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Influence of levan-producing acetic acid bacteria on buckwheat-sourdough breads

Tharalinee Ua-Arak, Frank Jakob*, Rudi F. Vogel

Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany

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ABSTRACT

Buckwheat sourdoughs supplemented with molasses as natural sucrose source were fermented with levan-producing *Gluconobacter (G.) albidus* TMW 2.1191 and *Kozakia (K.) baliensis* NBRC 16680. Cell growth, concomitant levan and low-molecular-weight metabolite production were monitored. Sourdough breads were prepared with different sourdoughs from both strains (24, 30 and 48 h fermentation, respectively) and analyzed with respect to bread volume, crumb hardness and sensory characteristics. During fermentation, levan, acetic and gluconic acids were increasingly produced, while spontaneously co-growing lactic acid bacteria additionally formed acetic and lactic acids. Sourdoughs from both strains obtained upon 24 h of fermentation significantly improved the bread sensory and quality, including higher specific volume as well as lower crumb hardness. Buckwheat doughs containing isolated levan, with similar molecular size and mass compared to *in situ* produced levan in the sourdough at 48 h, verified the positive effect of levan on bread quality. However, the positive effects of levan were masked to a certain extent by the impact from the natural acidification during fermentations. While levan-producing acetic acid bacteria are a promising alternative for the development of clean-label gluten-free breads without the need of additives, an appropriate balance between acidification and levan production (amount and structure) must be reached.

1. Introduction

Sourdoughs have long been used for the leavening of the doughs as well as for the acid and flavor formations in wheat and rye doughs (Arendt et al., 2007; Vogel et al., 1994). They are a mixture of flour and water fermented by lactic acid bacteria (LAB) and yeasts in a complex ecosystem, resulting in several biochemical processes such as acidification, proteolysis, synthesis of enzymes, antifungal compounds (Corsetti et al., 1998; Lavermicocca et al., 2000) as well as exopolysaccharides (EPS) (Korakli et al., 2001; Tieking et al., 2003). Such bacterial and enzymatic biochemical changes positively enhance the sensory, nutritional values and physical qualities of the baked products (Arendt et al., 2007; Gobbetti et al., 2014). Similar approaches have been used to improve the quality of gluten-free (GF) breads, which are generally made of GF flours and starches that lack the structural-forming gluten proteins, and therefore have poor sensorial and textural quality as well as low nutritional values (Moroni et al., 2009; Thompson, 2000). The presence of EPS produced naturally by the sourdough bacteria can exhibit great benefits to the development of GF bread structure (Tieking et al., 2003), which normally requires the addition of hydrocolloids such as xanthan gum or hydroxypropyl methylcellulose (HPMC) to mimic the structure forming impact of the missing gluten proteins (Anton and Artfield, 2008; Hager and Arendt, 2013; Lazaridou et al., 2007). The use of EPS-containing sourdough for the production of GF breads without additives would be ideal to meet the demands for high quality, additive-free, clean-label products by the celiac disease patients and other health-concerning consumers (Capriles and Arêas, 2014).

Levan is a homopolysaccharide (HoPS) of fructan type with β-(2 → 6) glycosidic linkages. Due to its uncommon properties such as low intrinsic viscosity from other polysaccharides (Arvidson et al., 2006), levan has been found useful in a wide range of applications including food (stabilizer, fat substitute), feed (prebiotics), cosmetics (whitener, moisturizer), and medicine (antioxidant, anti-inflammatory, anti-cancer activities) (Öner et al., 2016; Srikanth et al., 2015). In sourdough, levan is formed naturally *in situ* during a classical wheat/rye sourdough fermentation by

* Corresponding author.
E-mail address: frank.jakob@wzw.tum.de (F. Jakob).

