Microbiology Spectrum
American Society for Microbiology Press

Fungi as a Source of Food

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ABSTRACT In this article, we review some of the best-studied fungi used as food sources, in particular, the cheese fungi, the truffles, and the fungi used for drink fermentation such as beer, wine, and sake. We discuss their history of consumption by humans and the genomic mechanisms of adaptation during artificial selection.

INTRODUCTION

Since ancient times, humans have used fungi as food sources (1, 2). The edible sexual structures of basidiomycetes and ascomycetes (e.g., truffles), the so-called mushrooms, are produced mostly in wood because many fungi are tree symbionts or decayers of tree tissues. These fruiting bodies represent a rich source of proteins, with low fat content and otherwise nutritionally quite poor. In some soils, they accumulate pollution (heavy metals and radioactivity) and should only be eaten in moderate quantities. Some mushroom species are considered delicacies (e.g., truffles, boletus, morels), but cultivation attempts have been unsuccessful, with a few exceptions (e.g., *Morchella rufobrunnea*). Only a few saprobic species can be industrially produced, such as *Agaricus bisporus* (3), *Lentinus edodes* (shiitake), and *Pleurotus ostreatus*, with production having mainly taken place in Asia for thousands of years (4). Some other fungi, while not really cultured, are inoculated on trees grown in appropriate natural habitats to increase the production of fruiting bodies, such as for shiitake and oyster mushrooms, with, however, sometimes unpredictable success, as is the case for truffles (5).

A few plant-pathogenic fungi are also eaten, for example, the corn smut fungus, *Ustilago maydis*, which produces black tumors on maize. These tumors are considered a delicacy in Mexico, where it is called *huitlacoche* (6). These fungi can have great cultural significance (7), as do hallucinogenic mushrooms in many traditional cultures (8).

Fungi are also used for the production of fermented food and beverages in all traditional and indigenous cultures in the world. Examples include cheeses, bread, beer, wine, cider, rice, and soy sauce. Humans have exploited the natural abilities of fungi to ferment fruits and grains to produce alcoholic beverages and bread since as early as 6000 BCE (9–11) and for cheese since at least 7500 BCE (12). Yeasts are used for the fermentation of bread, wine, and beer, while filamentous fungi are used for the maturation of cheeses and soy sauce

Received: 13 October 2016, **Accepted:** 20 March 2017, **Published:** ••••••

Editors: Joseph Heitman, Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710; Barbara J. Howlett, School of Biosciences, The University of Melbourne, Victoria, NSW 3010, Australia; Eva Holtgrewe Stukenbrock, Environmental Genomics, Christian-Albrechts University of Kiel, Kiel, Germany, and Max Planck Institute for Evolutionary Biology, Plön, Germany

Citation: Dupont J, Dequin S, Giraud T, Le Tacon F, Marsit S, Ropars J, Richard F, Selosse M-A. 2017. Fungi as a source of food. *Microbiol Spectrum* 5(2):FUNK-0030-2016. doi:10.1128/microbiolspec.FUNK-0030-2016.

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(from soybeans using *Aspergillus oryzae*) (13) and the production of alcohol from rice (yielding sake, also using *A. oryzae*) (14). In the south of France, a plant pathogenic fungus, *Botrytis cinerea*, is used to concentrate sugar in grape berries before collection, yielding "noble rot," which is a sweet and expensive wine (15). Some other filamentous fungi and yeasts are used to ferment and preserve meat from food spoilage, in particular *Penicillium nalgiovense*, which forms the white crust on salamis (16).

Before Louis Pasteur's research, microorganisms and their importance were not recognized. Humans thus unintentionally used the ability of fungi to ferment and produce alcohol, which inadvertently led to artificial selection of the most effective fungal strains, thus modifying species such as beer yeasts and cheese fungi (17–19). Because these species have acquired new adaptive traits compared to their wild ancestors (17–24), they can be considered domesticated species. They thus constitute excellent models for studying rapid and recent adaptation (18).

FUNGI AND CHEESE: DOMESTICATION OF *PENICILLIUM* FUNGI

Cheese making by early Neolithic farmers was a major advance in food processing, allowing milk to be preserved in a nonperishable, transportable form and making it more digestible for adults, because cheese contains much less lactose than fresh milk does (25). The earliest evidences of cheese making date from the 6th millennium BCE in Poland, with findings of the presence of milk fat in sieve vessels (12), and from the early Bronze Age (ca. 3,800 years ago), with the discovery of residues of old cheese in tombs (26). The earliest cheeses were simple, made by adding lactic acid bacteria (LAB) to fresh milk to provoke milk curdling and then draining in sieve vessels. The use of different mechanical processes to drain the curd, i.e., carving, brewing, pressing, grinding, heating, and the maturing of these cheeses, appeared later and gave rise to the large variety of cheeses known today (soft cheeses, blue-veined cheeses, hard cheeses, uncooked firm cheeses, and cooked firm cheeses). Microorganisms play a major role in the cheese-making process, from the initial milk curdling by LAB to the maturation step by fungi (including yeasts and molds). Indeed, during ripening, microbiological and biochemical changes directly influence the development of the texture and flavor that make each kind of cheese unique. Primary biochemical changes include (i) lipolysis, in which LAB are the main actors by metabolizing lactose into lactate; (ii) proteolysis, which has a direct influence on flavor through the production of short peptides and amino acids, originating from six primary sources (e.g., the coagulant, the milk, starter LAB, nonstarter LAB), secondary starters (molds such as *Penicillium roqueforti* in blue cheeses), and Gram-positive bacterial microflora on the surface of smear cheeses (27); and (iii) metabolism of residual lactose, lactate, and citrate. Secondary biochemical changes include metabolism of fatty acids and of amino acids by molds such as *P. roqueforti* in blue cheeses and *Penicillium camemberti* or *Geotrichum candidum* in soft cheeses such as Camembert and Brie. Microbial interactions within a cheese are thus crucial to produce a cheese with the desired texture and flavor, making the cheese substrate a complex but very interesting ecosystem in which to study microbial community interactions (28, 29). Q1

Besides the emblematic cheese-making species *P. camemberti* and *P. roqueforti*, cheese producers widely use other important filamentous Ascomycota within the classes of Eurotiomycetes and Sordariomycetes (30). *Penicillium* species (Eurotiales) are used for the production of blue cheeses such as Roquefort and Fourme d'Ambert (*P. roqueforti*) and soft cheeses such as Camembert and Brie (*P. camemberti*). *Sporendonema casei* (Onygenales) is used for the production of firm cheeses such as cantal and Salers. Some *Scopulariopsis* species (*Scopulariopsis candida*, *Scopulariopsis flava*, and *Scopulariopsis fusca*, Microascales) are found in uncooked hard cheeses, including Tomme des Pyrénées and Ossau-Iraty, while *Fusarium domesticum* (Hypocreales) is inoculated for the production of Saint Nectaire and Reblochon. Along with Ascomycota, several Mucoromycotina species from the genus *Mucor*, including *Mucor racemosus*, *Mucor circinelloides*, and *Mucor lanceolatus*, are used for the production of uncooked hard cheeses such as Saint Nectaire and Tomme de Savoie (31). From an ecological point of view, some species are specific to the cheese environment (e.g., *F. domesticum*, *P. camemberti*, *S. casei*, *S. flava*, *M. lanceolatus*) while others are ubiquitous (e.g., *P. roqueforti*, *S. fusca*, *S. candida*). This leads to the questions of whether and how these species have adapted to this particular man-made, nutrient-rich ecological niche.

P. camemberti and *P. roqueforti* are ideal models for studying parallel adaptation because they are distantly related and have completely different domestication histories (32). *P. camemberti* is a human-created species, being a clonal lineage selected in the late 19th century for its white color and its fluffy, downy texture. It is thought to be derived from the blue-gray mold *Penicillium commune*, a species complex now split into

Penicillium biforme and *Penicillium fuscoglaucum* (32, 33). *P. camemberti* is found only in the cheese environment and is inoculated in soft cheeses such as Camembert and Brie; no genetic or morphological diversity has been found in this species so far. In contrast, *P. roqueforti* is a common food spoilage agent in refrigerated stored foods, meat, and wheat products because of its ability to tolerate cold temperatures, low oxygen concentrations, alkali, and weak acid preservatives; it also occurs in silage and even in forest soil and wood (34, 35).

