huang, S.-T., & Lo, Y.-C. (2019). *Dekkera bruxellensis*, a beer  
 1544 yeast that specifically bioconverts mogroside extracts into the intense natural sweetener  
 1545 siamenoside I. *Food Chemistry*, 276(1727), 43–49.  
 1546 <https://doi.org/10.1016/j.foodchem.2018.09.163>
- 1547 Wang, R. R.-C., Li, X.-M., & Chatterton, N. J. (1999). Loss of heterozygosity and accelerated genotype  
 1548 fixation in rice hybrids. *Genome*, 42(5), 789–796. <https://doi.org/10.1139/gen-42-5-789>
- 1549 Wedral, D., Shewfelt, R., & Frank, J. (2010). The challenge of *Brettanomyces* in wine. *LWT - Food*  
 1550 *Science and Technology*, 43(10), 1474–1479. <https://doi.org/10.1016/j.lwt.2010.06.010>
- 1551 Wertheimer, N. B., Stone, N., & Berman, J. (2016). Ploidy dynamics and evolvability in fungi.  
 1552 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1709).  
 1553 <https://doi.org/10.1098/rstb.2015.0461>
- 1554 Wiens, F., Zitzmann, A., Lachance, M.-A., Yegles, M., Pragst, F., Wurst, F. M., ... Spanagel, R. (2008).  
 1555 Chronic intake of fermented floral nectar by wild treeshrews. *Proceedings of the National*  
 1556 *Academy of Sciences*, 105(30), 10426–10431. <https://doi.org/10.1073/pnas.0801628105>
- 1557 Wolfe, K. H., & Shields, D. C. (1997). Molecular evidence for an ancient duplication of the entire yeast  
 1558 genome. *Nature*, 387(6634), 708–713. <https://doi.org/10.1038/42711>
- 1559 Woolfit, M., Rozpędowska, E., Piškur, J., & Wolfe, K. H. (2007). Genome Survey Sequencing of the  
 1560 Wine Spoilage Yeast *Dekkera* (*Brettanomyces*) *bruxellensis*. *Eukaryotic Cell*, 6(4), 721–733.  
 1561 <https://doi.org/10.1128/EC.00338-06>
- 1562 Yarrow, D., & Ahearn, D. G. (1971). *Brettanomyces abstinentis* sp.n. *Antonie van Leeuwenhoek*, 37(1),  
 1563 296–298. <https://doi.org/10.1007/BF02218499>
- 1564 Zeng, S.-W., Huang, Q.-L., & Zhao, S.-M. (2014). Effects of microwave irradiation dose and time on  
 1565 Yeast ZSM-001 growth and cell membrane permeability. *Food Control*, 46, 360–367.  
 1566 <https://doi.org/10.1016/j.foodcont.2014.05.053>
- 1567 Zepeda-Mendoza, M. L., Edwards, N. K., Madsen, M. G., Abel-Kistrup, M., Puetz, L., Sicheritz-Ponten,  
 1568 T., & Swiegers, J. H. (2018). Influence of *Oenococcus oeni* and *Brettanomyces bruxellensis* on  
 1569 Wine Microbial Taxonomic and Functional Potential Profiles. *American Journal of Enology and*  
 1570 *Viticulture*, 69(4), 321–333. <https://doi.org/10.5344/ajev.2018.17092>
- 1571 Zhu, L., Zhang, Y., & Lu, J. (2012). Phenolic Contents and Compositions in Skins of Red Wine Grape  
 1572 Cultivars among Various Genetic Backgrounds and Originations. *International Journal of*  
 1573 *Molecular Sciences*, 13(3), 3492–3510. <https://doi.org/10.3390/ijms13033492>
- 1574 Zuehlke, J., & Edwards, C. (2013). Impact of Sulfur Dioxide and Temperature on Culturability and  
 1575 Viability of *Brettanomyces bruxellensis* in Wine. *Journal of Food Protection*, 76(12), 2024–2030.  
 1576 <https://doi.org/10.4315/0362-028X.JFP-13-243R>
- 1577

