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International Journal of Rice Research

Research Article

Gallo M, et al. Int J Rice Res. IJRR-103.
DOI: 10.29011/IJRR-103. 000003

Rice Fermentation by Lactobacillus Paracasei CBA L74

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Citation: Gallo M, Nigro F, Passannanti F, Salameh D, Budelli A, et al. (2018) Rice Fermentation by Lactobacillus Paracasei CBA L74. Int J Rice Res. IJRR-103. DOI: 10.29011/IJRR-103. 000003

Received Date: 22 October, 2018; **Accepted Date:** 31 October, 2018; **Published Date:** 09 November, 2018

Abstract

In recent decades, the increase in economic wellbeing and in nutrition awareness have increased the attention of consumers towards the high-quality food. In particular, consumers pay attention to the effects of food on physiological functions, and to the connections between food components and the prevention of some pathologies. Potential solutions to the consumer interests are the functional foods. A wide spectrum of probiotic foods may be produced by bacterial fermentation of milk.

This contribution reports preliminary results regarding the development of a functional semi-finished food by fermentation of *Lactobacillus Paracasei* CBA L74 using rice flour as substrate. The process was investigated by tests carried out at a laboratory scale. Batch fermentations were carried out in a 1.5 L fermenter equipped with a thermostatic system. The medium was a suspension of rice flour and water.

The fermentation process was characterized in terms of pH, microbial population, primary (lactic acid) and secondary acid (butyric and acetic acids) concentration. The maximum bacterial charge of $3*10^8$ CFU/ml achieved during the process and the high production of lactic acid of 222,4 mg/L suggested that the conditions implemented throughout the fermentation process could be appropriate for the production of a rice flour based functional food.

Keywords: Batch Reactor; Fermentation; Functional Foods; Lactobacillus Paracasei; Mixing System

Introduction

From last scientific evidence, functional foods are acquiring more attention due to their beneficial effect on human health and prevention of diseases. The concept of functional foods includes foods or food ingredients that exert a beneficial effect on host health and/or reduce the risk of chronic diseases beyond basic nutritional functions [1]. Probiotic foods contain a single or mixed culture of microorganisms that affect beneficially the consumer's health by improving their intestinal microbial balance [2].

Fermentation is one of the oldest and most economical methods of producing and preserving food [3]. In addition, fermentation provides a natural way to destroy undesirable components, to enhance the nutritive value and appearance of the food [4]. An appropriate selection of the strain is necessary to efficiently control the different types of the metabolic end products [5-7].

Lactic acid fermentation is one of the most important food processing technologies to produce probiotic foods. Today most probiotics available on the market are dairy based providing several types of fermented milks and cheeses [8]. Several studies (conducted *in vivo* and *in vitro*) reported in the literature point out a potential positive effect of the fermented dairy based foods on the human health [9-11].

The food industry is directing new product development towards the area of functional foods and cereals could offer an alternative for the production of this type of foods. Cereals are widely harvested worldwide and contribute to over 60% of the world food production [12] providing dietary fibre, proteins, energy, minerals, and vitamins required for human health. Although they

are deficient in some basic components such as essential amino acids, fermentation may be a simple and economic way to improve their nutritional value. Furthermore, the non-digestible components in the cereal matrix may act as prebiotics and propose the cereal as a good substrate for the growth of probiotic strains [12,13].

There are some proofs that cereals may be a healthier option to produce new non-dairy probiotic foods. Indeed, they can overcome some of the disadvantages associated with fermented dairy products like lactose intolerance, allergy and the contribution to the increase of the cholesterol concentration in the blood stream [14].

The possible applications of cereals or cereal constituents in functional food formulations could be summarised: as fermentable substrates for growth of probiotic microorganisms, especially Lactobacilli and Bifidobacteria, as dietary fibre promoting several beneficial physiological effects, as prebiotics due to their content of specific non-digestible carbohydrates, as encapsulation materials for probiotic in order to enhance their stability. The possibility of fermenting a solid cereal based matrix has been already investigated, in particular in a study using water based pudding with 75% rice and 25% maize, with and without the addition of Litesse™ -a fibre with prebiotic properties. The growth and metabolism of four strains of lactobacillus was evaluated. Starting with a bacterial charge of 7 log CFU/g, the final charge of bacteria reached was 8 log CFU/g with a lactic acid production of 560-640 mg/Kg. The fermentation was carried out in glass jars for 12h with no agitation. Growth and metabolism of the selected strains of probiotic bacteria were not affected by the addition of Litesse™ [15].