P. roqueforti has occurred in blue cheeses since at least antiquity (36–38). The fungus was originally not inoculated during blue cheese production but appeared spontaneously. At the beginning of the 19th century, environmental spores of *P. roqueforti* were collected from rotten bread left on purpose to rot on shelves in caves. Later, breads were inoculated with *P. roqueforti* spore powder kept from previous inocula, and strains that yielded good cheese were selected. For the past 30 to 40 years, the inoculated strains have been much more carefully controlled. Inoculated spores now mostly come from sterile *in vitro* conditions and derive from monospore isolations. Such controlled cultivation aims to avoid sanitary issues and to render the cheese maturation process more repeatable and reliable (36–38). In contrast to *P. camemberti*, *P. roqueforti* shows substantial within-species genetic and phenotypic diversity, as well as genetic subdivision (39, 40). In particular, different genetic clusters of *P. roqueforti* seem to have been independently domesticated (41), and subclusters correspond exclusively to particular designation of origins, notably the Roquefort cheeses (41).

Recent studies compared the genomes of the two emblematic cheese species *P. camemberti* and *P. roqueforti* to eight other *Penicillium* species, four of which were isolated from the cheese environment but considered as contaminants by producers (*Penicillium paneum*, *P. fuscoglaucum*, *P. biforme*, and *P. nalgiovense*) (17, 19). The study also included a *Penicillium* species isolated as a contaminant in the meat environment (*Penicillium carneum*), the penicillin-producer *Penicillium rubens* and its close relative *Penicillium chrysogenum*, and the necrotrophic pathogen *Penicillium digitatum*, which is specialized to citrus. The genomic comparison revealed multiple horizontal gene transfers (HGTs) likely involved in cheese adaptation (17, 19). HGT is the process whereby genetic material is transferred between species by means other than vertical descent. In particular, two genomic regions of several hundreds of kilobases, named *Wallaby* (17) and *CheesyTer* (19), were shown to share a nucleotide identity of almost 100% among all cheese *Penicillium* species while being absent from noncheese species. These horizontally transferred regions (HTRs) were unique when compared to other HTR cases cited in the literature by (i) their size (*Wallaby* is ca. 600 kb long, encompassing 2% of the genome of *P. roqueforti*, and *CheesyTer* is 80 kb long), (ii) their eukaryotic origin (likely among cheese-associated *Penicillium* species), (iii) the number of species in which the same regions have been horizontally transferred, and (iv) the very recent date of the transfers, likely associated with the human history of cheese production (17, 19, 32, 42). These HTRs are flanked by copies of a particular family of transposable elements, namely, the *i* non-long terminal repeat (LTR) retrotransposons, that are always found clustered around putative HTRs in *P. roqueforti*. In contrast, the other transposable element families such as *copia* or *gypsy* LTR retrotransposons are widespread along the *P. roqueforti* genome (19). This supports the idea that *i* non-LTR retrotransposons may be involved in the horizontal transfer of these regions.

The gene content of these HTRs suggests that *Wallaby* and *CheesyTer* may play a role in the cheese adaptation. Indeed, two genes in *Wallaby*, named *paf* and *Hce2*, encode proteins previously described to be involved in interactions with other microorganisms (17). Genes coding for a lactose permease and a beta-galactosidase were found in *CheesyTer*, suggesting a role in the metabolism of the cheese substrate (19). These genes were found to be overexpressed during the first days of cheese maturation in an industrial Canadian Camembert-type cheese (19, 43). Experiments of growth and competition on different media using *P. roqueforti* strains have further supported a role of these HTRs in adaptation to cheese. Indeed, *P. roqueforti* was found to display polymorphism regarding the presence of *Wallaby* and *CheesyTer*: strains isolated from other environments than cheese did not carry either of the two HTRs, and among cheese strains, only a subset of strains carried the HTRs, and always either both *Wallaby* and *CheesyTer* or neither of them. Strains carrying the two HTRs grew significantly faster on cheese medium and significantly more slowly on minimal medium (19). Furthermore, strains carrying the HTRs showed a significant competitive advantage, both against *P. roqueforti* strains lacking the HTRs and against other *Penicillium* species also lacking the HTRs (19).

In conclusion, *Penicillium* fungi show footprints of rapid adaptation through frequent HGTs that have occurred under selection in the human-made cheese environments. This shows the importance of HGTs, in

particular in the food environments. Now that we know about the ability of microorganisms to exchange genes through nonvertical descent and even between distant species, the use of genetically modified organisms in the food chain should be avoided.

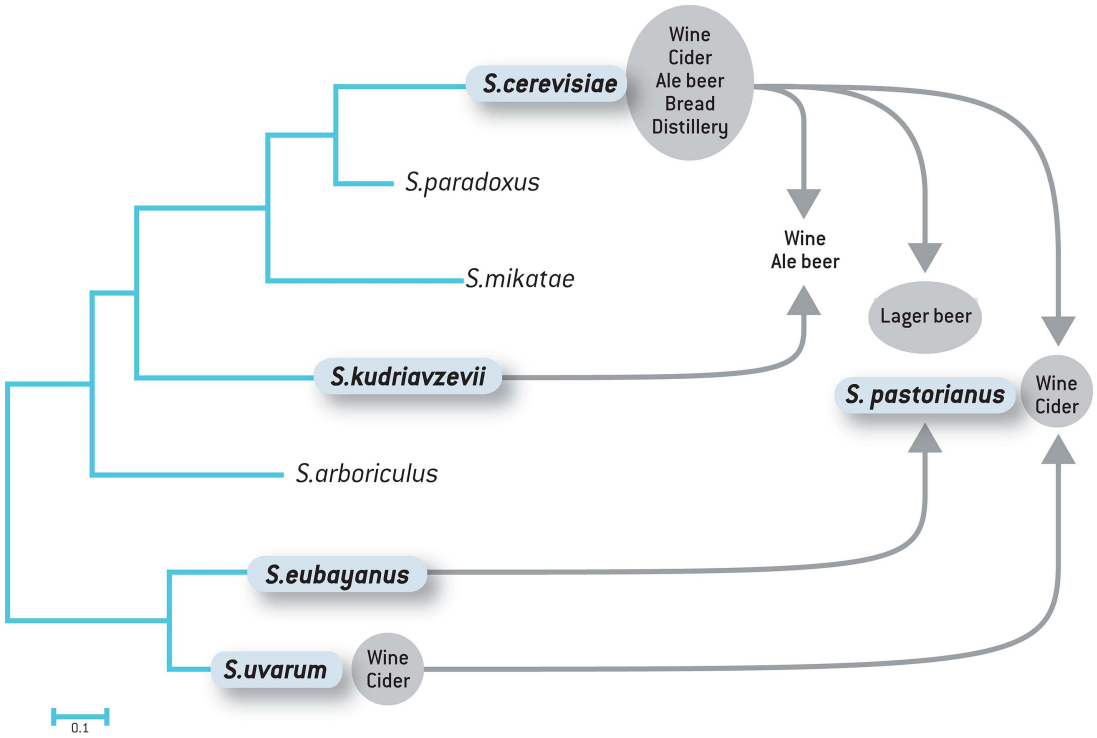
Finally, domestication of cheese fungi has impacted their mode of reproduction. Indeed, industrial companies replicate their strains exclusively clonally, without sex. This together with bottlenecks and strong selection may contribute to the degeneration of cheese strains in the form of reduced fertility as occurs in domesticated plant and animal species (44–47). Investigations of premating (i.e., prevention of syngamy between individuals) and postmating fertility (i.e., syngamy occurs but the progeny is not viable or fertile) between and within genetic clusters of *P. roqueforti* has suggested reduced fertility of cheese strains (48). This degeneration has occurred independently in two cheese lineages, representing convergent evolution on a very short timescale.

YEASTS IN THE WINERY, BAKERY, AND BREWERY

Humans have used *Saccharomyces* yeasts for thousands of years for brewing, baking, and wine making. Because yeasts are able to convert sugars into alcohol, they were used primarily as effective ways to preserve the quality and safety of foods and beverages, because high concentrations of ethanol are toxic for most other microbes. During food fermentation, yeasts convert sugars to ethanol and carbon dioxide under both anaerobic (fermentation) and aerobic conditions (known as the Crabtree effect) (49, 50).

