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Fermentability of Brown Rice and Rice Bran for Growth of Human *Lactobacillus plantarum* NCIMB 8826

*Premsuda Saman[1], Pablo Fuciños[2], José A. Vázquez[3] and Severino S. Pandiella[1]**

[1]School of Chemical Engineering and Analytical Science, The University of Manchester, Sackville Street, PO Box 88, Manchester M60 1QD, UK

[2]Department of Analytical and Food Chemistry, University of Vigo, r/As Lagoas s/n, Ourense-32004, Galicia, Spain

[3]Group of Recycling and Valorisation of Wastes, Marine Research Institute (CSIC), r/Eduardo Cabello 6, Vigo-36208, Galicia, Spain

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Summary

The growth on rice-based media of the probiotic strain *Lactobacillus plantarum* NCIMB 8826 isolated from the human gut has been investigated. Fermentation broths were obtained from the whole grain brown rice and rice bran of two Thai rice cultivars, RD6 (glutinous) and RD17 (nonglutinous). The rice used was not germinated and fermentations were carried out in a single step without growth supplementation. *L. plantarum* grew well in all tested broths, and a final biomass value of approx. 10.4 log CFU/mL was obtained. In addition, biomass production and substrate depletion were satisfactorily modelled using an unstructured mathematical model. There were no statistical differences observed among the four rice media. The results confirm that brown rice and rice bran are suitable substrates for the culture of the probiotic *L. plantarum* NCIMB 8826. Rice bran, currently a by-product of the traditional cereal processing industry, has shown similar fermentability to brown rice. This indicates that rice bran or rice bran extracts could be used in new probiotic food developments, while probably still maintaining other functional properties of the bran.

Key words: *Lactobacillus plantarum*, probiotic bacteria, cereal fermentability, brown rice, rice bran

Introduction

Rice is one of the basic components of the daily diet for nearly half of the world's population. Nutritionally it is an important source of carbohydrates, protein, iron, calcium, thiamine, riboflavin and niacin (*1*).

Consumers nowadays are increasingly demanding foods with not only acceptable organoleptic characteristics but also with health-promoting properties. A large number of individual food constituents are known or suspected to have a directly positive or negative effect on human health, and increasingly new foods have associations with different aspects of health. Food and beverages offering specific health benefits beyond basic nutrition are increasingly valued.

Probiotics are one of the fastest growing sectors within functional foods. Probiotic foods are fermented products containing sufficient number of a certain live microorganism that favourably modifies the intestinal microbiota of the host. The recently developed probiotics tend to be milk-based, although in recent years other substrates have been explored in new probiotic formulations. Amongst these substrates, cereals are becoming one of the most promising alternatives to milk due to their ability to support the growth of probiotic bacteria and their protective bile resistance effect (*2*).

*Corresponding author; E-mail: s.pandiella@manchester.ac.uk

Rice, and in particular bran rice, contains compounds like tocopherols, tocotrienols, anthocyanins, polyphenols, γ-oryzanol, enzymes, polyunsaturated fatty acids and resistant starch (*3–7*), which have shown positive effects as modulators of blood pressure, glycemia or serum cholesterol levels (*8,9*). It may also contain antioxidants that could help in the prevention of tumoral proliferation (*10,11*), and even nutraceutical prebiotics that could help control colitis by modification of the colonic microbiota (*12,13*).

Whole rice grains or fractions of the grain can also be modified to improve their nutritional value or to promote their functional properties. For centuries fermentation with lactic acid microorganisms has been a simple and inexpensive technique to preserve or upgrade the digestibility and stability of foods. Traditionally, the two main reasons to ferment rice-based foods have been firstly to prevent the action of unacceptable microorganisms, and secondly to create foods with better flavour, aroma and texture (*14*).

Despite the fermentability and functional potential of rice, there have been relatively few attempts to use this cereal as a substrate for the production of probiotic foods. The existing works in the literature refer to the use of pregerminated rice, white rice and rice bran supplemented with simple sugars or treated with exogenous enzymes to enhance saccharification and improve fermentability (*15,16*).

