BRAZILIAN JOURNAL OF MICROBIOLOGY XXX (2018) XXX–XXX

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

1 Food Microbiology

Selection of starter cultures for the production of sour cassava starch in a pilot-scale fermentation process

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ARTICLE INFO

Article history:
Received 18 May 2017
Accepted 5 February 2018
Available online xxx
Associate Editor: Solange I. Mussatto

Keywords:
Q1 Lactic acid bacteria (LAB)
Yeasts
Starter cultures
Fermentation
Bakery products

ABSTRACT

Sour cassava starch (*Polvilho azedo*) is obtained from a spontaneous fermentation conducted by microorganisms from raw materials and fermentation tanks. This product is traditionally used in the baking industry for the manufacture of biscuits and Brazilian cheese breads. However, the end of fermentation is evaluated empirically, and the process occurs without standardization, which results in products of inconsistent quality. Predominant microbiota from a cassava flour manufacturer was isolated in order to select starter cultures for the production of sour cassava starch in a pilot-scale fermentation process. Lactic acid bacteria (LAB) and yeasts were isolated, enumerated and grouped by Restriction Fragment Length Polymorphism (RFLP), and PCR fingerprinting, respectively. One isolate of each molecular profile was identified by sequencing of the rRNA gene. LAB were prevalent throughout the entire process. *Lactobacillus brevis* (21.5%), which produced the highest values of acidity, and *Lactobacillus plantarum* (13.9%) were among the most frequent species. *Pichia scutulata* (52.2%) was the prevalent yeast and showed amylolytic activity. The aforementioned species were tested as single and mixed starter cultures in a pilot-scale fermentation process for 28 days. *L. plantarum* exhibited better performance as a starter culture, which suggests its potential for the production of sour cassava starch.

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https://doi.org/10.1016/j.bjm.2018.02.001
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Please cite this article in press as: Penido FC, et al. Selection of starter cultures for the production of sour cassava starch in a pilot-scale fermentation process. *Braz J Microbiol*. (2018), https://doi.org/10.1016/j.bjm.2018.02.001

Introduction

Because cassava (*Manihot esculenta* CRANTZ) has a high starch content (approximately 80%), it is an important source of carbohydrates that can be sold fresh or processed into a variety of value-added products; cassava is characterized as a multipurpose crop.[1] In 2014, the estimated Brazilian cassava production was 23 million tons.[2] Although widely consumed, cassava has limitations due to its perishability, toxicity and low protein content.[3] The root of cassava contains cyanogenic glycosides that act as defence substances through the release of hydrogen cyanide, which is responsible for its toxicity. The traditional fermentations of cassava are quite suitable for the detoxification, preservation and development of products with desirable viscoelastic texture.[4] Lactic fermentation not only extends the shelf life of this root but also decreases its toxicity.[5]

Natural fermentation of wet starch extracted from cassava root is a traditional technology widely used in Latin America. Sweet cassava starch and sour cassava starch undergo the same process of starch extraction, but differ in fermentation time (from two to seven days and from 20 to 70 days, respectively) and therefore have different levels of acidity, maximum of 1% of acidity and 5% of acidity, respectively. Sour cassava starch stands out from sweet cassava starch and other flour in the preparation of bakery products for its unique expansion capacity, without the addition of baking soda and in the absence of gluten. Sour cassava starch is a typical Brazilian food generally produced by small and medium-sized rural industries.[6] As sour cassava starch is essentially handcrafted, even though it is produced in modern cassava flour manufacturers, it still has heterogeneous physicochemical and sensory quality. The succession of microorganisms from raw materials and fermentation tanks occurs naturally during cassava fermentation and results in a microbiota with a prevalence of lactic acid bacteria (LAB), such as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus brevis* and *Leuconostoc mesenteroides*.[7–10] In the production of sour cassava starch in Brazil, *Lactobacillus* occurs in association with yeasts, such as *Galactomyces geothricum* and *Issatchenkia* (now *Pichia*) sp.[6,11]