The present paper reports on a recent investigation regarding the fermentation process using cereal flour as a substrate to produce ingredients of functional foods. Rice flour was used as substrate for a culture fermentation of *Lactobacillus Paracasei* CBA L74. The fermentation was characterized in terms of pH, bacterial growth, lactic acid and secondary acids production. Also kinetic parameters, such as productivity, biomass and product yield were evaluated.

Materials and Methods

Strain and Substrate

The microorganism was *L. paracasei* CBA L74 (Heinz Italia SpA, Latina, Italy), a gram positive homofermentative, facultative anaerobic bacteria. International Depositary Accession Number was LMG P-24778. The bacterial stock was stored at -26° C. The lyophilized starter culture with a potential bacterial charge of 10^{11} CFU/ml was revitalized in MRS broth by incubation at 37°C for 24h. The fermentation substrate, rice flour, was provided by Heinz Italia s.p.a. and stored at 4°C.

Apparatus

Fermentation tests were carried out in a batch reactor of 1.5-L. The experimental laboratory system consisted essentially of four components: vessel, mixing system, thermal conditioning system and temperature measurement system. The vessel was cylindrical Pyrex (20 cm high, 10 cm ID) equipped with an external jacket that allowed the circulation of a service fluid necessary to maintain the entire apparatus at a constant temperature. The fluid was thermo regulated through a thermostatically-controlled water bath.

The mixing system was a stainless-steel impeller equipped with an inclined blade turbine and a Rushton turbine that were used to provide axial and radial flow to guarantee homogeneity of the matrix and avoid gradient temperature and microorganism concentration. The impeller was linked to a motor that allowed to adjust the stirring speed (in this case it was 180 rpm chosen for a substrate viscosity of about 1.15 [Pa. s]). The impeller had an input connected to a probe, useful for measuring automatically the temperature simultaneously to the process' progress.

Operating Conditions and Procedure

The working volume was set at 1-L. Substrate composition was 85% of water, 15% of rice flour. The fermentation tests were carried out at 37°C and the total time of process was approximately 24 h. Before each fermentation, the rice was dry heated at 121°C for 90 minutes whereas each component of the system was sterilized by a standard autoclave cycle (121°C for 20 minutes). Moreover, the rice suspension was tyndallized, in order to kill vegetative forms of bacteria. Before the start of fermentation, the suspension in the reactor was subjected to two thermal cycles each formed by subsequent heating (at 70°C for 30 minutes) and cooling (at 37°C for 30 minutes).

This heat treatment caused the gelatinization of the starch in the suspension hence the use of the impeller previously described to guarantee homogeneity of suspension. Once the system was stable at 37°C, the cells under active growth state were inoculated in the fermenter. During the 24h of the process, withdrawals were done to evaluate the bacterial growth rate, pH and the production of primary and secondary acids.

Analytical Methods

The viscosity of the suspension, after the tyndallization phase was determined vs shear rate using a stress-controlled rheometer (SR200, Rheometric Scientific) equipped with parallel plates geometry of 40mm diameter. The temperature was kept constant by using a Peltier apparatus, which ensures an accuracy of ±1°C. The gap of the measurements was kept fixed to1mm. Rheological tests were carried out at 30°C. For each sample, the same test was performed three times, by loading a fresh sample each time. The

presence of micro-agglomerates in the samples did not allow the use of the cone-plate geometry that would have been preferred to parallel plates in non-linear regime.

To analyse cell growth, production of organic acids and pH values, samples were withdrawn aseptically from the bioreactor at different times up to 24h. Samples of the fermented matrix were transferred on Petri Plate to monitor the growth of lactobacilli on MRS (De Man, Rogosa and Sharpe, oxoid) agar plates using the spread plate method. Specific agar plates such as Mac Conkey (Oxoid) and PCA (Oxoid) were used to control contaminant presence. All plates were incubated for 24h at 37°C.

Lactic acid concentration was measured by using an HPLC (Agilent technologies 1100 with Visible/UV detector). Agilent Zorbax C18 column (4,6mm x 150 mm and a pore size of 80A) was used. The flow rate was set at 0.8mL/min; the mobile phase was ammonium phosphate with a pH of 2.7 with detection at 218 nm. Butyric and acetic acids concentration was carried out by gas chromatography (Agilent Technologies 6890). Capillary Poraplot Q column (25m x 0.32mm) was used. The flow rate was 200 ml\min. The mobile phase was helium gas.