Saccharomyces species are ascomycetous yeasts (51) that are model systems for ecology, genetics, and evolution (52), particularly at the genomic level (53). Studies have focused primarily on domesticated forms: besides the famous *Saccharomyces cerevisiae*, other closely related species belonging to the *Sacharomyces sensu stricto* complex and several important interspecific hybrids have been isolated from fermented food (bread) and beverages (beer, wine, cider, and sake). Their relationships are shown in Fig. 1. The abundance and variety of hybrids in fermented products are intriguing (54) and suggest that such hybrids could have selective advantages over parental species (55). It is possible that stressful conditions in fermentation processes promote hybridization events (52). The persistence of these hybrids in the long term has been questioned (56), but there is some evidence that they undergo progressive

FIGURE 1 Relationships between *Saccharomyces* species and their industrial hybrids. Tree topology was obtained using a subset of 25,000 single nucleotide polymorphisms selected after genome alignment.



genome stabilization (54). Comparative genomic studies of hybrids and ideally of the progenitors of the hybrids are essential to unravel the process of domestication and the impact of domestication on genome architecture and function (57). The discovery of progenitors is sometimes challenging, as it was for the lager beer hybrid *Saccharomyces pastorianus*. This organism has been known to be an interspecific hybrid for more than 3 decades (see below for more details), but the second progenitor was only recently identified as *Saccharomyces eubayanus* (58). The fact that new food-associated *Saccharomyces* species are still being discovered points to our still-limited knowledge about the diversity of yeasts.

The origin and the natural environment of the baker's yeast *S. cerevisae* have long remained a mystery. Given their ability to ferment sugars, *Saccharomyces* yeasts have been sought in mature and rotted fruits, in sugar-rich plant exudates, and in nearby soils (59). It seems that oak trees in the Northern Hemisphere (59–63) and *Nothofagus* trees in the Southern Hemisphere (58) could be important in the natural life cycle of *Saccharomyces* yeasts. Currently, *S. cerevisiae* seems to have a global distribution in wild and human-associated environments (56). In contrast, other species such as *Saccharomyces paradoxus* and *Saccharomyces kudriavzevii* seem to be more limited in their geographical distribution, with strong spatial genetic subdivision between populations suggesting limited dispersal (56). Recently, extensive field surveys conducted in China revealed substantial and novel genetic diversity among wild isolates, suggesting East Asia as a possible center of origin of *Saccharomyces* yeasts (59, 63).

The life cycle of *Saccharomyces* yeasts is well documented in the laboratory (52), but little is known about their life cycle in natural populations. This is true even for the well-studied *S. cerevisiae*; how strains disperse and how and where they reproduce is unknown (64). Recent data have confirmed the importance of insects in their dispersal, e.g., fruit flies for *Candida* species, which are members of the same family (65). Social wasps host *S. cerevisiae* in their intestine and disperse them in the wild (66). More importantly, the wasp intestine provides environmental conditions favoring mating and outbreeding of *Saccharomyces* yeasts (67), an important finding that may explain the high levels of variability and mosaicism observed in *S. cerevisiae* populations. Genotyping and population genomic studies have indeed revealed a complex population structure of *S. cerevisiae* (68–72), with, on the one hand, well-delimited lineages of particular geographical or technological origins (wine, sake, beer) and, on the other hand, mosaic lineages, with recombinant genomes likely originating from admixture after human-associated dispersal. The mosaic lineages include clinical isolates (73), wine strains (74), bakery isolates, and some laboratory strains (64, 70). Strains also cluster according to their ploidy level and type of mating compatibility (75).

Altogether, these results suggest different domestication histories for beer, bread, and wine strains. The following sections focus on recent progress made in understanding the origin and the impact of domestication of *Saccharomyces* yeasts in different fermentation usages.

Wine Fermentation

The first evidence of wine production by humans is dated to the Neolithic period. The presence of tartaric acid and terebinth resin was detected in a pottery jar dated to 5400 to 5000 BCE in Hajji Firuz, Iran (10). Evidence was also provided that the yeast *S. cerevisiae* was responsible for wine fermentation in Egypt by at least 3150 BCE (76). Wine fermentation technologies expanded from Mesopotamia toward Europe and subsequently spread to the New World (77).

Wine alcoholic fermentation is a complex process involving many yeast genera and species that are part of the grape berries' microflora (78). The first stage of wine fermentation involves mainly non-*Saccharomyces* yeasts. However, due to its outstanding fermentative abilities in anaerobic conditions and to its high tolerance to ethanol, *S. cerevisiae* rapidly dominates alcoholic fermentation and is responsible for the degradation of the majority of sugars in alcohol. In the 1970s, the practice of inoculation with selected pure cultures of *S. cerevisiae* in the form of active dry yeast became widespread, and this has enabled better control and reliability of the fermentation process, contributing to the improvement of wine quality. In addition to *S. cerevisiae*, other *Saccharomyces* species and a growing number of interspecies hybrids involved in wine fermentation have been recently identified and characterized.

The fermentation of grape juice exposes yeast to a variety of stresses, including high osmolarity reflecting high sugar concentrations (180 to 260 g/liter), low pH (3 to 3.5), the presence of added sulfites (40 to 80 mg/liter), anaerobiosis, limiting amounts in nutrient (nitrogen, lipids, and vitamins), and ethanol toxicity. During this process, the majority of hexoses are converted into ethanol and CO_2, and a small fraction of sugars is used to synthesize the anabolic precursors required for biomass formation. Various by-products, including glycerol, carboxylic acids, aldehydes, higher alcohols,

esters, carbonyl compounds, and sulfur compounds, are also formed, deriving from the degradation of sugars, amino acids, and fatty acids. Moreover, yeast can also convert nonaromatic grape precursors into aromas (monoterpenes and thiols). These metabolites play a crucial role in the organoleptic balance of wines (79).

Wine fermentation comprises mainly a lag phase and a short growth phase followed by a stationary phase, during which most of the sugar (between 50 and 80%) is fermented. Nitrogen is the main limiting nutrient responsible for cell proliferation arrest, although the availability of other micronutrients such as lipids and vitamins can also be a limiting factor (80). Nutrient imbalance affects yeast fermentation capacity, resulting in stuck or sluggish fermentations, and can also affect the production of volatile compounds and the organoleptic balance of wine.

Today, more than 200 wine strains of *S. cerevisiae* are used to inoculate grape musts. These strains have been selected from spontaneous fermentations or selected in the vineyard environments according to particular fermentative capacities, tolerance to stresses, particularly ethanol and sulfites, and the limited production of undesirable compounds such as H_2S and acetate.

Diversity of wine yeasts and evidence of adaptive evolution to the wine environment

In the past decade, various studies based on multigene sequencing (59, 66, 68), microsatellite analyses (69), tiling array hybridization (81), low-coverage whole-genome sequencing (70), or restriction-site-associated sequencing (Rad-seq) (82) have provided deep insights into the population structure and evolutionary history of *S. cerevisiae*. Genome analyses of *S. cerevisiae* isolates from different sources have led to the identification of five distinct lineages based on their technological and geographic origin (West African, Malaysian, North American, sake, and European/wine). Strains with mosaic genomes resulting from crosses between these lineages have also been identified (70, 81). These studies and earlier findings (68–70, 81–83) have shown that wine yeasts belong to a distinct phylogenetic group with low diversity and have suggested an initial domestication event followed by an expansion of the population favored by human activity. A recent study increased the number of collections of natural lineages, especially oak-associated wild isolates, and used population genomics analyses to analyze a balanced number of anthropic and natural *S. cerevisiae* strains (84). This study identified a new Mediterranean oak population that seems to be the closest wild relative to the wine lineage known to date. The estimated divergence time between the two populations is consistent with the first historical evidences of wine making. Furthermore, three horizontally acquired regions largely widespread in wine yeasts and containing genes relevant to wine making (24) are absent in the wild Mediterranean oak group. These results suggest that the common ancestor of these two populations provided the wild genetic stock that underwent domestication and gave rise to wine yeasts (84, 85).

An increasing number of studies in the past decade have focused on the understanding of the genetic and functional underpinnings of human-driven selection of wine yeasts over millennia. Wine yeast strains demonstrate better resistance to chemical compounds such as copper (86, 87) used in vineyards or sulfites (88, 89) added in grape must. Several wine yeast strains also have the ability to use xylose as a carbon source (90) or different types of di-tripeptides as nitrogen sources (22, 91, 92), and recent data suggest that they might produce more fruity aromas than do strains from other origins (93). Identifying the variations contributing to the properties of wine yeast and deciphering the mechanisms that participate in these evolutionary processes remain major challenges. Several molecular mechanisms may have contributed to the adaptive evolution of wine yeast genomes, including single nucleotide polymorphisms, chromosomal rearrangements, gene copy number variations, introgressions, HGT, and interspecific hybridization (21, 94–96). A hallmark of adaptation has been detected in the genome of so-called flor yeasts. Flor yeasts are closely related to wine strains but have distinct capacities such as the ability to form a biofilm on the surface of wine after fermentation and to develop oxidative metabolism in the presence of a high ethanol concentration and a low amount of fermentable sugar, mainly fructose (97). Flor strains have acquired two mutations in the promoter and the coding regions of the gene *FLO11* encoding a glycosylphosphatidylinositol-anchored cell surface glycoprotein (flocculin) required for pseudohyphal growth and biofilm formation (98, 99), resulting in enhanced cell adhesion. In addition, flor strains possess a fructophylic allele of the *HXT3* hexose transporter previously identified in an industrial strain (100) and a high-affinity fructose transporter, *FSY1*, which may provide an adaptive advantage after alcoholic fermentation when traces of fructose remain in the fermented must but glucose is exhausted (101).

