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Evaluation of functionally important lactic acid bacteria and yeasts from Nigerian sorghum as starter cultures for gluten-free sourdough preparation

A.O. Ogunsakin [a, d], V. Vanajakshi [b], K.A. Anu-Appaiah [c], S.V.N. Vijayendra [c], S.G. Walde [d], K. Banwo [a], A.I. Sanni [a], P. Prabhasankar [d, *]

[a] Department of Microbiology, University of Ibadan, Ibadan, Nigeria
[b] Department of Food Safety and Analytical Quality Control Laboratory, CSIR - Central Food Technological Research Institute, Mysuru 570020, India
[c] Department of Microbiology and Fermentation Technology, CSIR - Central Food Technological Research Institute, Mysuru 570020, India
[d] Flour Milling, Baking and Confectionery Technology Department, CSIR - Central Food Technological Research Institute, Mysuru 570020, India

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ABSTRACT

There is an increasing prevalence of celiac disease, which is gradually encroaching into Africa and Nigeria ranks first among sorghum-producing African countries. It is important to select starter cultures that can improve the functional properties of peculiar flour such as sorghum for the development of novel gluten-free diets. Therefore, this study aimed at screening of functionally important lactic acid bacteria (LAB) and yeasts as starter cultures for the development of sorghum-based sourdough. A total of 103 LAB and 20 yeast isolates obtained from Nigerian sorghum flour were screened for functional properties essential for sourdough preparation. Three LAB isolates were selected based on proteolytic activities, exopolysaccharide production and acidification properties, while one yeast strain was selected based on its gas production ability, low pH and acetic acid tolerance. *Pediococcus pentosaceus* SA8, *Weissella confusa* SD8 and *P. pentosaceus* LD7 showed highest proteolytic activity (24.7 mm), exopolysaccharide production (753.3mg/100 ml) and acidification property (0.25 g/100 g lactic acid) respectively. *Saccharomyces cerevisiae* YC1 had vigorous gas production (CO_2 > 20 mm at 8 h), tolerated pH 2.5 and 0.1% acetic acid, and had highest leavening activities (60.87% at 1 h). The selected LAB and yeast strains possessed promising attributes as functional starter cultures for the production sourdoughs breads.

1. Introduction

Sorghum is about the largest cultivated crop in the northern Guinea savanna areas of Nigeria, which ranks first among African sorghum-producing countries. Sorghum provides a good basis for gluten-free breads and other baked products for celiac patients (Ogunsakin, Banwo, Ogunremi, & Sanni, 2015).

In modern biotechnology of baked goods, sourdough is widely used as a natural leavening agent because of the many advantages it has over baker's yeast. These include better taste, shelf life, eating characteristics and the development of the characteristic flavour, resulting in products with high nutritional and sensory quality than the conventional yeast-leavened breads. Sourdough fermentations are characterized by the mutual effects of LAB and yeasts. The organic acids, exopolysaccharides (EPS), and enzymes produced by LAB metabolites during sourdough fermentation have positive effects (Carnevali, Ciati, Leporati, & Paese, 2007; Tieking & Gänzle, 2005). The ability of some yeasts to bring a rapid and efficient conversion of sugars into alcohol and CO_2 gives them an historical edge over other microorganisms (Gèlinas, 2009).

The choice of starter cultures is a major determinant of the final quality of sourdough as a cereal-based product; therefore, it is important to select dominant strains that out-compete contaminants to be applied for specific sourdough fermentations in order to improve nutritional and rheological properties of peculiar flour like sorghum. For yeast intended for use in the sourdough fermentation environment, high CO_2 production, low pH tolerance, and the ability to grow in the presence of acetic acid are among considered physiological characteristics. Whereas, EPS production, proteolytic activity and acidification properties are desirable in LAB to be used

* Corresponding author.
E-mail address: psankar@cftri.res.in (P. Prabhasankar).

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as starter cultures (Leroy & De Vuyst, 2004; Van der Meulen, Scheirlinck, Van Schoor, Huys, & Vancanneyt, 2007; Vrancken, Rimaux, Vuyst, & Mozzi, 2010).

The aim of the present study is to select promising LAB and yeast strains from the microbiota of spontaneously fermenting sorghum sponge to be employed as starter cultures in sourdough preparation.

2. Materials and methods

2.1. Acquisition and processing of raw materials

A commercial flour variety of white sorghum seeds were obtained during 2015 season from a local retail market in Ibadan, South-West Nigeria and transferred in polythene sacs to processing area at ambient temperature (27±2 °C). The sorghum grains were germinated and processed into flour as described by Ogunsakin et al. (2015).

2.2. Fermentation of sorghum batter and physicochemical analyses of fermenting sponge

Sorghum flour and tap water were mixed in the ratio of 1:1(w/v) and fermented naturally at 27±2 °C for 48 h. The temperature and pH were determined as described by Ogunsakin et al. (2015). The amount of lactic acid produced in the fermenting batter was determined using the standard titration procedure according to A.O.A.C. (1990). A 10 ml of aliquots (triplicates) were pipetted and titrated against 1N NaOH to phenolphthalein end-point. Each sponge of 1N NaOH is equivalent to 90.08 mg of lactic acid. The level of leavening of the fermenting batter was determined by placing a short meter rule along the side of the beaker containing the batter and its level was read. The difference between the initial reading and the final reading was recorded as the leavening level.