The wide range and complexity of cassava spontaneous fermentation microbiota are the main factors responsible for the lack of homogeneity and low product quality. The use of selected strains is an important alternative because it provides less variation in the content of chemical compounds, shorter fermentation time, higher yield and sensorial quality.[12,13] The LAB, which are used as natural or selected starter cultures in fermented foods, are able to acidify and enhance the flavor. Furthermore, the LAB can protect food from the development of pathogens due to the formation of antimicrobial compounds.[14,15]

Although sour cassava starch is widely consumed in Latin America and gained prominence in the preparation of bakery products with a low level or the absence of gluten, there are few studies on its fermentation process. Therefore, the study of starter cultures contributes significantly to the understanding and optimization of sour cassava starch production. The present work aimed to select the starter cultures with the appropriate characteristics for the production of sour cassava starch in a pilot-scale fermentation process.

Materials and methods

Collection of samples from cassava flour manufacturer

A total of 16 samples of 100 g each were collected from a cassava flour manufacturer in the municipality of Formiga, Minas Gerais (MG) state, Brazil, on days 0, 5, 12, 19, 26, 33 and 40 of a 56-day spontaneous fermentation from a fermentation tank with usable capacity of 16,000 L. These samples were transported to the laboratories of Food Microbiology and Industrial Microbiology and Biocatalysis (Faculdade de Farmácia, Universidade Federal de Minas Gerais, MG, Brazil) on ice and processed within 24 h. The processing consisted of weighing twenty-five grams of each sample in sterile flasks, diluted in 225 mL of 1 g L^{-1} peptone water, and preparing serial decimal dilutions.[16]

Identification of lactic acid bacteria found on samples from cassava flour manufacturer

Appropriate decimal dilutions were spread on de Man, Rogosa and Sharpe (MRS; Acumedia, Lansing, MI, USA) agar containing 0.1 g L^{-1} cycloheximide[16] and incubated in anaerobic jars of 2.5 L (Permution, Curitiba, Brazil) at 37 °C for 48 h. After growth, one colony of each morphotype was counted and purified for later identification. Each isolate was Gram stained and then subjected to the catalase test.[17]

DNA was extracted with an adaptation of the method described by Hoffman and Winston.[18] The colonies previously grown on MRS agar were resuspended in 100 μL of Tris–EDTA (TE). Then, 100 μL of phenol–chloroform–isoamyl alcohol (25:24:1) and 0.3 g of glass beads were added to the suspension. Tubes containing this mixture were homogenized by a vortex shaker (QL-901, Biomixer, Santa Clara, CA, USA) for three to 4 min and centrifuged at 13,000 rpm for 5 min (Eppendorf, Hamburg, Hamburg, Germany). After that, the supernatant was transferred to another tube. Then, a volume of 960 mL L^{-1} ethanol corresponding to the volume of the supernatant recovered was added to the tube. The tubes were homogenized by inversion and centrifuged at 13,000 rpm for 2 min. The liquid phase was discarded, the tubes were dried overnight, and the DNA resuspended in 50 μL of TE. The DNA concentration was determined by NanoDrop ND 1000 Spectrophotometer (NanoDrop Products, Wilmington, DE, USA). The DNA of lactic acid bacteria was subjected to PCR amplification of the 16S rRNA gene using the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′).[19] All LAB isolates were grouped by Restriction Fragment Length Polymorphism (RFLP) by digestion with restriction enzymes *Msp*I, *Hinf*I and *Hae*III (Promega Corporation, Madison, WI, USA) according to the modified methodology of Brightwell et al.[20] For the digestion reaction, 2 μL of 10× buffer, 2 μL of bovine serum albumin (BSA) only for the *Msp*I enzyme, 1 μL of enzyme, DNA ≤1500 ng/μL and water q.s.p. 20 μL. The tubes were incubated at 37 °C for 3 h. The restriction fragments obtained were separated

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