A striking example of yeast adaptation to current wine-making environments is the response of these fungi to chemicals used in the vineyard and winery environments. Elevated copper tolerance in the European wine

and sake lineages has been associated with a copy number variation of *CUP1*, which encodes a copper-binding metallothionein (87). The recent resequencing of the genome of 100 yeast strains revealed that some strains of the wine/European cluster could possess up to 18 copies of the *CUP1* gene (102). The acquisition of this trait can be associated with the use of copper sulfate in vineyards, which has been used as a fungicide against powdery mildew since the 1880s (86).

Genomic variations have also been gained by wine yeasts as a response to the use of sulfite. Two reciprocal translocations, between chromosomes VIII and XVI or XV and XVI, have been detected among wine yeasts. These translocations result in the overexpression of *SSU1*, encoding a sulfite pump and thus conferring a high level of sulfite resistance (89, 103, 104).

Several signatures of introgressions with potential adaptive roles have been identified in wine yeasts. A large *S. paradoxus* introgressed region, identified in commercial *S. cerevisiae* wine yeast strains (105), contains the *S. paradoxus SUC2* gene, which encodes sucrose-hydrolyzing invertase, and a gene similar to *S. cerevisiae HPF1*, which encodes a glucan alpha-1,4-glucosidase that, when overexpressed, reduces protein haze formation in white wines (106). The cryotolerant species *Saccharomyces uvarum* is also found in wine fermentation. A recent study of the population structure and diversity of this species revealed multiple introgressions from other *Saccharomyces* species, especially from *S. eubayanus* (107). These introgressed regions are enriched in functions involving nitrogen metabolism, suggesting that these regions might confer an advantage under nitrogen-limiting wine fermentation conditions.

Horizontal gene transfer also significantly contributes to the adaptation of wine yeasts. Three large genomic regions, called A, B, and C, acquired through independent HGT events from distant yeast species were identified in the genome of the commercial *S. cerevisiae* wine yeast EC1118 (24) and were furthermore shown to be widespread among wine yeasts and some mosaic genomes (24, 71, 108). These genomic islands contain 39 genes encoding potentially important metabolic functions in wine making (sugar and nitrogen metabolism), suggesting a role in adaptation to the wine environment. The yeasts *Zygosaccharomyces bailii* and *Torulaspora microellipsoides* were identified as the donors of regions B and C, respectively (22, 24). For region C, the transfer was dated to approximately 2,000 years ago (22). Evidence was provided of an initial insertion of a 158-kb genomic fragment from *T. microellipsoides* into the *S. cerevisiae* genome, followed by several rearrangements including gene losses and gene conversion between two tandemly duplicated *FOT* genes encoding oligopeptide transporters (22). Interestingly, *FOT* genes are strongly conserved in wine yeasts. These genes confer a strong competitive advantage during grape must fermentation by increasing the amount and diversity of oligopeptides that yeast can utilize as a source of nitrogen, thereby improving biomass formation, fermentation efficiency, and cell viability (22).

Thus, the acquisition of *FOT* genes has favored yeast adaptation to the nitrogen-limited wine fermentation environment. Furthermore, *FOT*-mediated uptake of peptides rich in glutamate substantially affects central carbon and nitrogen metabolism, resulting in decreased acetic acid production and increased ester formation, which might improve the organoleptic balance of wines (109).

Wine hybrids: characterization and advantages

An increasing number of interspecific hybrids have been identified in the wine environment, including *S. cerevisiae*/*S. kudriavzevii* (110–117), *S. cerevisiae*/*S. uvarum* (113, 118–120), and *S. cerevisiae*/*S. kudriavzevii*/*S. uvarum* (23, 110). These hybrids present several advantages in wine fermentation such as increased tolerance to various stresses and robustness compared with the parents (54, 114, 115, 121–123). *S. kudriavzevii* and *S. uvarum* are better adapted to growth at low temperatures compared to *S. cerevisiae*, whereas *S. cerevisiae* is more alcohol-tolerant. The natural hybrids between these species have adapted to growth under ethanol and temperature stress through the inheritance of competitive traits from one or another parental species (124). These hybrids have potential value under conditions of fermentation of white wines at low temperatures (10 to 15°C) that minimize the loss of aromatic volatile compounds. Hybrids of *S. cerevisiae* × *S. kudriavzevii* produce more esters, fusel (>2 carbon) alcohols (112, 115, 121), and thiols derived from nonaromatic precursors present in grapes than *S. cerevisiae* does (125, 126). The abundance of these hybrids could reflect an adaptive advantage, but it is also possible that stressful conditions trigger hybridization events (52).

Improvement of wine yeast

Although many benefits to wine production have been gained through the selection of strains with suitable properties, efforts can still be deployed to improve wine yeasts for various traits, e.g., improvement of stress tolerance, fermentative performance, aroma properties, or low ethanol production. Non- genetically modified

organism strategies based on rational genetic strategies to exploit existing natural diversity have proven successful in the past decade to develop strains with improved traits. These strategies build on the identification of desired traits in the yeast genome by quantitative trait locus analyses. Quantitative trait loci controlling the formation of acetic acid, aroma compounds, SO_2 production, nitrogen utilization, or ethanol resistance have been identified (127). Once quantitative trait loci have been identified, the desired alleles can be transferred into a new strain by breeding using marker-assisted allele transfer. In addition, several strains with improved phenotypes have been developed using adaptive laboratory evolution approaches, which involve the propagation of a microbial population under defined selective conditions for prolonged periods of time and the selection of better-fitted mutants. These strategies have allowed the development of wine yeast strains overproducing esters, which are major determinants of the fruity character of wines (128) and strains with reduced ethanol yield (129, 130). The construction of interspecific hybrids to generate new combinations of genes conferring new properties has also been performed (131, 132).

Baker's Yeasts

S. cerevisiae is also known as baker's yeast. Bread-making scenes have been discovered on reliefs in several tombs dating from ancient Egypt, and the analysis of pottery containing remains of cereals and bread has allowed the investigation of ancient Egyptian baking methods (11). However, the origin and expansion of the process of leavened bread is poorly documented, and it is unclear whether yeasts came from cereals or from the process of fermenting beer (133). In countries that produce beer, ale yeast was used in bread production, and a portion of the yeasted dough was recycled by blending it with fresh water and flour for formation of the next dough. The production of baker's yeast exclusively for bread dough production has been dated from the late 19th century. Initially, this was done by production of distiller's yeast on mashed grains, and later with the less expensive source of assimilable sugar, molasses (134).

Most of the bread consumed today is leavened with the commercial baker's yeast, *S. cerevisiae*. However, the consumer demand for sourdough bread is increasing, and some bakers use sourdough containing natural lactic bacteria and yeasts as an alternative to commercial yeasts (see reference 135 for a review). The diversity of yeast strains is high in French natural sourdoughs, with *Kazachstania* species dominating and *S. cerevisiae* being an uncommon species (136). This suggests that *S. cerevisiae* does not compete well with other sourdough and yeast species.

Baker's yeast provides the gas required to leaven the dough through the alcoholic fermentation of sugars, in particular maltose, but yeast also influences the bread texture and taste. In contrast to beer and wine, bread dough is a solid fermentation occurring in a reduced level of free water, and the cellular physiology of the yeasts is likely different than in liquid fermentation. The study of the yeast transcriptome throughout the bread dough fermentation process has revealed three phases during which protein- and RNA-coding genes are differentially expressed (137). At the onset of fermentation, expression of glucose-regulated genes changes dramatically, and the osmotic stress response is activated. The middle fermentation phase is characterized by the induction of genes involved in amino acid metabolism. Finally, in the last phase, cells suffer from nutrient depletion and activate pathways associated with starvation and stress responses. Altogether, the results have shown that cells embedded in a solid matrix such as bread dough suffer severe osmotic stress.

