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Yeast diversity of sourdoughs and associated metabolic properties and functionalities

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ABSTRACT

Together with acidifying lactic acid bacteria, yeasts play a key role in the production process of sourdough, where they are either naturally present or added as a starter culture. Worldwide, a diversity of yeast species is encountered, with *Saccharomyces cerevisiae*, *Candida humilis*, *Kazachstania exigua*, *Pichia kudriavzevii*, *Wickerhamomyces anomalus*, and *Torulaspora delbrueckii* among the most common ones. Sourdough-adapted yeasts are able to withstand the stress conditions encountered during their growth, including nutrient starvation as well as the effects of acidic, oxidative, thermal, and osmotic stresses. From a technological point of view, their metabolism primarily contributes to the leavening and flavour of sourdough products. Besides ethanol and carbon dioxide, yeasts can produce metabolites that specifically affect flavour, such as organic acids, diacetyl, higher alcohols from branched-chain amino acids, and esters derived thereof. Additionally, several yeast strains possess functional properties that can potentially lead to nutritional and safety advantages. These properties encompass the production of vitamins, an improvement of the bioavailability of phenolic compounds, the dephosphorylation of phytic acid, the presence of probiotic potential, and the inhibition of fungi and their mycotoxin production. Strains of diverse species are new candidate functional starter cultures, offering opportunities beyond the conventional use of baker's yeast.

1. Introduction

Sourdoughs are the result of the spontaneous or starter culture-initiated fermentation of mixtures of flour (fractions) and water by lactic acid bacteria (LAB) and yeasts (Corsetti and Settanni, 2007; De Vuyst and Neysens, 2005; De Vuyst et al., 2009, 2014; Gobbetti et al., 2014; Minervini et al., 2014). The prokaryotic LAB are usually present in at least one log count higher than the eukaryotic yeasts (Gobbetti, 1998; Ottogalli et al., 1996). The major metabolic activities of the sourdough microbiota are acidification (LAB), flavor formation (LAB and yeasts), and leavening (yeasts and heterofermentative LAB species). Many reviews have dealt with LAB occurring and active in sourdoughs (Corsetti and Settanni, 2007; De Vuyst and Neysens, 2005; De Vuyst and Vancanneyt, 2007; De Vuyst et al., 2009, 2014; Ehrmann and Vogel, 2005; Gänzle and Gobbetti, 2013; Gänzle et al., 2007, 2008; Gobbetti et al., 2005, 2014; Huys et al., 2013). Although many papers have dealt with yeasts in sourdoughs too, few reviews have focused on their occurrence and activity in sourdoughs (Daniel et al., 2011; Gullo et al., 2003; Huys et al., 2013; Rossi, 1996). Therefore, the present review summarizes the major aspects of species diversity and technological functionality of sourdough yeasts.

2. Sourdough yeasts and their diversity

Yeasts are unicellular fungi with a typical vegetative growth by budding or fission. Sexual reproduction occurs without a fruiting body. In sourdough, they have to withstand a specific and stressful microbial environment, characterized by a low pH, low oxygen tension, and carbohydrates (mainly maltose) that have to be shared with the LAB communities (De Vuyst et al., 2014). Fermentative yeasts adapted to such ecosystems typically belong to the phylum Ascomycota, subphylum Saccharomycotina, class Saccharomycetes, order Saccharomycetales, family Saccharomycetaceae (Huys et al., 2013).

In general, sourdough yeasts are enumerated on and isolated from Wallerstein Laboratories (WL) nutrient agar medium, yeast extract-peptone-dextrose agar (YPDA) medium, Sabouraud dextrose agar, yeast extract-glucose (YG) agar, and yeast and malt extract agar, supplemented with chloramphenicol as antibacterial agent, after incubation at 25–30 °C for 2–3 days (Huys et al., 2013). The first sourdough-specific yeast species isolated from spontaneous sourdoughs (*in casu* a San Francisco sourdough) was *Saccharomyces exiguus* (Sugihara et al., 1971), reclassified later as *Candida milleri* and then as *Kazachstania exigua*, and most probably closely related to *Candida humilis* (De Vuyst et al., 2014; Huys et al., 2013). It forms a trophic relationship with the

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sourdough-specific LAB species *Lactobacillus sanfranciscensis* in that the yeast and LAB species are maltose-negative and maltose-positive, respectively (Gobbetti, 1998; Gobbetti et al., 1994a; Kline and Sugihara, 1971).