1578 [Data Accessibility](#)

1579 Phenotypic data files (figure 2): Avramova et al. (2018) DOI: 10.3389/fmicb.2018.01260

1580 Genomic data (figure 1B): Eberlein et al. (2021) DOI: 10.1101/gr.275380.121

1581 [Author contribution](#)

1582 JH, CE, JS and WA conceived the original outline of the review, with substantial inputs from all  
1583 authors. All authors screened the literature and selected the subset of publications to be included.

1584 JH, CE, JS and WA drafted a first version. MDL helped more specifically drafting the central  
1585 metabolism section, IMP and CMS the aroma/biofilm/technological part, and PB helped with the

1586 aroma section. JH, CE and WA contributed to the figures. All authors extensively revised the

1587 manuscript.

1588 Tables and Figures

1589 Figure 1. Genetic position and population structure of *Brettanomyces bruxellensis*.

1590 1A. Neighbor-joining tree of the *Brettanomyces* genus.

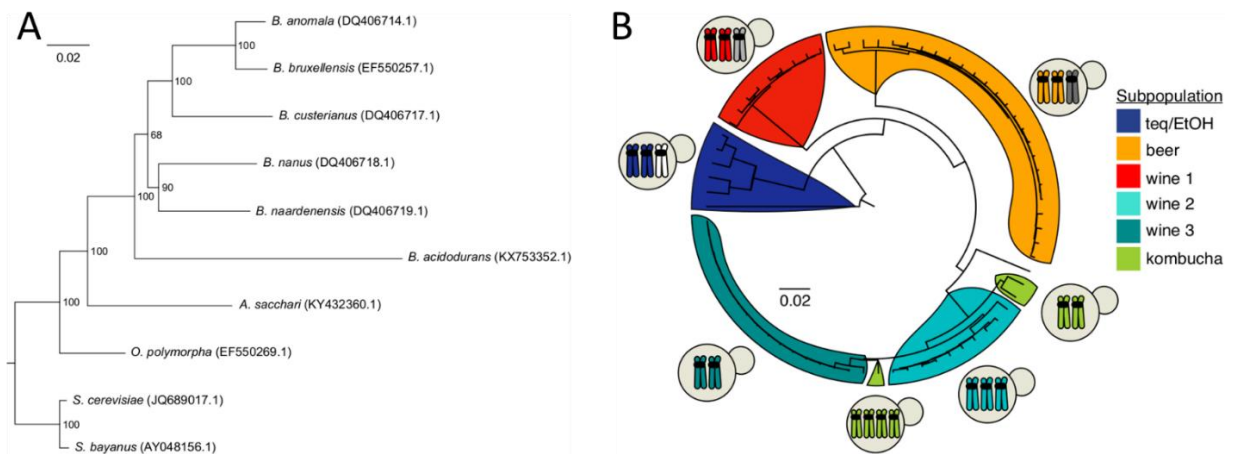
1591 The distance tree comprises the six *Brettanomyces* species, *Allodekкера sacchari* (the closest sister  
 1592 species to *Brettanomyces* clade), *Ogataea polymorpha*, *Saccharomyces cerevisiae* and  
 1593 *Saccharomyces bayanus* as outgroups. 26S ribosomal RNA gene sequences were used. A global  
 1594 alignment with free end gaps was prepared using Geneious (Prime 2020.2) and the default setting for  
 1595 multiple alignments. The Neighbor-joining tree was built with the Tamura-Nei Model of genetic  
 1596 distances. The final tree represents a consensus of 1,000 resampled trees obtained with an extended  
 1597 majority rules method. The consensus supports of the nodes are given in %. Sequences data was  
 1598 downloaded from NCBI.

1599 1B. Genetic diversity within *Brettanomyces bruxellensis* species.