Baker's yeasts have lower genetic diversity and a higher number of chromosomes than other *S. cerevisiae* lineages used for wine, sake, and beer fermentations (138). But unlike brewing yeasts, which are allotetraploid, baking yeasts are autotetraploid, displaying four complete chromosome sets arising from the same species. Usually transitory, the autotetraploid status may have occurred very recently and/or may have been maintained by human selection because of metabolic benefits for fermentation (75). The genome analysis of three bread strains has shown the mosaic nature of their genomes, with some regions very similar to those present in wine strains; some others being more related to sake, oak, or palm wine yeast genomes; and some that were unique (70, 138). This suggested that baker's yeasts have arisen from crosses between wild lineages that had appropriate fermentation properties (138).

Improvement of Baker's Yeast

The evolution of bakery technologies has highlighted a need to improve yeasts for their tolerance to various stresses, including high sugar concentration (in sweet dough), drying, and freezing (in relation to the increase of production of dry and frozen dough, respectively), as well as for their performance in the fermentation of dough sugars and for their growth rate and biomass yield relevant to the production of the baker's yeast itself (138, 139). Different genetic engineering strategies have

been applied, in particular manipulation of the small osmolyte pathways, including those of glycerol, proline, and trehalose (137, 138). Experimental evolution has been used to enhance the freeze-stress tolerance of industrial baker's yeast cells by generating adaptations to growth at low temperature (140). Two new baker's yeast strains obtained by hybridization between commercial baker's yeasts have shown improved fermentation abilities and cryoresistance (141).

Brewing Yeasts

Beer is the most widely consumed alcoholic beverage in the world, with a total consumption volume of 176.4 billion liters in 2014, corresponding to total revenues of $464.4 billion (142).

Water, hops, malt, and yeasts are the four main ingredients for manufacturing beers (143). Hops give beer its typical bitterness and aroma. Malt is obtained by inducing the germination of grains, mainly barley grains, for a period of time necessary for enzymes to degrade complex molecules, such as starch and proteins, into forms that can be assimilated by yeasts. To stop the process, water is removed by fresh air or heating, to obtain green to pale or dark dried malts, respectively. This manipulation of the process impacts the beer's characteristics, in particular the color of pale to dark beers. Barley or malt is roasted to make the typical dark stout beers.

Generally, the yeasts used for beer production belong to the *Saccharomyces* genus. Depending on their physiological characteristics, and on the quality of other ingredients, two main types of beers are produced, i.e., ale or lager-style beers. Ales are brewed at a relatively high temperature (15 to 26°C) with top-fermenting *S. cerevisiae* strains, which rise to the surface of the fermenting wort after cell aggregation during the flocculation process. Lager beers are brewed at lower temperature (5 to 14°C) with bottom-fermenting yeasts that have a tendency to sediment after flocculation and sink to the bottom of the fermenters. Ales have been brewed since ancient times, possibly as early as 6000 BCE (144). In contrast, lager beer production was limited to cool seasons but expanded with the invention of the refrigerating machine by Linde in 1871, allowing the necessary cool temperatures for bottom fermentations to be achieved during all seasons. Another major advance was the development of pure cultures of yeasts. Previously, brewmasters selected best batches and yeasts were reutilized from batch to batch. At the time, problems in the end product such as bitter taste and bad odor frequently occurred. In 1883 E. C. Hansen, who worked at the Carlsberg Laboratory in Copenhagen, developed an effective technique to obtain pure cultures of yeasts. This technique was essential to standardize beer production by selection of desirable yeasts and to the standardization of yeast cultures all over the world (145). Today lager beer production dominates the market.

A fascinating and ongoing research story began when the lager yeast, whose current name is *S. pastorianus* (syn. *Saccharomyces carlsbergensis*) (146), was discovered to be an interspecific hybrid showing high similarity in nucleotide sequence to both *S. cerevisiae* and *Saccharomyces bayanus* (147). A microsatellite analysis suggested that the ancestral *S. cerevisiae* parent of the hybrid came from the ale-brewing process, because ale and lager beer yeasts clustered together and were distinct from bread and wine yeasts (69). This result was confirmed by sequence data (148, 149). The second parental species was suggested to be closely related to *S. bayanus* (23, 150, 151) until the discovery of a better candidate, the cryophilic species *S. eubayanus*, first isolated in Patagonia (58) and recently also in China (63). *S. pastorianus* strains have greater mitochondrial DNA sequence similarity to the Tibetan strain BaiFY1 than to the type strain CBS12357 isolated in Patagonia (152), in agreement with previous genomic data (63). These data suggest a common origin of the domesticated *S. pastorianus* strains with the wild Tibetan strains and support a scenario of continental migration of *S. eubayanus* from China to Europe along the Silk Road (153), rather than by overseas transport from Patagonia to Europe as originally suggested (58). It is thought that the hybridization event probably occurred quite recently, about 500 years ago (153). To elucidate the date and circumstances of the process, more isolates from more diverse origins in the world and particularly along the hypothesized migration routes are needed for population genomics studies.

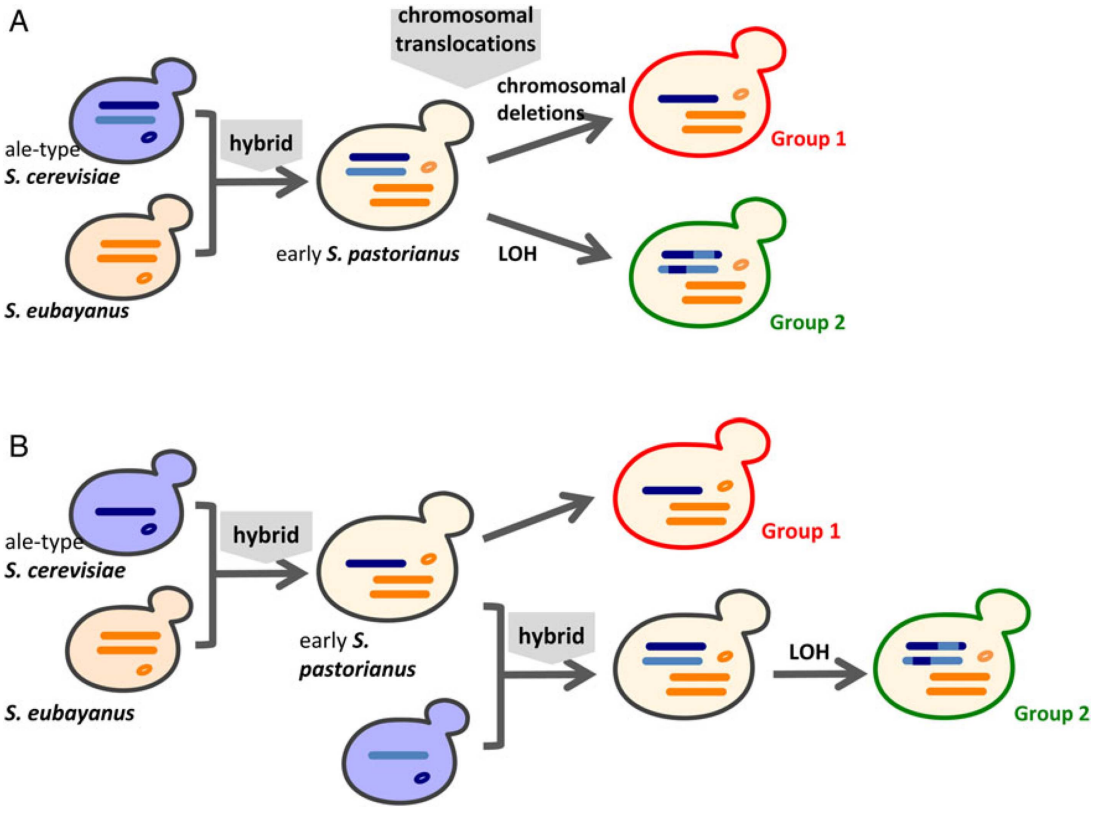
The genetic diversity in *S. pastorianus* populations is much lower than that in ale yeasts (154). Two groups were identified in *S. pastorianus*, called Saaz (or group I) and Frohberg (group II), after the locations in Bohemia and Germany in which original strains were derived (145). Each group has specific brewing abilities and produces different flavors. Saaz strains have a greater cold tolerance than Frohberg strains but relatively poor fermentation performance due to their inability to utilize maltotriose (155, 156). This deficiency may have contributed to favor the use of Frohberg strains in modern brewing companies (155). Not surprisingly, genetic differences were found between the two groups, including

genome rearrangements, copy number variations, ploidy differences, mitochondrial and nuclear DNA sequence polymorphisms (148, 152), and transposon distribution (157). More precisely, the genomic content inherited from hybridizing ancestral species appeared different in the two *S. pastorianus* groups. Saaz strains are triploid, containing less than one haploid genome of *S. cerevisiae* and more than a diploid *S. eubayanus* genome (156), while Frohberg strains are tetraploid, containing one full diploid *S. cerevisiae* genome and one full diploid *S. eubayanus* genome (149, 152, 156, 158).