This work aims to evaluate the ability of brown rice and rice bran to support the growth of probiotic bacteria in a single step process, without the addition of hydrolytic enzymes or growth promoters. Whole brown rice and rice bran obtained by debranning from two different rice varieties (glutinous and nonglutinous) will be used. The biomass formation and chemical changes during fermentation will be monitored in all cases.

Materials and Methods

Milling of brown rice to produce brown rice flour

Two rice varieties, RD17 (nonglutinous rice cultivar from Pathumthani, Thailand) and RD6 (glutinous rice cultivar from Sisaket, Thailand) were used in this work. The grains were milled using a laboratory hammer mill 3100 (Perten Instruments, Segeltorp, Sweden) equipped with a 0.8-mm sieve. After cooling, the whole rice flour was stored in sealed bags at –30 °C to prevent contamination and to stop the action of cereal enzymes.

Debranning of brown rice grains to produce bran fraction

The debranning was carried out in a Satake TM05 test mill (Satake Engineering Co, Hiroshima, Japan) with an abrasive wheel (sieve size no. 40) rotating at 1450 rpm. Pearlings obtained after 20 s of abrasive polishing (bran fraction) were stored in sealed bags at –30 °C for later use.

Fermentation monitoring

Microorganism and inocula

Lactobacillus plantarum NCIMB 8826, originally isolated from human intestine, was used for the fermentation of all rice broths. The strain was maintained at 4 °C and subcultured monthly on MRS agar slants (Oxoid Ltd, Cambridge, UK).

Colonies isolated from MRS agar plates were precultured twice in MRS liquid broth at 37 °C, first for 24 h and then for 18 h, to ensure that all the cells were harvested from the early stationary phase. The bacterial suspensions were then used to inoculate the rice media at 1 % (by volume). In all cases, the initial microbial concentration was approx. 7.5 log CFU/mL.

Fermentation procedures

Shake flask fermentations were performed in duplicate using 500-mL screw-capped glass bottles with 400 mL of medium. Suspensions (3 %, by mass per volume) in distilled water were made from the whole grain flour and bran of the nonglutinous and glutinous rice varieties. The resulting slurry was then autoclaved (15 min, 121 °C) to sterilise the media and to gelatinise the rice starch. Culture media were inoculated and incubated at 150 rpm and 37 °C for 48 h. Samples were regularly taken for total cell counting, and the centrifuged fermented media (5000×*g*, 10 min, 4 °C) were stored at –20 °C for chemical analysis.

Cell enumeration

Viable cells were enumerated using the method of Collins (*17*). Decimal dilutions of fermentation broths were prepared using sterile Ringer's solution. A volume of 12 μL was dropped onto 3- to 4-day-old MRS agar plates and then incubated at 37 °C for 2 to 3 days. Viable cell counts were calculated as log colony forming units (CFU) per mL. Dilutions with less than 10 or more than 130 colonies were discarded.

Chemical analyses

The concentration of soluble free amino nitrogen (FAN) during fermentation was assayed by European Brewery Convention (EBC) ninhydrin colorimetric method (*18*). The protein content was calculated by multiplying the total Kjeldahl nitrogen by a factor of 6.25. Total reducing sugars (TRS) were assayed by the 3,5-dinitrosalicylic acid method (*19*).

Mathematical models

In order to describe and compare the kinetics of lactic acid bacteria on rice media, an unstructured mathematical model was used (*20*):

$$X=\frac{X_{max}}{1+\exp\left[2+\frac{4\cdot v_{max}}{X_{max}}\cdot(\lambda_X-t)\right]} \qquad /1/$$

$$S=S_0+\frac{X_0}{Y_{X/S}}-\frac{1}{Y_{X/S}}\cdot\frac{X_{max}}{1+\left(\frac{X_{max}}{X_0}-1\right)\cdot e^{-\frac{4\cdot v_{max}}{X_{max}}\cdot t}}-$$

$$-\frac{m_S\cdot X_{max}^2}{4\cdot v_{max}}\cdot\ln\left[\frac{X_0\cdot\left(e^{\frac{4\cdot v_{max}}{X_{max}}t}-1\right)+X_{max}}{X_{max}}\right] \qquad /2/$$

where X is biomass concentration expressed as log CFU/mL, and S is the total reducing sugars concentration (in g/L). The definition and units of the model parameters and variables are given in the list of abbreviations.