1600 *Brettanomyces bruxellensis* subpopulations are represented by different colors and named from  
 1601 previous reports (Avramova, Cibrario, et al., 2018; Gounot et al., 2020). The tree was built from whole  
 1602 genome Illumina short-read sequencing of 71 *B. bruxellensis* isolates aligned to the reference genome  
 1603 *B. bruxellensis* (Fournier et al., 2017) and 24,313 genetic variants evenly distributed across the genome  
 1604 (Eberlein et al., 2021). The ploidy level of each population was schematized, two chromosomes  
 1605 represent diploid groups, three chromosomes represent triploids ones. Light grey, dark grey and white  
 1606 chromosomes represent the independent haploid acquired genomes from unknown sister species.

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1608

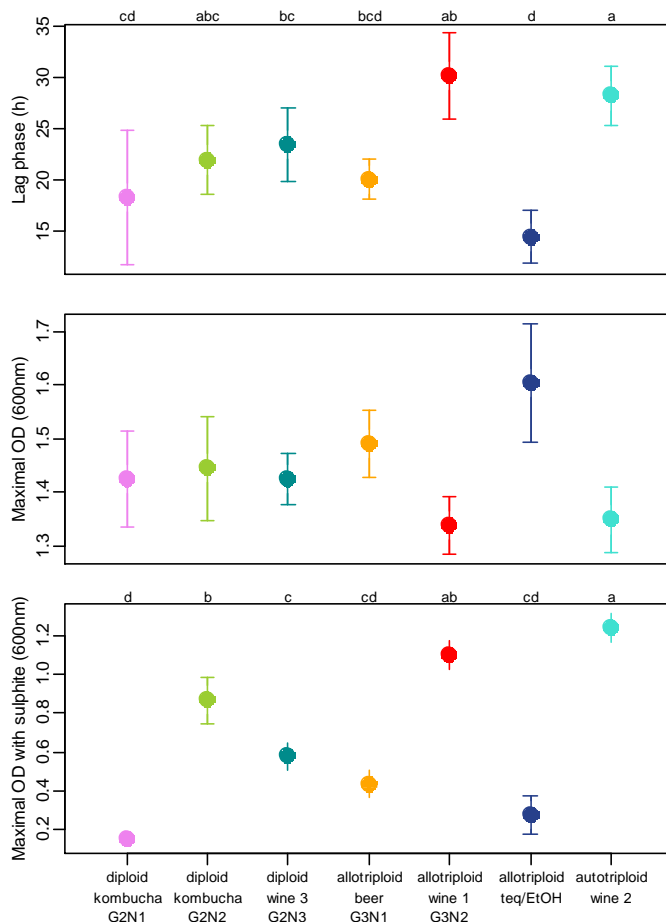


1609

1610

1611 Figure 2. Growth parameters for different subpopulations of *B. bruxellensis*.

1612 Genetic groups as described by previous reports (Avramova, Cibrario, et al., 2018; Gounot et al., 2020).  
1613 Growth data was taken from Avramova et al. (Avramova, Vallet-Courbin, et al., 2018). The growth  
1614 parameters correspond to the lag phase (hour), maximum OD (600 nm) without or with sulphite (0.6  
1615 mg.L<sup>-1</sup> of molecular SO<sub>2</sub>). For each genetic group, mean values are represented by a circle, and error  
1616 bars correspond to standard error. Top letters represent significance groups as defined by Kruskal-  
1617 Wallis test when significant (p-value < 0.05). P-values were 0.0054, 0.11 and 5.4 10<sup>-10</sup> for lag phase,  
1618 maximum OD without or with sulphite respectively.