To date, it is not known whether the Saaz and Frohberg lineages arose from single or separate hybridization events. The differences between the two groups suggest separate hybridization events, in particular the high level of variation in telomeric sequences and LTR-transposon copy number and location (157), the difference in ploidy (148) and in single-nucleotide variants (152), the different rates of evolution in the two subgenomes since hybridization (159), and the pattern of presence/absence variation of the lager-specific genes that are located in the subtelomeric regions (160). Baker et al. (159) suggested that hybridizations may have involved nearly identical *S. eubayanus* parents but more diverse *S. cerevisiae* parental strains. Two classes of *S. cerevisiae* parental strains were identified as a Foster-O-like ale yeast and a stout yeast, respectively, for the groups I (Saaz) and II (Frohberg) hybrid lineages (160). The main argument favoring a single hybridization event at the origin of the two groups is that the two lineages share at least three chromosomal translocations between *S. cerevisiae* and *S. eubayanus* genomes, with identical breakpoints at the nucleotide level (152, 156).

Okuno et al. (152) proposed two scenarios with at least one common hybridization event to reconcile all the data (Fig. 2). Scenario A proposes hybridization

FIGURE 2 Two hypotheses regarding origins of *S. pastorianus* based on shared chromosomal translocations and differences in ploidy between groups 1 and 2. **(A)** Hybridization between diploid Sc and Se types occurred before chromosomal translocations, whereas chromosomal deletions occurred only in ancestral group 1 strains. **(B)** After hybridization between haploid Sc and diploid Se types and chromosomal translocations, ancestral group 2 strains gained another Sc type (i.e., a second hybridization event occurred). Chromosomal deletion or loss of heterozygosity (LOH) explains single nucleotide polymorphisms observed when comparing reference genomes.



between diploid parental strains as suggested in references 144 and 156 and chromosomal deletion to explain the massive reduction of the *S. cerevisiae* genome in the Saaz type. Scenario B involves a hybridization event between haploid *S. cerevisiae* and diploid *S. eubayanus* leading to a triploid hybrid ancestral Saaz group. To explain the tetraploid Frohberg type, an additional hybridization between the F_1 hybrid and another *S. cerevisiae* strain was suggested, as proposed by Baker et al. (159) and Monerawela et al. (160). The instability in the nascent allopolyploid hybrid genome may have contributed to rapid evolution of the yeast to tolerate the conditions prevalent in the brewing environment (153), particularly the fermentation temperatures, and to the selection of new biochemical activities (85). As a result, amplification and deletion of genes and/or pseudogenization would have occurred at rearrangement breakpoints on different chromosomes in lager hybrids. An increase in the number of copies of genes involved in maltose utilization has been observed on chromosome II. Several breakpoints have led to changes in flocculation genes, particularly the elimination of the *S. cerevisiae* copy of the *FLO5* gene, but the apparent retention of the *S. eubayanus* version of the gene known as Lg-*FLO1* (148, 161). The Frohberg genome was shown to carry inactive copies of both *S. cerevisiae* and *S. eubayanus SUL1* sulfate transporters while retaining functional versions of the two *SUL2* genes, which are more efficient under fermentation conditions (58), sulfite formation being important in lager brewing because it is an antioxidant and flavor stabilizer (139).

The impact of domestication has also been investigated in terms of selective pressure that may have occurred on genes, and particularly on metabolic genes that are important for brewing (159). A global increase in the fixation of nonsynonymous substitutions in the hybrid *S. eubayanus* subgenome of *S. pastorianus* in comparison to the genome of a wild strain of the parental *S. eubayanus* species was observed and was interpreted as a relaxation of purifying selection during domestication (159). Some genes showed footprints of positive selection, with particularly high rates of protein sequence evolution in both Saaz and Frohberg lineages. However, different nonsynonymous changes have been acquired in the two lineages, indicating convergent adaptations to the same environment (159). Examples include genes encoding global transcription factors or factors required for the expression of enzymes specialized for ethanol oxidation rather than fermentation, as well as genes involved in the regulation of other genes related to the utilization of ethanol (159).

Novel lager brewing yeasts

The recent discovery of *S. eubayanus* opened up the possibility of using this species alone as a lager brewing strain (153, 155) and to create novel artificial hybrids (162, 163). The best of these novel hybrids have shown growth at a broader range of temperatures, a high fermentation capacity in laboratory-scale lager beer fermentations, and desirable aromatic profiles that were significantly different from the profiles produced by the currently exploited lager yeasts. Hybrids previously obtained by breeding lager yeasts with *S. cerevisiae* ale strains have shown improved stress resistance, as seen in the ale parent, including improved survival at the end of fermentation (164).

A. oryzae in Japanese fermentations

A. oryzae has been used for hundreds of years in Japanese fermentations to produce traditional alcoholic drinks, sauces, and condiments. It is one of the most potent secretory producers of proteins among filamentous fungi, and its use is now extended to the commercial production of enzymes in modern biotechnology (165, 166). In Japan, it is used to prepare koji, a starter for secondary fermentations, using solid-state cultivation, a process thought to have originated in China 3,000 to 2,000 years ago (167). The commercialization of koji dates back to the 13th to 15th century, in Heian and Muromachi periods (167). The long history of extensive use in the food industries placed *A. oryzae* on the FDA list of Generally Recognized as Safe (GRAS) organisms in the United States (168). For making koji, *A. oryzae* spores are spread onto steamed rice. After ~2 days of growth, the resulting *A. oryzae*-rice mixture (koji) is mixed with soybeans or additional steamed rice and water and fermented mainly by yeasts to produce miso, shoyu, and sake (169, 170). The fungus has an important role in the degradation of proteins and starch contained in the raw material for fermentation and contributes to the color, flavor, and aroma of the fermented foods. Starch is converted into simple sugars by a biochemical process called saccharification.

A. oryzae was first isolated from koji in 1876 by H. Ahlburg. It appeared morphologically almost indistinguishable from *A. flavus* (171) and nearly identical when analyzed by DNA/DNA hybridization (172). Unlike *A. flavus*—which produces the toxic and carcinogenic aflatoxins, a serious problem worldwide in agricultural commodities such as maize, peanuts, tree nuts, and cotton seeds (173)—*A. oryzae* is a nontoxigenic species (174). However, using multilocus genotyping and sequencing analysis, *A. oryzae* strains were shown

to form a monophyletic clade nested within *A. flavus*, closely related to atoxigenic *A. flavus* strains. This finding suggests that *A. oryzae* may have evolved by domestication from a single lineage of *A. flavus* selected for its particular properties (173, 175). This hypothesis is in agreement with previous studies based on SNPs and evolutionary analyses (168, 174). The close evolutionary relationship is reflected in their genomes, which are nearly identical in size and gene content (176, 177). These two genomes are larger than those of *A. fumigatus* and *A. nidulans* due to the expansion of metabolic gene families that may have been acquired by HGT (13).

Genes in expanded families include those encoding secretory hydrolases, transporters, and primary and secondary metabolism. These latter families represent the most expanded gene classes, and they are involved in the degradation of the vast amount of compounds to which the fungi are exposed during fermentation for *A. oryzae*, or in the fields for *A. flavus*, and in the excretion of toxic compounds (13, 167). The fact that these expanded gene families are present in both *A. oryzae* and *A. flavus* indicates that they expanded before domestication (167). *A. flavus* and *A. oryzae* also share the aflatoxin biosynthetic gene cluster, but in *A. oryzae* the gene cluster sequences contain deletions, frameshift mutations, and base pair substitutions (166, 178). No sequence derived from aflatoxin biosynthetic genes was found in the large-scale collection of expressed sequence tags obtained from cDNA libraries from nine biologically or industrially important cultures of *A. oryzae* (179). This indicated the lack of transcription of the *aflR* gene, which encodes a transcription factor positively regulating expression of aflatoxin biosynthetic genes in *A. flavus*. A comparison of the transcriptomes and proteomes of *A. oryzae* and *A. flavus* when grown on rice showed a global downregulation of biosynthetic genes for secondary metabolites in *A. oryzae*, and particularly of the aflatoxin pathway (166), explaining its inability to produce the toxin. Interestingly, aflatoxin is toxic to *S. cerevisiae* (180), suggesting that the toxicity loss of *A. oryzae* might have been driven by its cooperation with yeast during fermentation for making sake and/or by selection by humans of nontoxic strains (166). Putative adaptations to a starch-rich diet were also found in *A. oryzae*. Several genes involved in carbohydrate primary metabolism, such as those encoding for α-amylase or maltase glucoamylase involved in the catabolism of starch, were significantly upregulated in *A. oryzae*.

