Food Microbiology 38 (2014) 26–35

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products

Marianne Perricone, Antonio Bevilacqua*, Maria Rosaria Corbo, Milena Sinigaglia

Department of the Science of Agriculture, Food and Environment, University of Foggia, Italy

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form
30 July 2013
Accepted 14 August 2013
Available online 22 August 2013

Keywords:
Multifunctional starter
Multivariate analyses
Sourdough
Survival into the gut
Yeasts

ABSTRACT

The main topic of this research was to select some suitable functional starter cultures for cereal-based food or beverages. This aim was achieved through a step-by step approach focused on the technological characterization, as well as on the evaluation of the probiotic traits of yeasts; the technological characterization relied on the assessment of enzymatic activities (catalase, urease, β-glucosidase), growth under various conditions (pH, temperature, addition of salt, lactic and acetic acids) and leavening ability. The results of this step were used as input data for a Principal Component Analysis; thus, the most technologically relevant 18 isolates underwent a second selection for their probiotic traits (survival at pH 2.5 and with bile salts added, antibiotic resistance, antimicrobial activity towards foodborne pathogens, hydrophobic properties and biofilm production) and were identified through genotyping. Two isolates (*Saccharomyces cerevisiae* strain 2 and *S. cerevisiae* strain 4) were selected and analyzed in the last step for the simulation of the gastric transit; these isolates showed a trend similar to *S. cerevisiae* var. *boulardii* ATCC MYA-796, a commercial probiotic yeast used as control.

1. Introduction

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material and produce a fermented food, conducting its fermentation and assuring food safety, shelf-life, technological and economic feasibility criteria (Champagne and Møllgaard, 2008).

Apart from these classical technological traits, the selection of a starter should focus on the QPS status (Qualified Presumption of Safety) of the microbial cultures (i.e. they should not produce toxic compounds, or be responsible of the spreading of antibiotic resistance) (Bevilacqua et al., 2012).

A new trend in food microbiology is the use of multifunctional microorganisms, i.e. a starter culture able to perform another function (e.g. probiotic activity, reduction of toxic compounds, production of vitamins) (Bevilacqua et al., 2012); some examples of food processed by multifunctional microorganisms are the table olives containing probiotic microorganisms (Lavermicocca et al., 2005) or sausages fermented by *Lactobacillus acidophilus* and *Bifidobacterium animalis* (Holko et al., 2013). Yeasts have a long history of safe and technological use in baking and brewing and nowadays they are the backbone of many commercially important sectors (foods, beverages, pharmaceuticals and industrial enzymes). *Saccharomyces cerevisiae* is the most common yeast for food fermentation and possess the QPS status (Moslehi-Jenabian et al., 2010); moreover, many authors reported its beneficial effects on human health (Weichselbaum, 2009; Czerucka et al., 2007).

The term probiotic includes a large range of microorganisms, mainly bacteria but also yeasts (Binetti et al., 2013); probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amount (Rivera-Espinoza and Gallardo-Navarro, 2010). Maintaining the viability (the recent trend is to have 1 billion viable cells per portion – i.e. 100 g of product) (Settanni and Moschetti, 2010) and the activity of cultures in foods to the end of shelf-life are two important criteria that must be fulfilled in order to provide efficacious probiotic food products. In order to exert health benefits on the host, probiotics must be able to grow and persist in the human intestine (Rivera-Espinoza and Gallardo-Navarro, 2010).

Orally ingested probiotics must survive harsh conditions during their passage through the intestinal tract to be able to influence the human gut microflora. Ingested strains do not become established members of the normal intestinal flora but generally persist only

* Corresponding author.
E-mail addresses: antonio.bevilacqua@unifg.it, abevi@libero.it (A. Bevilacqua).

0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.08.006

for the period of consumption and for a relatively short period thereafter (Weichselbaum, 2009).

Traditionally dairy fermented products have been considered as the best carrier for probiotics. However, the use of milk-based products may be limited by allergies or cholesterol diseases; therefore, several raw materials, including cereals and cereal-based beverages, have been extensively investigated to determine if they are suitable substrate to produce novel non-dairy functional foods (Rivera-Espinoza and Gallardo-Navarro, 2010).

Many authors focused on the selection of some strains of lactic acid bacteria as functional and probiotic microorganisms; few data are available on functional yeasts. Silva et al. (2011) reported on the probiotic characterization of yeasts from Portuguese brined olives and proposed some strains as suitable functional starters for this kind of food, whilst Pedersen et al. (2012) studied the probiotic traits of some yeasts isolated from *Fura*, a traditional African fermented cereal.

Many traditional cereal foods contain yeasts in a living form (biza, tarhana, shalgam, Fura, brembali, burukutu, etc.) (Pedersen et al., 2012; Blandino et al., 2003); therefore, the use of probiotic/functional yeasts for these products or for other traditional cereal foods could be a suitable way for their valorization. Thus, the main topic of this research was the technological and probiotic characterization of 49 isolates from Altamura sourdough (Southern Italy) to select some promising strains/isolates to be used as functional starter cultures; the specific aims of the research were: a) the evaluation of the technological traits of the isolates, focusing on the enzymatic activities, growth as a function of pH, temperature, salt, lactic and acetic acids, and leavening activity; b) analysis of the probiotic traits; c) studying the behavior of the most promising strains under conditions simulating the gut.