The pH was measured in culture samples using a bench-top pH-meter mod. Expandable IonAnalyzer EA920-Orion Research. The substrate concentration (starch) consumed by lactobacillus during fermentation was measured by an enzymatic kit- Analytical method according to AOAC method 996.11, AATC method 76.13 and ICC standard method No.168.

1.1. Kinetic Parameters

In addition, the process was characterised in terms of: productivity, biomass and product yield. The biomass production throughout the fermentation process (*P* [mg/h], Productivity of Fermentation Process, PFP) was calculated according to the Eq. (1):

$$P = \frac{v\,tot * x_{biomass}}{t_{fermentation} + t_{dead}} \qquad (1)$$

Where v_{tot} [L] is the total volume of suspension, $x_{biomass}$ [mg/L] is the biomass produced during process; to switch from CFU/ml to mg/ml the conversion factor was calculated experimentally after 24 h of incubation, *L. Paracasei* CBA L74's growth was evaluated through spread plate method and through spectrophotometer examinations to verify if the final bacterial charge was comparable in both methods, after which the culture was centrifuged and the pellet obtained was weighed and the ratio between the weight of the latter and the number of CFU/ml read on the plates was $1*10^{-9}$. $t_{fermentation}$ [h] the time of process, and t_{dead} [h] the dead time of the fermentation process.

The amount of biomass produced to the amount of substrate consumed (Y_{sx}, Biomass Yield, BY) was calculated according to Eq (2):

$$Y_{sx} = \frac{\Delta X}{\Delta S} = \frac{X_{fin} - X_0}{S_0 - S_{fin}} \qquad (2)$$

Where X_0 [mg/L] is the initial concentration of biomass, X_{fin} [mg/L] is the final concentration of biomass, S_0 [mg/ml] is the initial concentration of substrate, S_{fin} [mg/L] is the final concentration of substrate. The amount of lactic acid produced respect to the amount of substrate consumed (Y_{sp}, Product Yield in Lactic Acid, LAPY) was calculated according to Eq (3):

$$Y_{sp} = \frac{\Delta P}{\Delta S} = \frac{P_{fin} - P_0}{S_0 - S_{fin}} \qquad (3)$$

Where P_0 [mg/L] is the initial concentration of lactic acid, P_{fin} P_{fin} [mg/L] is the final concentration of lactic acid, S_0 S_0 [mg/L] is the initial concentration of substrate, $S_{fin}S_{fin}$ [mg/L] is the final concentration of substrate.

Result

Considering the difficulties of working with high viscosity, firstly, we had to study the fluid dynamics parameters to guarantee the best condition of homogeneity. The viscosity of suspension analysed ranged from 1÷3 [Pa. s] for a range of 60÷400 [rpm], with a shear rate ranging from $125s^{-1}$ to $837s^{-1}$. The innovation of our work was the improvement of the process thanks to the use of a closed batch reactor equipped with an adequate mixing system.

In relation to the high values of viscosity measured, in fact, two kind of turbines have been selected: an inclined blade turbine and a Rushton turbine in order to obtain both axial and radial movement. Secondly, as indicators of a good performance of the fermentation, high values of CFU counts and high production of lactic acid were taken into account.

The trend of the growth curve is reported in (Figure 1) whereas lactic and secondary acids production is represented in (Figure 2). All values reported in growth and organic acids production curves, are the result of a triplicate mean value. As shown in (Figure 1), starting with an initial bacterial charge of $3,7*10^6$ CFU/ml, the maximum growth is achieved at 17h with a bacterial charge of $3*10^8$ CFU/ml; at the end of the fermentation $2*10^8$ CFU/ml was reached. This data indicated that the exponential phase start at 4h and ends at 17h.

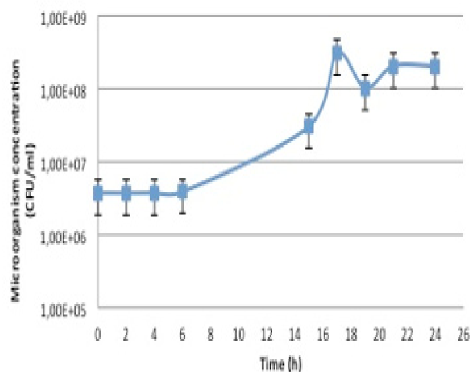

Figure 1: Growth curve.

