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Biomass production and small-scale testing of freeze-dried lactic acid bacteria starter strains for cassava fermentations

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ABSTRACT

Based on their predominance in *Gari* fermentations, as well as suitable technological properties, *Lactobacillus plantarum, Lactobacillus fermentum, Weissella paramesenteroides* and *Leuconostoc mesenteroides* strains were investigated for their suitability for development as starter strains for this African traditional fermented cassava product. The strains were grown in optimized growth media in 2 L fermenters, harvested and freeze dried, and then tested in lab-scale cassava mash fermentation trials for their ability to ferment the cassava. The strains performed well and rapidly increased the titratable acidity from 1.1 to 1.3% at 24 h to 1.3–1.6% at 48 h. The benefit of including starter cultures was that it lowered the pH of the product much faster and to lower levels than in the uninoculated control fermentation. The results furthermore indicated that especially the *L. plantarum*-group strains could be produced as starter strains at low cost. Overall, the results of this study showed that starter strains could be easily and economically produced, and thus represent a feasible possibility for further development for application in the field.

1. Introduction

Lactic acid bacteria (LAB) occupy a central role in many vegetable, meat, dairy and cereal fermentations (Leroy & De Vuyst, 2004). There has been a recent trend to select wild-type strains from traditional products in order for them to be used as starter cultures in fermentation processes (Beukes, Bester, & Mostert, 2001; De Vuyst et al., 2002). A reason for this could be that pure cultures isolated from traditional fermented foods diverge strongly from comparable strains used as bulk starters in terms of their diversity of metabolic activities, and that the strains isolated from the traditional fermentation as predominant isolates are well adapted for growth in the fermentation substrate (Klijn, Weerkamp, & de Vos, 1995).

When LAB are isolated for development as starter cultures, they need to demonstrate an ability to be produced on a large scale, to withstand the freeze drying process and to maintain their functional activity, before they are considered practicable for industrial applications (Carvalho et al., 2002). Such industrial starter preparations furthermore require the use of cheap raw materials in order to be economically feasible. Freeze drying is currently the most suitable and widely used technique for LAB preservation (Carvalho et al., 2002; Zayed & Roos, 2003). The freeze-drying process imposes environmental stress on the bacterial cells, such as freezing, drying, long-term exposure to low water activities and rehydration. Intrinsic resistance of strains, initial concentration of the microorganisms, growth conditions, drying medium, protective agents used, freezing rate, storage conditions (temperature, atmosphere, relative humidity) and rehydration are all important factors that determine microbial survival (Andersen, Fog-Petersen, Larsen, & Skibsted, 1999; Carvalho et al., 2002; Morgan, Herman, White & Vesey, 2006).

Fermentation processes in Africa, such as the fermentation of cassava for *Gari* production, usually take place at a household level. Little use, if any, is made of starter cultures and most of these fermentations rely on back-slopping techniques to start the fermentation. Back-slopping involves the use of a residue ('starter

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dough') from a previous fermentation batch of acceptable quality for inoculation of a fresh batch (Holzapfel, 1997). However, retention of product characteristics over time may prove difficult due to changes in microbial types. Quality, safety and acceptability of traditional fermented foods may be significantly improved by using starter cultures that are selected on the basis of multifunctional properties which include technological properties, as well as possibly functional (probiotic) properties (Holzapfel, 2002). For the production of *Gari* it is also of utmost importance to develop a process that is not only industrially applicable, but also economically feasible.

This study focused on the assessment of selected predominant LAB strains isolated from *Gari* fermentations to be produced as starter cultures and their ability to withstand a freeze-drying process. Furthermore, the starters were evaluated for their suitability as starters by their capabilities for rapid substrate acidification in addition to linamarin degradation, thereby enhancing both product quality and safety.

2. Materials and methods

2.1. Strains and culture conditions

Seventeen predominant LAB strains (9 *Lactobacillus plantarum*, 3 *Lactobacillus pentosus*, 2 *Lactobacillus fermentum*, 2 *Weissella paramesenteroides* and 1 *Leuconostoc mesenteroides* ssp. *mesenteroides*, Table 1) isolated from cassava fermentations during the production of *Gari*, were previously identified (Kostinek et al., 2007) and preselected on the basis of technological characteristics as potential starter strains. These characteristics included production of β-glucosidase, which may aid in the detoxification of cyanogenic glucosides, in addition to showing antimicrobial activity i.e., presumptive bacteriocin activity (Table 1). All strains were routinely grown in de Man, Rogosa and Sharpe (MRS) (Merck, Darmstadt, Germany) broth at 30 °C for 18 h under aerobic conditions. Stock cultures of these were stored in MRS broth containing 20% glycerol (Merck, Darmstadt, Germany) at −80 °C. Stored vials for the selected strains were thawed as needed, and used as seed inoculum.

