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Characterization of lactic acid bacteria isolated from wheat bran sourdough

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ABSTRACT

Spontaneously fermented foods, such as sourdough, represent a source of lactic acid bacteria with potential interesting functional and technological properties as well as a potential source of probiotics. The choice of the starter cultures has a critical impact on the palatability, processability and nutritional attributes of fermented products. The aim of this study was to characterize the predominant microbial species previously isolated from a sourdough-like spontaneous fermented wheat bran. Lactic acid bacteria, such as *Leuconostoc mesenteroides, Leuconostoc citreum, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus sakei, Lactobacillus plantarum* and *Pediococcus pentosaceus*, were phenotypically characterized by their growth and acidification rate, carbohydrate metabolism, antifungal activity, exopolysaccharide production, as well as safety. The strains were also tested for xylan- and phytate-degrading activities. Moreover, probiotic properties, such as acid and bile tolerance, anti-listeria activity and adhesion ability to Caco-2 cells were examined. *L. plantarum* CE42, CE60 and *P. pentosaceus* CE65, CE23 showed interesting technological application potential due to their antifungal activity and exopolysaccharide production. Some strains also exhibited phytate degrading activity and could be exploited to improve mineral bioavailability of fermented products. Moreover, *P. pentosaceus* CE65 seems to be a candidate for use as probiotic.

1. Introduction

The demand for faster, more efficient, controllable and large-scale fermentation has resulted in a careful selection of starter microorganisms to guarantee the reproducibility of fermentation at industrial scale and to obtain a product with specific properties (Carnevali, Ciati, Leporati, & Paese, 2007). The choice of starter culture has critical impact on the final quality of fermented foods. Fermentation with well-characterized cultures, yeast or lactic acid bacteria (LAB), could be a potential tool to improve the palatability, processability and the nutritional value of fermented cereal products or high-fiber ingredients, such as sourdough bread, fermented wheat bran and whole-meal flours (Salmenkallio-Marttila, Katina, & Autio, 2001).

The main criteria used to select microbial starters are desirable technological, sensory and nutritional aspects. Technological factors of interest for fermentation are growth and acidification rate (Coda et al., 2010), synthesis of antimicrobial compounds (Messens & De Vuyst, 2002), antifungal activity (Coda et al., 2013) and exopolysaccharide production (e.g. glucan and fructan) (Galle & Arendt, 2014). Among nutritional properties, degradation of anti-nutritional factors (e.g. phytic acid) and increased availability of functional compounds (e.g. soluble fiber, soluble arabinoxylans, free phenolic acids, bioactive peptides) are desirable (Katina & Poutanen, 2013). Technologically interesting potential starter strains are usually selected from the food matrix they are going to be used for. Some recent studies have shown that the use of selected autochthonous LAB to ferment sourdough is a suitable biotechnological approach to exploit the potential of cereals and pseudo-cereals in bread making (Coda et al., 2010). Moreover, fermentation of bran with yeasts and LAB or with enzymes has been shown to improve loaf volume, crumb structure and shelf life, as well as nutritional properties of bread supplemented with

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fermented bran (Katina et al., 2012; Salmenkallio-Marttila et al., 2001).

LAB could also be exploited because of their probiotic properties in order to contribute to the health and wellbeing of the hosts by maintaining or improving their intestinal microbial balance (Asahara et al., 2004). In the case of baked products, the probiotic cultures should be added to the final product and/or their survival and viability should be guaranteed throughout the process steps involved in the manufacture and during the storage conditions (Soukoulis et al., 2014). The aim of the current study was to characterize the 13 LAB strains previously isolated from a spontaneous fermented sourdough-like wheat bran (Manini et al., 2014) for their potential use as starter cultures in food applications such as wheat bran fermentation and sourdough bread, and as potential probiotics.

2. Materials and methods

2.1. Microorganisms

Thirteen LAB strains were previously isolated from spontaneously fermented wheat bran sourdough-like and identified by phenotypic and molecular techniques (Manini et al., 2014). The following species were found: *Lactobacillus brevis* (n = 2), *Lactobacillus plantarum* (n = 3), *Lactobacillus curvatus* (n = 1), *Lactobacillus sakei* (n = 1), *Leuconostoc mesenteroides* (n = 2), *Leuconostoc citreum* (n = 2), and *Pediococcus pentosaceus* (n = 2) (Table 1). As control, *Lactobacillus rhamnosus* GG was used as well as two recently isolated potential probiotic strains *L. plantarum* Q823 and *Lactobacillus casei* Q11 (unpublished results).

2.2. Growth and acidification rate

The fermentation capacity of the strains was evaluated measuring microbial counts, pH and Total Titratable Acidity (TTA) using wheat bran (raw, untreated) (mean particle size 475–633 μm -Molino Quaglia, Vighizzolo D'Este, PD, Italy) as a substrate.