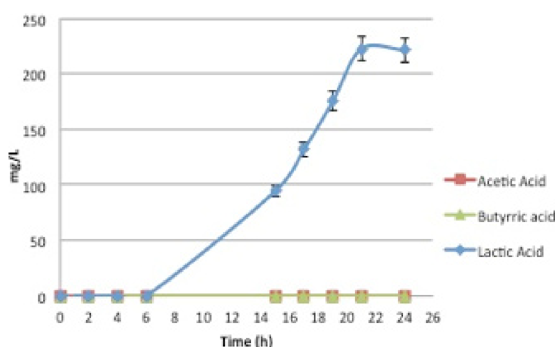

Figure 2: Organic acids.

The highest concentration of lactic acid achieved, at 24h, was 221.6 mg/L, as shown in (Figure 2) corresponding to the lowest value of pH registered at 3.67 as shown in (Figure 3). Moreover, the presence of other acids (butyric and acetic) was not detected, indicating that the fermentation process was conducted in sterile conditions and that *Lactobacillus paracasei CBA L74* is an obligatory homo-fermentative producing high levels of lactic acid.

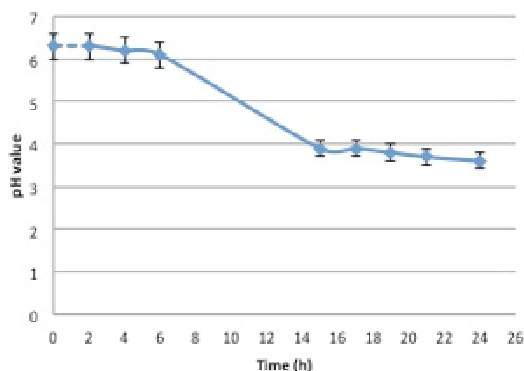

Figure 3: pH value.

The concentration of substrate was studied too, in particular, starch concentration was analysed at different times during fermentation process. From enzymatic analysis, it is possible to have an initial starch concentration of about 67 mg/ml, this concentration decreases during fermentation reaching a quantity of 19 mg/ml at the end of the fermentation.

In these conditions, *L. paracasei* CBA-L74 was able to grow in the rice flour- water suspension in batch fermentation. The productivity of the fermentation was calculated using the Eq. (1). The process achieved a productivity PFP of 5.76 mg/h. The biomass and product yield were calculated using Eq (2) and (3) respectively. The process achieved a biomass yield, BY of 0.026, and a product yield, LAPY of 0.177.

This particular lactobacillus has shown a good performance in gelatinized substrates with final bacterial concentration of 10^8 which is higher than that requested by probiotic standards. In fact, based on the literature available, a minimum level of more than 10^6 viable probiotic bacteria per millilitre or gram of food product is accepted [16].

Similar results to ours have been collected by [17] that studied the performance of L. Helveticus in a multi cereal beverage where the optimal substrate consisted of 50% malt, 25% rice and 25% maize. In its study, Jing Ai added, before the start of fermentation, α-amylase and glucoamilasi, to avoid the gelatinization of the starch; then the matrix was filtered obtaining a liquid matrix.

The latter was then autoclaved at 121°C 15 minutes. The fermentation was carried out 37°C for 24 hours with no pH control reaching a cell concentration of 8.43 log CFU/ml and a production of 1.46 mg/ml of lactic acid at the end of the fermentation.

Discussion

This process demonstrated the efficiency of the fermentation at a laboratory scale. As seen we were able to perform a lactic fermentation, obtaining a product with bacterial charge sufficient to produce a potential functional food. The innovation of our work was the improvement of the process thanks to the use of a closed batch reactor equipped with a mixing system adequate to viscosity of product, by which we obtained homogeneity of the conditions. The fermentation was performed on a rice suspension, a rich source of vitamins, prebiotics, minerals but with a high viscosity.

The product of fermentation process is characterized by a starch concentration of 19 mg/ml with a substrate consumption of about 48 mg/ml, a cell concentration of 8.43 log CFU/ml and a production of 1.46 mg/ml of lactic acid at the end of the fermentation. In conclusion, with a starch consumption of about 70% it is possible to have an increase of cell concentration of two log CFU/ml associated with a lactic acid production, obtaining a product with probiotic potential. Future prospects will be the

improvement of the process and the verification of efficiency of this process in pilot and industrial scale.

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