2.2. Biomass production in 2 L fermenters

Biomass production for all 17 strains was carried out in 2 L Biostat B (B. Braun Biotech International, Melsungen, Germany) fermenters. A working volume of 1.4 L was used, which comprised 1.3 L growth medium and 100 ml of inoculum. Inocula were prepared by adding 100 μl of the selected preserved strain to 100 ml of MRS broth (approx. 1×10^7 CFU/ml), in a 250 ml Erlenmeyer flask and were incubated aerobically at 30 °C for 16 h without agitation.

The raw material composition of the various media used in the fermentations is outlined in Table 2. MRS medium is referred to as medium 1. Medium 2 was MRS medium containing additional 20 g/L of glucose. Medium 3, which was specific for the biomass production of *L. plantarum* and *L. pentosus* strains, and which is often used at the Walloon Center for Industrial Biology, University of Liège for these purposes, contains 10 g/L corn steep liquor (CSL) (Roquette, Lestrem, France). Media 4 and 5 had minor variations to medium 3. Media 4 and 5 had no meat extract, but a higher yeast extract (1 g/L) content (Table 2).

To test starter culture growth conditions prior to the fermentations in the 2 L fermenters, the 17 selected strains were grown in static flask cultures. This was either done as a scale-up and also as a pilot experiment. All *L. plantarum* and *L. pentosus* strains were grown using media 3, 4 and 5 (Table 2), while all of the *L. fermentum*, *W. paramesenteroides* and *L. mesenteroides* ssp. *mesenteroides* were grown using all 5 media compositions (results not shown). The reason for this was that the media for *L. plantarum* and *L. pentosus* were already optimised from previous work on different strains of these species (result not shown). Parameters for these were thus only adjusted to the use of CSL products that were obtained locally in South Africa (African Products, Germiston, South Africa). For the *L. fermentum, W. paramesenteroides* and *L. mesenteroides* ssp. *mesenteroides* strains optimisation of the media was required. Individual fermentation medium compositions were chosen based on the cell counts, OD_{660} and biomass produced in these small-scale pilot experiments for each strain (results not shown). The fermentation medium selected for each strain is shown in Table 2.

The media used were sterilised in the fermenter at 121 °C for 15 min. Glucose monohydrate was autoclaved separately and added aseptically to the fermenter. The set point pH was 5.6, temperature 30 °C, stirrer speed 100 rpm and airflow 0.3 sLpm (standard litres per minute). The pH was adjusted with 1 N HCl or 1 N NaOH. The fermentation was run until glucose was depleted or for ~24 h. Samples (2 mL) were taken every 2–4 h for determinations of pH, glucose concentration, optical density at 660 nm and the viable cell count (CFU/ml). Biomass was estimated using the dry weight

Table 1
Morphological and biochemical properties of selected strains used for starter culture development.

Isolate BFE No.	Country of sample origin	Morphology	Presumptive bacteriocin activity[a]	β-glucosidase activity	Presumptive identification/group	Genotypic identification
BFE 6620	Benin	Rods	+	−	Obligatory heterofermentative rods	*L. fermentum*
BFE 6625	Benin	Rods	−	−	Obligatory heterofermentative rods	*L. fermentum*
BFE 7589	Benin	Rods	+	+	*L. plantarum*-group	*L. pentosus*
BFE 7596	Benin	Rods	+	+	*L. plantarum*-group	*L. pentosus*
BFE 6748	Benin	Rods	−	+	*L. plantarum*-group	*L. pentosus*
BFE 6793	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 6710	Benin	Rods	−	+	*L. plantarum*-group	*L. plantarum*
BFE 6739	Benin	Rods	−	+	*L. plantarum*-group	*L. plantarum*
BFE 6688	Benin	Rods	−	+	*L. plantarum*-group	*L. plantarum*
BFE 6711	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 6713	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 7685	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 7687	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 7688	Benin	Rods	+	+	*L. plantarum*-group	*L. plantarum*
BFE 7601	Benin	cocci	−	+	*Leuconostoc/Weissella*	*W. paramesenteroides*
BFE 7608	Benin	cocci	−	−	*Leuconostoc/Weissella*	*W. paramesenteroides*
BFE 7668	Kenya	cocci	−	+	*Leuconostoc/Weissella*	*L. mesenteroides* ssp. *mesenteroides*

[a] Positive results indicates a zone of inhibition of at least 2 mm against the indicator organism *W. paramesenteroides* DSM 20288 as detected by spot on lawn test.