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cereal-related LAB strains such as *Lactobacillus reuteri* (Galle et al., 2012; Schwab et al., 2008) or *L. sanfranciscensis* (Kaditzky et al., 2008; Korakli et al., 2001; Tieking et al., 2003), and also in the unconventional sourdoughs by acetic acid bacteria (AAB) such as *Kozakia baliensis* DSM 14400 and *Neoasaia chiangmaiensis* NBRC 101099 (Hermann et al., 2015). Mixed results on the effect of *in situ* EPS on the quality of GF sourdough breads were shown by different studies, for example, Galle et al. (2012) reported the reduction in the crumb hardness and prolongation of the shelf-life of sorghum sourdough breads in the presence of *in situ* fructan by *L. reuteri* Y2, while no positive influence was found by levan from *L. reuteri* LTH5448 on sorghum breads (Schwab et al., 2008) or from *L. sanfranciscensis* on wheat breads (Kaditzky et al., 2008). One influence of EPS on the bread quality was the structure of the EPS particles, in which the EPS with higher molar mass resulted in the most improved moisture content, baking loss and crumb firmness of the buckwheat-rice breads, as shown by the work of Rühmkorf et al. (2012b), or the higher increase of loaf volume and more reduction of crumb hardness in the wheat breads by Jakob et al. (2012).

Although LAB are the typical microorganisms in sourdough (De Vuyst and Neysens, 2005), few studies have also reported the identification of AAB strains in some wheat, rye or maize sourdoughs together with LAB (Chaves-Lopez et al., 2016; Minervini et al., 2012; Scheirlinck et al., 2008; Vogelmann et al., 2009). Since all the sourdough and sourdough bread studies have been focusing only on the LAB, it would be appealing to pay attention to the sourdough from AAB to broaden the possibility of GF bread development. AAB are gram-negative, obligate aerobes that are well-known for the incomplete oxidation of sugars and alcohols into organic acids (Deppenmeier et al., 2002), making them valuable in various food and biotech industries (Macauley et al., 2001; Sengun and Karabiyikli, 2011). Due to their strict oxygen requirement for growth, fermentation of AAB in the dough system can be challenging (Hermann et al., 2015). An addition of sucrose in the sourdough fermentation with AAB was patented to enhance their competitiveness in non-sterile environment (Brandt, 2013) and simultaneously to serve as a source for levan production. We have selected molasses as an alternative, natural source of sucrose, since it has already been used for the nutritional improvement of breads (Bakr, 1997; Filipcev, 2011; Simurina et al., 2012) and the additional micro-nutrients in molasses can be beneficial for cell growth during the fermentation.

In a previous study, we have demonstrated the possibility of using selected strains of AAB in the GF sourdough production, which contained *in situ* levan in high amounts with uniquely high molar size and mass as compared to those observed in LAB (Ua-Arak et al., 2016). The aim of this study was to evaluate the potential of levan-producing AAB on sourdough production using molasses as a natural source of sucrose for the improvement of GF bread quality toward the naturality and clean labeling approach.

2. Material and methods

2.1. Cultivation and levan production

The strains *Gluconobacter (G.) albidus* TMW 2.1191 and *Kozakia (K.) baliensis* NBRC 16680 were cultivated aerobically in sodium gluconate (NaG) medium modified from Adachi et al. (1979) containing 20 g/L sodium gluconate, 3 g/L yeast extract, 2 g/L peptone from casein, 3 g/L glycerol, 0.2 g/L $MgSO_4 \cdot 7H_2O$ and 10 g/L mannitol (pH 6.0). For agar plates, 20 g/L agar was added. Pre-cultures in liquid NaG medium were grown to the mid-exponential growth phase at 30 °C, 200 rpm and used as starter cultures for subsequent sourdough fermentations (initial cell count ca. 3×10^7 CFU/g dough). For the production of levan in laboratory medium, 300 mL of NaG medium containing 80 g/L sucrose in 2-L Erlenmeyer flask was cultivated with *G. albidus* for 32 h at 30 °C, 200 rpm.