2. Materials and methods

The research was divided in some steps, as follows: a) yeast isolation from Altamura dough; b) enzymatic and technological characterization of the isolates; c) Principal Component Analysis to select the most relevant technological isolates; d) probiotic characterization of the relevant isolates; e) Principal Component Analysis to select the most promising strains; f) simulation of the transit into the gut for the most promising strains.

2.1. Yeast isolation

Eight different samples of sourdough from Altamura (Apulian Region, Southern Italy) were collected in two different periods (January and April); 10 g of each sample were diluted with 90 mL of a sterile saline solution (0.9% NaCl) and homogenized through a Stomacher (Bagmixer, Interscience, St Nom la Bretêche, France) for 60 s. The homogenates were serially diluted in saline solution and plated onto the following media:

1. MRS agar (Oxoid, Milan, Italy), added with 0.17 g/L of cycloheximide (Sigma–Aldrich, Milan) and incubated at 30 °C for 2–4 days under anaerobic conditions for the evaluation of lactic acid bacteria.
2. WL Nutrient Medium (Oxoid) and Yeast Universal Medium (YM) (Yeast Extract, 3.0 g/L; Malt Extract, 3.0 g/L; Peptone from soybeans, 5.0 g/L; Glucose, 10.0 g/L; agar, 15 g/L), incubated at 25 °C for 2–4 days for the evaluation of yeasts.
3. Violet Red Bile Glucose Agar (VRBGA) (Oxoid), incubated at 37 °C for 18 h to count *Enterobacteriaceae*.

Yeasts, showing the typical appearance of *Saccharomyces* and *Candida* (white-to-yellow colonies) on WL Nutrient Medium and the typical cell appearance at the microscope, were randomly selected and labeled with a numeric code, ranging from 1 to 50, and maintained on Sabouraud Agar slants (Bacteriological Peptone 5 g/L; Tryptone 5 g/L; Glucose 20 g/L; agar, 15 g/L), stored at 4 °C and monthly transferred. Before each assay, the isolates were grown in Sabouraud broth (SAB) (30 °C for 48 h) up to 7 log CFU/mL.

2.2. Technological characterization

2.2.1. Catalase activity

Catalase activity of yeasts was evaluated by adding 3% (v/v) of hydrogen peroxide (Sigma–Aldrich) onto the cultured colonies, according to the Whittenbury method (1964). The results were expressed as follows: "−" (no activity), "+" (weak activity) and "++" (strong activity).

2.2.2. Urease activity

This test was performed in the Christensen's urea agar (Oxoid), containing phenol red as pH indicator. After yeast inoculum, plates were incubated at 25 °C for 2–7 days; colour turning to purple highlighted urea hydrolysis and pH increase.

2.2.3. β-glucosidase activity

β-glucosidase activity was detected on a *medium* containing arbutin (hydroquinone-β-D-glucopyranoside) (Biochemicals, Solon, Ohio) as the only carbon source (Arbutin, 5 g/L; Yeast Extract, 10 g/L; Ferrous Chloride, 200 mg/L; agar, 15 g/L). After streaking the isolates onto the surface of the *medium*, yeasts were incubated at 25 °C for 7 days; β-glucosidase activity was evidenced by a brown halo around the colonies (Caridi et al., 2005). Although this assay is qualitative and not quantitative, the diameter of the browning halo is strictly related to the enzymatic activity; thus, he results were expressed as follows: "−" (no halo), "+" (weak browning zone, 1–3 mm) and "++" (well-defined browning zone, >3 mm).

2.2.4. Growth at different pH values, NaCl amounts and different temperatures

Sterile tube containing 10 mL of SAB broth, adjusted to different pHs (2.5, 3.0, 3.5, 4.0, 9.0, 9.5) or added with NaCl (2.0%, 4.0%, 6.0% w/v), were inoculated with approximately 3 log CFU/mL of yeasts and incubated at 10–15–25–37 °C for 2 days; the level of the *inoculum* was checked by plate count. Aliquots of not-modified SAB broth (pH 6.0), inoculated with yeasts and incubated at 25 °C, were used as positive controls.

Microbial growth was evaluated after 24 and 48 h as absorbance at 600 nm with a spectrophotometer UV–VIS DU 640 Beckman (Fullerton, California, USA).

The analyses were performed in duplicate and data were modeled as Growth Index (GI) (Bevilacqua et al., 2009):

$$GI = (Abs_s/Abs_c)*100$$

where Abs_s is the absorbance of the samples at different pHs, temperatures and NaCl concentrations; whereas Abs_c the absorbance of the positive control. GI values were classified as follows (Bevilacqua et al., 2009):

GI < 25% yeast inhibition
25% < GI < 75% partial inhibition
GI > 75% growth similar to the control

2.2.5. Evaluation of the leavening ability

The assay was performed with the isolates from sourdough and with a strain of *S. cerevisiae* (labeled C), isolated from a commercial