Numerical and statistical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between the observed and model-predicted values, using the non-linear least squares (quasi-Newton) method provided by the macro Solver add-in of the Microsoft Excel XP spreadsheet. Statistica v. 6.0 software (StatSoft, Inc, Tulsa, OK, USA) was used to evaluate the significance of the estimated parameters by fitting the experimental values to the proposed mathematical models, and the consistency of these equations.

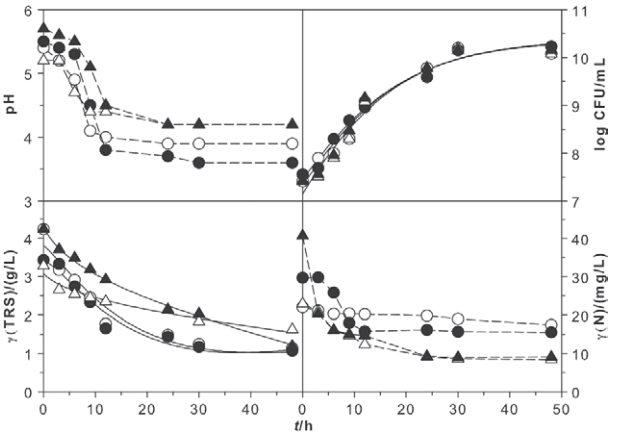

Fig. 1. Fermentation of *Lactobacillus plantarum* in rice media. Solid lines represent the mathematical models used to fit the experimental data represented by points. Dashed lines show the experimental profiles. ● glutinous bran, ○ glutinous rice, ▲ nonglutinous bran, △ nonglutinous rice. TRS=total reducing sugars, N=nitrogen

Results

The capability of the different rice fermentation broths to support the growth of *Lactobacillus plantarum* was investigated. Fig. 1 shows the growth of *L. plantarum* and the chemical changes during fermentation of the brown rice and rice bran fractions of the two rice varieties used. The numerical values of the kinetic parameters obtained from fitting the experimental data to the unstructured mathematical models as well as the statistical analysis of the equations and parameter validation are summarised in Table 1. According to these results, all media were able to provide a considerable growth of the bacteria, reaching a maximum cell concentration of approximately 10.4 log CFU/mL in 30 h. The statistical analysis shows that the differences between the tested media were not significant ($p>0.05$) in all of the kinetic parameters (v_{max} and X_{max}) defined in Eq. 1.

The pH dropped slightly through fermentation. The biggest changes were observed during the first 12 h, after which the pH remained nearly constant. The fractions from the glutinous rice variety showed larger pH drops than the nonglutinous ones. The pH drops (ΔpH) of the glutinous rice bran and brown rice were 1.9 and 1.5, respectively. The broths with nonglutinous rice gave ΔpH of 1 and 1.5 for the brown rice and rice bran fraction, respectively. In both varieties, the rice bran showed larger pH drops. The production of lactic acid was concomitant with this trend (data not shown).

The evolution of TRS was adequately described by the proposed model in Eq. 2 (see statistical analysis in Table 1). The highest biomass yield per sugar uptake ($Y_{X/S}$) was obtained in the nonglutinous rice media followed by nonglutinous bran, glutinous bran and glutinous rice.

Table 1. Parametric estimations corresponding to the kinetic models (1 and 2), applied to the cultures of *L. plantarum* in the rice media

Variables	Rice fermentation media			
	Glutinous bran	Glutinous rice	Nonglutinous bran	Nonglutinous rice
Growth (X)	values±CI	values±CI	values±CI	values±CI
X_{max}/(log CFU/mL)	10.409±0.488	10.372±0.710	10.364±0.600	10.379±0.752
X_0/(log CFU/mL)	7.433±0.308	7.311±0.481	7.199±0.443	7.147±0.527
v_{max}/(log CFU/(mL·h))	0.191±0.072	0.201±0.110	0.215±0.100	0.208±0.116
F (df_1=3, df_2=5; α=0.05)	9359.19	3840.02	4591.22	3162.25
p-value	<0.0001	<0.0001	<0.0001	<0.0001
R (obs-pred)	0.9923	0.9832	0.9871	0.9821
Sugars (S)	values±CI	values±CI	values±CI	values±CI
S_0/(log CFU/mL)	3.559±0.415	3.984±0.453	4.143±0.175	3.051±0.309
$Y_{X/S}$/[(log CFU/mL)/(g/L)]	0.851±0.399	0.720±0.288	1.870±0.674	2.557±2.277
m_S/[(g/L)/(log CFU/(mL·h))]	n.s.	n.s.	0.001±0.000	n.s.
F (df_1=3, df_2=5; α=0.05)	297.56	294.72	2918.42	581.25
p-value	<0.0001	<0.0001	<0.0001	<0.0001
R (obs-pred)	0.9799	0.9711	0.9976	0.9758