2.2. Sourdough fermentations

Sourdoughs (dough yield 350) were prepared with organic buckwheat flour (Bauck GmbH & Co. KG, Rosche, Germany), tap water and 35% (flour base) of sugarcane molasses (August Töpfer & Co., Hamburg, Germany) as described in Ua-Arak et al. (2016). The 300 g doughs in 2-L Erlenmeyer flasks were inoculated with pre-cultures and incubated at 30 °C, 200 rpm for up to 72 h. To prepare sourdoughs for bread makings, buckwheat doughs were fermented for 24, 30 or 48 h and stored at −20 °C. Chemically acidified control doughs were prepared by adding 20 μg/g flour chloramphenicol and 10 μg/g flour erythromycin, and were acidified with 100% acetic acid, 90% D,L-lactic acid and 50% gluconic acid before incubation without inoculation. Different amounts of organic acids were added to obtain the concentrations similar to the acids formed in the real sourdoughs by *G. albidus* at 24, 30 and 48 h, respectively (see Results, Table 1). All fermentations were carried out in triplicate.

2.3. Cell counts, pH and strain identification

The bacterial cell counts of AAB and LAB were determined as described earlier (Ua-Arak et al., 2016). Sourdoughs were serially diluted with 0.1% peptone-salt solution (1 g/L peptone from casein, 8.5 g/L NaCl, pH 7.0) and subsequently plated in duplicate on NaG agar plates containing 65 mg/L penicillin G to suppress the Gram-positive bacteria. In the case of *K. baliensis*, sucrose (40 g/L) was also added in the NaG agar plates (NaGS) to improve the cell growth and increase the colony differentiation. For LAB counts, the modified de Man, Rogosa and Sharpe medium (mMRS, pH 6.2) (Stolz et al., 1995) containing 10 g/L maltose, 5 g/L glucose, 5 g/L fructose, 15 g/L agar and 3 g/L 2-phenyl ethanol to suppress the Gram-negative bacteria (Lilley and Brewer, 1953) was used. To monitor the changes during sourdough fermentations over 72 h, cell counts, with the limit of detection of 100 CFU/mL (ca. 68 CFU/g dough), and

Table 1
Biochemical characteristics of sourdoughs fermented by levan-producing AAB (averaged from 6 individual sourdoughs).

Strains	Time [h]	pH	Sugars [mmol/kg flour]			Organic acids [mmol/kg flour]			Levan [g/kg flour]
			Sucrose	Glucose	Fructose	Acetic acid	Lactic acid	Gluconic acid	
G. albidus	24	4.09 ± 0.06^{ad}	112.69 ± 33.19^{ac}	41.92 ± 15.71^{a}	250.65 ± 18.53^{a}	91.80 ± 22.43^{a}	73.09 ± 24.41^{a}	187.17 ± 18.04^{a}	8.68 ± 3.28^{ab}
	30	3.91 ± 0.08^{be}	77.74 ± 56.24^{a}	9.76 ± 6.36^{b}	179.71 ± 48.08^{a}	175.47 ± 20.11^{a}	140.12 ± 21.26^{ac}	171.03 ± 28.48^{a}	11.58 ± 5.22^{bc}
	48	3.64 ± 0.03^{cf}	25.89 ± 63.41^{a}	3.94 ± 6.11^{b}	78.57 ± 87.88^{b}	646.39 ± 156.97^{b}	211.18 ± 23.62^{bd}	148.29 ± 25.79^{a}	13.91 ± 3.61^{b}
K. baliensis	24	4.23 ± 0.06^{a}	269.33 ± 74.62^{b}	47.56 ± 24.97^{a}	257.98 ± 21.34^{a}	90.83 ± 64.55^{a}	167.72 ± 46.32^{bc}	55.30 ± 33.92^{b}	2.52 ± 0.62^{a}
	30	4.00 ± 0.11^{bd}	214.83 ± 109.93^{c}	23.60 ± 25.59^{ab}	240.69 ± 36.47^{a}	148.99 ± 53.42^{a}	230.00 ± 51.63^{d}	57.26 ± 41.32^{b}	5.88 ± 1.59^{ac}
	48	3.78 ± 0.14^{ef}	146.82 ± 73.66^{ac}	5.95 ± 14.57^{b}	226.02 ± 74.83^{a}	452.81 ± 129.65^{c}	310.46 ± 62.20^{e}	45.91 ± 34.32^{b}	12.65 ± 2.38^{b}

*Different letters in the same column indicate significant differences among values ($p < 0.05$).