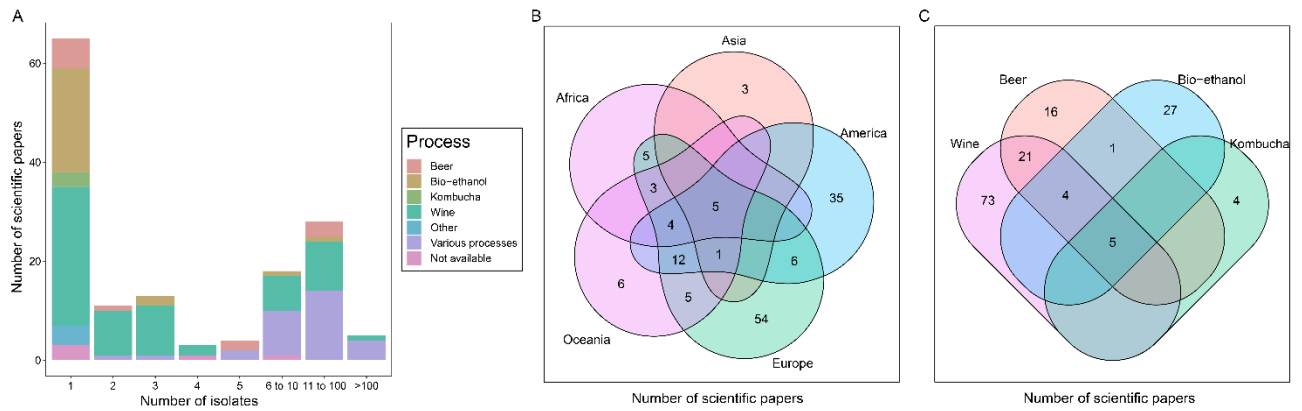
1619

1620 Figure 3. Representativity and diversity of *Brettanomyces bruxellensis* collections studied in the  
 1621 literature.

1622 3A: Histogram of the number of isolates of *B. bruxellensis* used in the studies referenced in  
 1623 supplemental Table 3, with their process/substrate of isolation.

1624 3B: Venn diagram of the number of studies including isolates of *B. bruxellensis* from various  
 1625 geographical origins.

1626 3C: Venn diagram of the number of studies including isolates of *B. bruxellensis* from various process  
 1627 origins.



1628

1629 Table 1. Whole-genome sequencing of *B. bruxellensis* isolates.

1630 <sup>1</sup>ND stands for ‘not determined’ by the corresponding publication.

Publication	Isolates ID (or number of isolates)	Niche; origin of isolation	Country/region of isolation	Ploidy <sup>1</sup>
Woolfit et al. (2007)	CBS 2499	Wine	France	ND
Piškur et al. (2012)	CBS 2499	Wine	France	Diploid
Curtin et al. (2012)	AWRI 1499	Wine	Australia	Triploid
Crauwels et al. (2014)	ST05.12/22 = VIB X9085	Beer	Belgium	Diploid
Valdes et al. (2014)	LAMAP 2480	Wine	Chile	ND
Borneman et al. (2014)	AWRI 1608	Wine	Australia	Triploid
	AWRI 1613	Wine	Australia	Diploid
Crauwels et al. (2015)	ST05.12/26 = MUCL 49865	Beer	Belgium	Diploid
	ST05.12/48	Beer	Belgium	Diploid
	ST05.12/53	Beer	Belgium	Triploid
	ST05.12/59 = CBS 6055	Dry ginger ale	United States of America	Triploid
Olsen et al. (2015), Jiang, et al. (2019)	CBS 11270	Bioethanol	Sweden	Diploid
	CBS 2796	Wine	Germany	ND
Fournier et al. (2017)	9 strains	Wine, beer, bioethanol	Europe, Oceania, Africa	Diploid, triploid
	53 strains	Wine, beer, bioethanol, soft drink	Europe, Oceania, Africa, America	Diploid, triploid
Colomer et al. (2020)	64 strains	Beer, bioethanol, wine, kombucha, tequila	Europe, Oceania, Africa, America	Diploid, triploid
Eberlein et al. (2021)	71 strains	Beer, bioethanol, wine, kombucha, tequila	Europe, Oceania, Africa, America	Diploid, triploid

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