2.3. Enumeration and isolation of microbial isolates

At the intervals of 0, 6, 8, 12, 24, 36 and 48 h, 1.0 g of the fermenting batter was taken and homogenized in 9.0 ml sterile peptone water for about 30s. The mixture was serially diluted in 0.1% sterile peptone water by the method of Meynell and Meynell (1970, p. 347). From these, appropriate 10 fold dilutions were pour-plated on de Mann Rogosa Sharpe (MRS) agar (LAB M, Lancashire, U.K.) and incubated (48 h, 37 °C). Cell number of presumptive LAB was estimated. Distinct colonies were isolated randomly from plates and purified by streaking on MRS agar, checked for morphology, Gram-staining and catalase test. Similarly, presumptive yeasts were cultivated on Glucose Yeast Peptone Agar (GYPA) (LAB M, Lancashire, U.K.) supplemented with chloramphenicol with incubation period of 72 h (37 °C) (Minervini et al., 2012). Pure cultures were maintained on appropriate agar slants and kept as stock cultures under refrigeration temperature (4 °C) and maintained in appropriate broth containing 15% glycerol (v/v) (−40 °C).

2.4. Screening of presumptive LAB isolates for their functional properties

One hundred and three presumptive LAB were screened for functional properties important in sourdough bread and potential starter cultures were selected based on the following criteria:

2.4.1. Proteolytic activity

Proteolytic activity was assayed on MRS agar plates containing 2% skimmed milk. LAB strains were grown in MRS broth overnight at 37 °C and the cell suspension were spot inoculated on agar plates and incubated at 37 °C for 72 h. Proteolytic activity was determined by measuring the diameter of the clear zone around the inoculated spots (mm) after addition of 1N HCl according to modified method of Tafti, Peighambardoust, and Hejazi (2013).

2.4.2. Exopolysaccharide production

A 500 μl of overnight-grown culture was inoculated into 50 ml MRS broth supplemented with 3% sucrose in Erlenmeyer flasks and incubated at 37 °C for 48 h. The broth culture was centrifuged for 15 min at 8000 *g* and the supernatant was separated. To this, one-two volumes of chilled isopropyl alcohol were added with stirring to recover the exopolysaccharide. This was kept in refrigerator overnight. The exopolysaccharide formed was carefully collected in aluminum boat and dried at 100 °C to constant weight. The amount produced was expressed as g/100 ml of broth medium (Vijayendra, Palanivel, Mahadevamma, & Tharanathan, 2008).

2.4.3. Acidification properties

Presumptive LAB isolates were tested for acidification properties in skimmed milk medium. A volume of 5 ml of the 10% skimmed milk medium was inoculated with 0.2 ml of overnight grown LAB broth culture and incubated at 37 °C for 48 h. At 24 h interval, the percentage total titratable acidity (TTA) produced by the LAB strains was determined after diluting 1 ml of the fermented skim milk medium in 9 ml distilled water. The mixture was homogenized and titrated against 0.1 N NaOH using phenolphthalein as an indicator (Ali & Mustafa, 2009). TTA was determined using the Kramer and Twigg (1970, pp. 155–205) formula.

$$\text{Total Titratable Acidity } (g/100g) = \frac{Vol.of\ NaOH}{\text{Vol.of sample}} \times 0.9$$

2.4.4. Pathogenicity test

Selected LAB isolates were tested for gelatinase, lecithinase and hemolysis ability. A medium supplemented with 2% gelatin, Baird-Parker agar (HiMedia, Mumbai, India) enriched with 5% egg yolk emulsion and 3% potassium tellurite, and 7% defibrinated sheep blood agar (HiMedia, Mumbai, India) were used to check gelatin hydrolysis, lecithinase activity and hemolytic activity respectively (Shobharani & Halami, 2014).

2.5. Screening of presumptive yeasts for their functional properties

Twenty yeast isolates were screened for functional properties important in sourdough bread and potential starter cultures were selected based on the following criteria;

2.5.1. Gas production ability

Respirometry tubes for the experimental procedure consisted of a test tube containing an inverted test tube and the level of CO_2 produced was determined by measuring the gas level within the Durham tubes using a meter rule at 2 h, 4 h, 6 h and 8 h according to Scholander, Claff, Andrews, and Wallach (1952).

2.5.2. Ability to grow in the presence of acetic acid and low pH tolerance

Modified media containing Yeast Nitrogen Base (YNB) medium 6.2 g/L and Glucose 5 g/L was used to determine the ability of yeasts to grow in the presence of acetic acid and to tolerate low pH (2.5) was determined following the method of Vrancken et al. (2010) with slight modifications. The growth of yeast cells was measured spectrophotometrically by UV-absorption at Optical