An overnight culture (1% v/v) of each test strain was individually inoculated into a sample (100 g) of wheat bran dough (15% w/v of bran and 85% of water) and incubated for 8 h at 30 °C. The microbial counts were evaluated before and after fermentation. The LAB were determined on de Man, Rogosa and Sharpe agar (MRS agar) (LAB M, Lancashire, UK) and the yeasts were evaluated on Plate Count Agar (PCA) (LAB M). Plates were incubated at 30 °C for 48–72 h pH and TTA developments were measured during fermentation. A sample (10 g) of bran was taken every 2 h and suspended in 100 mL of distilled water. For the determination of TTA, this suspension was titrated with 0.1 M NaOH to a final pH of 8.5, detected by a pHmeter (PHM 250, Radiometer, Copenhagen); TTA was expressed as mL of 0.1 M NaOH needed to achieve the final pH of 8.5. All samples were analyzed in duplicate.

Table 1
Lactic acid bacteria isolated from a sourdough-like fermented wheat bran.

Microorganism	Strain	Isolation source
L. brevis	CE94	Wheat bran sourdough
L. brevis	CE85	Wheat bran sourdough
L. plantarum	CE42	Wheat bran sourdough
L. plantarum	CE60	Wheat bran sourdough
L. plantarum	CE84	Wheat bran sourdough
L. curvatus	CE83	Wheat bran sourdough
L. sakei	CE47	Wheat bran sourdough
Ln. mesenteroides	CE52	Wheat bran sourdough
Ln. mesenteroides	CE48	Wheat bran sourdough
Ln. citreum	CE88	Wheat bran sourdough
Ln. citreum	CE54	Wheat bran sourdough
P. pentosaceus	CE65	Wheat bran sourdough
P. pentosaceus	CE23	Wheat bran sourdough
L. rhamnosus GG (control)	GG	GIT of a healthy human
L. plantarum (control)	Q823	Quinoa seeds
L. casei (control)	Q11	Quinoa seeds

2.3. Technological properties and antibiotic resistance

2.3.1. Carbohydrate metabolism and gas production assessment

Carbohydrate fermentation profiles of the strains were determined by using API 50 CH system (BioMèrieux, Marcy-l'Etoile, France). The test was performed according to the Manufacturer's instructions. Moreover, each pure culture was further characterized by Durham tube method in MRS broth at 30 °C for 24 h for detecting gas production.

2.3.2. Phytase activity

The LAB strains were preliminary inoculated in MRS broth and incubated at 30 °C for 24 h. Strains were then grown at 30 °C for 24–48 h in modified Chalmers broth without neutral red and with 1% of sodium phytate (Sigma–Aldrich, Milan, Italy). The phytase activity was determined on modified Chalmers agar plates without $CaCO_3$ and with 1% of phytic acid calcium or sodium salt (Sigma–Aldrich) as described by Anastasio et al. (2010).

2.3.3. Xylanase activity

For the screening of xylanase producing microorganisms, an agar medium was prepared by adding 0.1% (w/v) of the dyed substrate (Remazolbrilliant Blue R treated Azo-Xylan – birchwood), (Megazyme International Ireland Ltd, Co. Wicklow, Ireland), as the only carbon source, to a sodium phosphate buffer, 100 mM, pH 6 and/or a sodium acetate buffer, 100 mM, pH 4.5 (to test the activity at different pH).

LAB were preliminary inoculated in MRS broth and incubated at 30 °C for 24 h. The cell cultures were inoculated in wells made in triplicate in the agar plates and examined, after 48 h of incubation at 30 °C, for clearing zones around the holes.

2.3.4. Antifungal activity

The antifungal activity of the strains was determined using the overlay method described by Magnusson and Schnürer (2001), slightly modified.

The molds *Aspergillus oryzae* ATCC 66222 and *Aspergillus niger* 25541 were used for this test. A spore suspension was prepared by growing the molds on PCA at 30 °C for 3–4 days and then collecting the conidia by vigorously shaking the slants with sterile peptone water.

LAB were inoculated as two 2-cm-long lines on MRS plates and incubated at 30 °C for 48 h. The plates were then overlaid with 10 mL of malt extract soft agar (3% malt extract, 1.5% bacto peptone, 0.75% agar) inoculated with fungal spore suspension. After solidification, the plates were incubated aerobically at 30 °C for 48 h. The plates were examined for zones of inhibition around the bacterial streaks.

2.3.5. Exopolysaccharides

LAB strains were plated on different MRS agar plate with glucose, sucrose, raffinose, maltose, lactose and starch as the only carbon source. Plates were incubated for 2 days at 30 °C. Duplicate plates containing 25–250 colonies were scored for mucoid properties (scale of ++ = excess EPS to – = no visible mucoid). Colonies were scored as ropy if strings of 5 mm or more were detected when the colony was touched once with a wire inoculating loop (Ruas-Madiedo & De Los Reyes-Gavilán, 2005).