Domestication is an artificial selection process that can leave footprints of selective sweeps in genomes as a reduction in polymorphisms in genomic regions encompassing the selected variants. Selective sweeps have been detected in *A. oryzae*, mainly on genes and pathways involved in primary and secondary metabolism. The most relevant genes within selective sweeps are involved in protein and peptide degradation and in carbohydrate metabolism, including a glutaminase gene that catalyzes the hydrolysis of carbon-nitrogen bonds of l-glutamine to produce glutamic acid, a widely used enhancer of food flavor found in considerable quantities in sake (166). The transition to domestication has often been associated with a relaxation of selective constraints and population bottlenecks (46) leaving footprints in the genomes, such as an excess of nonsynonymous substitutions (often deleterious). Surprisingly, this has not been observed in *A. oryzae*; in contrast, the ratio of nonsynonymous to synonymous substitutions and the percentage of estimated deleterious substitutions are lower in *A. oryzae* than in *A. flavus*, and this observed lack of selective relaxation has been discussed with regard to population genetics and the ecology of domestication events (181).

In conclusion, *A. oryzae* is probably the result of a single domestication event from an atoxigenic lineage of *A. flavus*, which sake makers may have selected as a safe mutant, allowing yeast growth in fermented products (57). In *A. oryzae*, the genomic mechanisms of adaptation during domestication appear to have involved mainly changes in secondary metabolism.

TOWARD THE DOMESTICATION OF THE BLACK TRUFFLE (*TUBER MELANOSPORUM* VITTAD.): A GLIMPSE INTO A 2-CENTURY-LONG SAGA

True truffles are hypogeous fungi belonging to the genus *Tuber* (Ascomycota, Tuberaceae), which contains the most famous and prized edible mushrooms (182). The Piemont white truffle (*Tuber magnatum* Picco) and the Périgord black truffle (*T. melanosporum* Vittad.) are the most valuable, the black truffle being the most anciently cultivated in the Western part of the Mediterranean basin (183). Unfortunately, the production of *T. melanosporum* drastically declined during the 20th century, and there are still difficulties in increasing production, despite a sustained planting effort since the 1970s.

The mutualistic biology of truffles requires their association with a plant for accomplishing their sexual reproduction, and in particular for producing the edible fruiting body that results from a mating event (184, 185). Vegetative life depends on the edification of short-

lived structures called ectomycorrhizae that connect mycelia to shrubs or trees from various families (e.g., *Fagaceae*, *Salicaceae*, and *Betulaceae*) (186). In spontaneous truffle grounds, the Périgord black truffle mainly associates with oaks but also with Mediterranean shrubs in the *Cistaceae* (187), while it naturally establishes and fruits in early forest stages during secondary succession, mainly in the opened vegetation called "garrigues" (188).

The worldwide notoriety of *T. melanosporum* has fueled the development of unprecedented planting efforts and the introduction of the species in America, Australia, New Zealand, and other countries. As a consequence, truffle growers stimulated the development of highly inventive, but often empirical, techniques to induce truffle production in a wide range of ecological contexts. However, the obligate association between *Tuber* mycelia and trees of few compatible species, combined with the impossibility of producing fruiting bodies *ex situ*, has made the management of truffle growth complex. When remaining aloof, truffle growers act as blind gardeners who indirectly work on truffles through arboriculture practices (e.g., pruning) and agriculture (e.g., tillage or sowing) management methods, over years, with particularly uncertain outcomes.

Here we present a three-step history of the attempts to cultivate truffles, with a focus on the Périgord black truffle. We first deliver a comprehensive assessment of the 2-century-long empirical approach developed in spontaneous truffle grounds since the beginning of the 19th century, with special attention paid to the diversity of practices. Furthermore, we present the tremendous contribution provided by the agronomic approach developed in the 1970s, based on the use of inoculated plants and irrigation practices. We finally discuss the recent development of a global truffle-growing method that includes the input of metagenomics tools and ecological modeling.

The Early Attempts: Empiricalism and Observation in Spontaneous Truffle Grounds

It is likely that the truffle was first gathered as a wild product, although direct reports are lacking. An understanding of its exact ecology was probably delayed by the inconspicuous nature of its vegetative stage. Truffles were harvested and much appreciated by the Romans, although the identity of the species collected 2 millennia ago remains unclear. Pliny the Elder, for example, stated in his *Naturalis Historia* (*Natural History*) that the most valued truffles came from Africa (making a possible reference to the distantly related genus *Terfezia*) and that truffles were formed "when there have been showers in autumn, and frequent thunder-storms,… thunder contributing more particularly to their development; in some places the formation of them is attributed to waterstorms" (189). Yet empirical methods, as in many uses involving microbes, took into account the subterranean link to trees well before any idea of mycelium or mycorrhizae was reached (Table 1). Q7

The emergence of the concept of mycorrhizae itself is tightly linked with truffles. After the Prussian minister of agriculture commissioned a mycologist and botanist, A. B. Frank, to study the origin of truffles to promote their cultivation, Frank in 1885 described the morphology of the relationship of the fungus with tree roots and coined the word *mycorrhiza* (190, 191). He also pointed out the need for "an underlying limestone," i.e., the clear calcicolous ecology of the fungus. Before and after this discovery, many empirical methods already used inoculations on trees (Table 1), but without an established method.

Agronomy Considerations: Controlled Inoculation and Irrigation in Planted Truffle Grounds

The first prerequisite to optimize the chance of successfully producing truffles is the production of seedlings with a mycorrhizal relationship with the right fungus. The first successful attempt to synthesize truffle ectomycorrhizae was realized by Bruno Fassi, Mario Palenzona, and Anna Fontana in Italy (192–196). In 1970–1971, a collaboration started between these Italian scientists and two French scientists, Jean Grente and Gérard Chevalier (197). In 1972, Grente and Chevalier extended this inoculation technique on a large scale and contributed to the creation of the AGRITRUFFE society, exploiting a know-how license INRA/ANVAR. In 1973, AGRITRUFFE put on the market about 10,000 seedlings mycorrhizally colonized with *T. melanosporum* (198). Nowadays in France, 300,000 seedlings (oaks, hazel, hornbeams, pines, etc.) mycorrhizal with *T. melanosporum* are produced in 18 nurseries, and their quality is controlled by INRA or the CTIFL (Centre Technique des Fruits et Légumes) (199). This large-scale inoculation technique was developed first in France and then spread to Italy and Spain and is now used worldwide. At the same time, many truffle growers still use home-made inoculations.

The development of efficient techniques to control *T. melanosporum* inoculation is not the only agronomic contribution of the past decades. Water management during the first 3 or 4 years following planting is crucial

TABLE 1 Two centuries of domestication of the Périgord black truffles and production in France