A review of the yeast composition of 287 sourdoughs published since 1971 reveals the following seven yeast species (irrespective of the way they were identified) that are most encountered in spontaneously developed stable sourdoughs (in decreasing order of abundance): *Saccharomyces cerevisiae*, *C. humilis* [synonym (syn.) *C. milleri*], *Wickerhamomyces anomalus* (syn. *Pichia anomala* and *Hansenula anomala*; anamorph *Candida pelliculosa*), *Torulaspora delbrueckii* (anamorph *Candida colliculosa*), *K. exigua* [syn. *S. exiguus*; anamorph *Candida* (*Torulopsis*) *holmii*], *Pichia kudriavzevii* (syn. *Issatchenkia orientalis*; anamorph *Candida krusei*), and *Candida glabrata* (updated version of Huys et al., 2013).

Some of these yeast species have to be considered as generalists, able to thrive in a wide range of microbial ecosystems, as is for instance the case for *W. anomalus* (Daniel et al., 2011); others are well adapted to the sourdough ecosystem and its environment, as is for instance the case for *C. humilis* and *K. exigua* (Gullo et al., 2003; Lhomme et al., 2016). Although more research is needed to verify whether the species diversity encountered in baking environments worldwide is sourdough-specific or not, the overall situation is known to differ somewhat from the one encountered during the backslopping of sourdoughs under experimental laboratory conditions, as documented hereafter.

2.1. Region-specific bakery sourdoughs

In Table 1 the yeast species diversity of different bakery sourdoughs analyzed worldwide is included. The six most encountered yeast species are *S. cerevisiae, C. humilis*, *T. delbrueckii, W. anomalus, K. exigua*, and *P. kudriavzevii*. A single sourdough often harbors only one or two yeast species at a given time. For instance, Italian bakery sourdoughs often harbor *C. humilis* and/or *S. cerevisiae* as dominating yeasts. Certain French organic sourdoughs harbor *Kazachstania bulderi* and *C. humilis*, whereby *S. cerevisiae* is rare (Lhomme et al., 2016). Belgian bakery sourdoughs maintained for several years harbor *S. cerevisiae* and *W. anomalus* as the prevailing yeast species (Vrancken et al., 2010).

The widespread occurrence of *S. cerevisiae* may be linked to the presence of baker's yeast in the bakery environment (Minervini et al., 2015; Vrancken et al., 2010). Baker's yeast is mainly used as a leavening agent and became an alternative for the use for years of sourdough, especially in the case of fast and industrial bread production. Different types of baker's yeast are on the market, with respect to not only their shelf-life [yeast cream (liquid), granulated yeast (small granules), compressed yeast (block yeast), and dried yeast (active dry, instant dry, and dry frozen yeast)] but also their osmotolerant properties (suitable for baked goods with high carbohydrate concentrations) and activity retention at low temperatures (use of frozen doughs) (Guerzoni et al., 2013).

2.2. Backslopped sourdoughs performed under aseptic laboratory conditions

Table 1 also describes the yeast species diversity of different laboratory sourdoughs produced under aseptic conditions. As flour is the sole non-sterile component, the resulting species diversity is somewhat different from that of the actual bakery ecosystems mentioned above. The five yeast species most encountered in spontaneous backslopped laboratory sourdoughs are *S. cerevisiae*, *C. glabrata*, *Kazachstania unispora* [syn. *Saccharomyces* (*Torulopsis*) *unisporus*], *W. anomalus*, and *C. humilis*. Thus, *S. cerevisiae* not only reflects the use of baker's yeast in bakeries as mentioned above, but also occurs this yeast species in laboratory sourdoughs obtained through spontaneous backslopping. Indeed, molecular data of a large strain diversity of *S. cerevisiae* in single sourdoughs do suggest an autochthonous flour origin of this yeast species in sourdough (Meroth et al., 2003a; Pulvirenti et al., 2001, 2004; Succi et al., 2003). However, *S. cerevisiae* and to a lesser extent *C. humilis* are less frequently found in laboratory sourdoughs than in bakery sourdoughs. Backslopped laboratory wheat sourdough fermentation processes are often dominated by *C. glabrata* and *W. anomalus* (Vrancken et al., 2010). Together with *S. cerevisiae*, *C. glabrata* dominates laboratory teff sourdoughs too (Moroni et al., 2011). *Candida glabrata, K. unispora*, *P. kudriavzevii*, and *S. cerevisiae* have been identified in different combinations in rye sourdoughs backslopped in the laboratory for 56 days (Bessmeltseva et al., 2014). Both *S. cerevisiae* and *W. anomalus* are maltose-positive yeasts and to a certain extent tolerant toward a low pH and high osmotic pressure. Growth of *C. glabrata* relies on glucose, which could imply a trophic relationship with maltose-positive LAB species. *Kazachstania unispora* as well as *Kazachstania barnettii* have been reported seldomly, but both species belong to the *Kazachstania* clade (Vrancken et al., 2010; Moroni et al., 2010, 2011; Bessmeltseva et al., 2014; Lhomme et al., 2016). In backslopped laboratory sourdoughs, *T. delbrueckii* seems to occur sporadically, whereas *P. kudriavzevii* seems to be present mostly in nonwheat sourdoughs. The latter yeast species is most competitive in the presence of oxygen and thus in less dense doughs (Vogelmann and Hertel, 2011). The former yeast species is poorly competitive when it grows slowly because of unfavorable environmental conditions (Pacheco et al., 2012). No yeasts have been found in laboratory sourdoughs made from oat and the pseudocereal buckwheat for reasons yet unknown, albeit that the antimicrobial activity of flavonoids and tannins may play a role (Moroni et al., 2010, 2011; Vogelmann et al., 2009). In the case of the application of a yeast starter culture, *S. cerevisiae* often prevails (Table 1).

2.3. Yeast intra-species heterogeneity

A large strain diversity of *S. cerevisiae* occurs in single sourdoughs (as it is the case in many other fermented food ecosystems harboring this yeast species), given its phenotypic and genotypic intra-species diversity and thus making applications strain-dependent (Landry et al., 2006). Also, heterogeneity occurs within the species *C. humilis* (Huys et al., 2013).

3. Yeast properties of technological relevance

Baker's yeast strains ferment the flour carbohydrates (sucrose, glucose, fructose, and maltose) present in the dough to carbon dioxide and ethanol through glycolysis [production of ATP and reducing power ($NADH + H^+$)] and further pyruvate reduction (recycling of NAD^+) (Fig. 1). Production of glycerol, albeit at the cost of ethanol, also allows regeneration of NAD^+ (Fig. 1). Carbon dioxide causes the leavening of the dough. Although ethanol has an impact on dough properties too, the greater part evaporates during baking. The concentration of fermentable carbohydrates, mainly maltose, depends on their release from starch by flour amylase activity and, hence, starch hydrolysis by flour enzymes and carbohydrate consumption by yeasts should be balanced to achieve successful sourdough fermentation (Guerzoni et al., 2013). However, in sourdough, often carbohydrate and nitrogen source (amino acids) limitation occurs.

Whereas *S. cerevisiae* prefers maltose as energy source, sourdough-specific yeasts such as *C. humilis* and *K. exigua* are maltose-negative and form a trophic relationship (nutritional mutualism) with the strictly heterofermentative *L. sanfranciscensis*, thereby hydrolyzing sucrose and glucofructans (elaborating glucose and fructose), consuming glucose and providing amino acids (Gobbetti, 1998; Gobbetti et al., 1994a,b). This mutualistic interaction is mainly based on the ability of *L. sanfranciscensis* to use maltose as the preferred energy source, thanks to its constitutive expression of a dedicated, energy-efficient maltose phosphorylase. This enzyme splits maltose, taken up through maltose:H^+ symport, into glucose-1-P (enabling its further metabolism without ATP expenditure) and glucose, the latter being elaborated into