1564: Alphonso Ciccarello, following the ideas of the Arab philosopher Almadel, suggests inoculating trees with crushed ascospores.
1726: Richard Bradley suggests the same method, but for *Tuber aestivum* (Wulfen) Spreng.
Before 1750: Georges Louis Leclerc, Comte de Buffon, inoculates the roots of adult hornbeams in Montbard (Côtes d'Or) with crushed ascocarps of *Tuber melanosporum* and gets truffles 2 years later.
About 1750: Jean-Pierre-François Ripert de Monclar sows acorns in Saint-Saturnin-les-Apt (Vaucluse) and gets black truffles 10 years later.
1790: Pierre II Mauléon sows acorns (*Quercus pubescens* Willd) in Grand-Ponçay (Vienne) and harvests truffles 10 years later.
Between 1808 and 1815: Joseph Talon has the same idea and sows acorns in his fields at Saint-Saturnin-les-Apt (Vaucluse).
Around 1830: 100 to 200 hectares of artificial truffle orchards in the Vienne department and between 300 and 400 hectares in the Drôme and Vaucluse departments are created.
1847: Auguste Rousseau sows acorns (mainly *Quercus ilex*) in a 7-hectare (ha) area near Carpentras (Vaucluse) in previously cultivated soil and irrigates his orchard. He obtained large harvests reaching 70 kg/ha in the best years.
From 1860: 380,000 ha of eroded and overgrazed lands are reforested in southeast France. The Périgord black truffle naturally occurred in these plantations. The Mont Ventoux and the Lubéron mountain became the largest "truffle orchard" in the world.
1866: Bedel creates the "truffle sylviculture," a sylviculture on poor soils with low tree density allowing small-scale production of Périgord black truffles, probably less than 0.5 kg/ha.
From 1863: Beginning of the *Phylloxera* crisis, which destroyed French vineyards, and then in the late 19th century, disease of the silkworm. Numerous vineyards or fields of mulberry trees were replaced by managed truffle orchards, mainly in southeast France. Acorns were also replaced by seedlings cropped under productive trees or produced in nurseries and more or less having mycorrhizal associations with *T. melanosporum* ascospores, using crushed ascocarps but without soil disinfection. In the Drôme and Vaucluse departments, deep soils are used, allowing production reaching several kilograms per hectare in good years.
1885: Discovery and description of the ectomycorrhizae by Albert Bernard Frank.
1894: Dangeard describes ectomycorhizae of *T. melanosporum* on *Quercus pubescens*.
1900s: Peak of French truffle production, between 500 and 1,000 tons per year.
1914–1918: First World War. National production decreases.
1920–1939: Production is stabilized around 300 tons.
1939–1945: Second World War. Another decrease in production.
1945–1946: Only 66 tons produced.
1947–1960: Stabilization at around 200 tons per year.
1960–1980: Gradual decrease of production, probably resulting from rural exodus.
1980 to present: Stabilization of production around 50 to 60 tons per year.

for the seedlings and the mycorrhizae (200, 201). To favor the formation of new roots and good development of the *T. melanosporum* mycorrhizae, it is necessary to irrigate the seedlings during summer and to eliminate weed competition by manual hoeing, soil tilling, or mulching (200, 201).

There is increasing evidence that inoculation of the trees with ascospores throughout the truffle orchard life can increase the production of fruiting bodies. During the first years, such inoculations may allow the renewal of *T. melanosporum* mycorrhizae and decrease the chance of dominance of other ectomycorrhizal (ECM) fungi on roots of the host. Three to 5 years after planting, inoculation with ascospores may enhance sexual reproduction, which may be limited by exclusion of one mating type by the other at the mycorrhizal level (202–204) (see below). The current view is that truffle spores added by dispersal of fruiting body pieces act as male contributors to initiate reproduction and thus fruiting body formation: the so-called truffle-trapping designs, mixing spores with soil, often enhance the production of fruiting bodies (205), although the mechanisms, either genetic or linked to soil disturbance, remain unclear.

The Time for a Global Approach: from Metagenomics to Ecological Modeling

Since the early 1990s, the development of metagenomics tools and the *in situ* use of isotope (e.g., ^{13}C and ^{15}N) labeling experiments has opened up new research perspectives on fungal biology and ecology. The belowground description of truffle grounds based on the barcoding of ECM root tips provided valuable insights into the understanding of the fungal compartmentation in truffle grounds. During the past decade, the sequencing of root apices has been widely applied to truffle plantations and spontaneous systems to provide comprehensive views of below-ground distribution of truffles in *Tuber aestivum* (206), *Tuber borchii* (207), *Tuber macrosporum* (208), and *T. magnatum* (209) grounds. Molecular tools have been developed that are aimed at determining the distribution of *T. melanosporum* in soils and the abundance of black truffle mycorrhizae within ECM communities in planted orchards (e.g., 5, 210) and

in its typical habitat (188), where *T. melanosporum* is part of diversified ECM communities, with clear vegetative affinities for oaks as compared to companion shrubs (*Arbutus unedo*, *Cistus* spp.).

In situ labeling experiments have clarified the nutrition of *T. melanosporum* and answered a controversial question debated in handbooks until the 2010s regarding truffle cultivation. The natural abundance of ^{13}C and ^{15}N in tissues of the host and the fungus (211) reflects that *T. melanosporum* behaves like a true ECM fungus that does not exhibit a saprotrophic strategy during the development of its ascocarps, as speculated by some authors (see reference 183 for a review). Subsequently, a labeling experiment of tree photosynthates unambiguously demonstrated sugar transfers from host leaves to fine roots that act as a conduit and transfer carbon to *T. melanosporum* mycorrhizas and finally to the ascocarp, which accumulates host carbon 200 days after the labeling (212). A soil labeling experiment confirmed that *T. melanosporum* did not exhibit saprotrophic capacity for carbon and that carbon used by ascocarps is mainly provided by the host (213). These discoveries have paved the way for the development of pruning calendars that take into account the periods of carbon storage in the wood of the host (winter) and avoid any harmful management practice in periods of carbon demand by the fungus (summer).

The sequencing of the 125-Mb-long genome of *T. melanosporum* (214) was another essential step on the road to truffle domestication. One of the most striking features of this sequencing was the analysis of the region that governs the fungal sexual cycle, showing that *T. melanosporum* is a heterothallic species with two alternative genes at a single *MAT* locus. The provision of these genes revealed the unique population structure of *T. melanosporum* in planted orchards (202). Indeed, the black truffle shows markedly segregated patterns of spatial distribution of individuals based on their mating type, with long-lived maternal individuals vegetatively established in truffle grounds and paternal individuals remaining cryptic before fecundation (203), leading to the idea that germinating spores may be paternal individuals and not mycelia (185, 215, 216). This finding suggests that competition occurs between mycelia of different mating types at the establishment stage and highlights possible mechanisms explaining the absence of fruit body production in large parts of the orchards. Further research is needed to identify paternal reproductive structures in *Tuber* spp. and to detect the ephemeral dikaryotic structure derived from parental mating.

Toward a Domestication of Truffles?

For a long time, the domestication of truffles has been a critical issue for the economy of rural Mediterranean societies (217), and complete control of the life cycle of the fungus is not achieved yet. It is now a spatial planning challenge for stakeholders in many regions of the world who aim to implement a truffle-based development of landscapes (218). Current harvests of Périgord black truffle in its natural Mediterranean habitat are well below production levels in the early 20th century, perhaps as a result of marked changes in landscapes and rural desertification. Exceptional production levels obtained in plantations outside Europe, mainly in Australia, are promising for those who invest, but these countries are too remote to supply the world demand because of the impossibility of controlling all the factors involved during the truffle biological cycle. However, the unprecedented rhythm of scientific knowledge accumulation and the extremely efficient toolbox developed by laboratories give great hope that the synergy between truffle growers and researchers will lead to increasingly understood and controlled practices in the coming years.

CONCLUSION

Fungi have been used safely in food production, even unconsciously, for thousands of years since early agricultural practices, and there is also a long and notable tradition of wild edible fungi in different cultures all over the world. However, only a fraction of edible mushrooms are eaten, and a minute portion of fungi are used in food processing. Fungal biodiversity is evidently a resource pool for food but also for more value-added uses. Fungi are gaining popularity in the context of a global need for new sources of food, and some species are already exploited to supply new-generation bio-based products in proteins or dietary fibers. Moreover, fungal enzymes have huge potential to produce higher-value products as food and feed ingredients from biological raw materials and agro-industrial waste. In addition, they are valuable as good models for studying the genomic processes of adaptation and domestication, with their often small genomes and experimental tractability (18).

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Author Queries

Q1: Since the items listed (coagulant, milk, starter LAB, nonstarter LAB) add up to four, not six primary sources, we have changed "i.e." ("that is") to "e.g." ("for example"). Are there two other sources that can be named?
Q2: Is “long terminal repeat” correct for LTR?
Q3: "expensive" correct rather than "expansive"?
Q4: "billion" correct for "bn"?
Q5: Please check sense of sentence beginning "The instability..." We changed "in the selection" to "to the selection," meaning that the instability contributed to (a) rapid evolution of the yeast and (b) selection of new biochemical activities. As meant?
Q6: In the sentence beginning "The mutualistic biology...," OK to revise "imposes" (their association) to "requires"?
Q7: This publication does not permit Boxes. OK to call it a table?
Q8: Ref. 127: Published yet? Please add inclusive page numbers for the chapter and also the publisher’s location (city, country).
Q9: Ref 189: Please provide publication information for the edition you are citing from.