CI=confidence intervals (α=0.05), F=Fisher test (df_1=model degrees of freedom and df_2=error degrees of freedom) using a significance level (α) of 0.05, R=correlation coefficient between observed and predicted data, n.s.=not significant

The initial FAN concentration in the fermentation broths was higher for the rice bran than for the brown rice. In addition, the lowest consumption of nitrogen took place in the glutinous rice media (4.59 mg/L), while the highest was observed in nonglutinous bran (31.68 mg/L). Thus, no clear correlation between the observed FAN evolution and the corresponding cell growth and TRS consumption was observed through fermentation.

Discussion

Lactobacillus sp. has been described to have complex requirements for growth and fermentation (*21*), while brown rice and rice bran have carbohydrates, minerals, vitamins and other essential nutrients. They also contain a variety of active compounds that could lead to the development of novel functional foods and ingredients. However, most carbohydrates in rice are in the form of polysaccharides and the availability of the carbon sources for nonamylolytic microorganisms is limited.

Rice and rice bran from processing wastes have previously been used to grow lactic acid bacteria for the production of lactic acid (*22*). Rice broths have also been used to support the growth of probiotic cultures (*15,16*). In some of these works a preliminary enzymatic treatment was made to increase the availability of fermentable sugars. In other cases the fermentation media were supplemented with growth enhancers. Direct culture of probiotic strains, isolated from the human gut, on non-modified brown rice and rice bran has not been reported before and could maintain other functional properties unaltered in the raw material.

Four fermentation media prepared from brown rice and rice bran of two different rice cultivars were carried out, and the evolution of probiotic bacteria was monitored. The main compositional and physicochemical parameters measured through fermentation were pH, FAN and TRS. Fig. 1 shows that all media produce a similar growth of *L. plantarum* (over 3 log CFU/mL increment) and the maximum cell population is in all cases beyond the threshold for a minimum probiotic effect (10^6 CFU/mL) (*23*). The FAN and TRS profiles show that there are not major nutritional limitations. The statistical analysis indicates that there are not significant differences among the tested rice and rice bran fractions.

The biomass levels obtained in this work are higher than the ones obtained by Helland *et al.* (*15*). In that case, a number of lactic acid bacteria strains were grown in water-based rice and corn puddings to produce a novel probiotic food. They found that *L. rhamnosus* GG was the strain that expressed a higher growth with increments in cell populations of 1 log CFU/mL over a 12-hour period with 1 % (by mass) fructose supplement. Our experiments show an increase of biomass of 1.5 log CFU/mL during the same period. However, our results are in agreement with Trachoo *et al.* (*16*), who were able to increase the biomass of lactobacilli over 2.5 log CFU/mL in 24 h using a germinated rice broth. The different behaviour observed in these three works could be due to the composition of the media used for fermentation. In the work of Trachoo *et al.* (*16*) and in this work, germinated or nongerminated brown rice or rice bran were used, whereas Helland *et al.* (*15*) used commercial rice flour. The differences in growth could be related to the use of the bran. The outer layers of the grain have been reported to contain essential nutrients that could promote bacterial growth (*24*).

Conclusions

The obtained results support the hypothesis that brown rice or rice bran media contain the essential nutrients to support the growth of lactobacilli and can directly be used as fermentation substrates of probiotic bacteria. The reached biomass levels are above the minimum required in a probiotic formulation. Rice bran or extracts of rice bran could be used as an alternative source for non-dairy probiotics, which could increase the added value of what is currently a by-product of the cereal processing industry.

Abbreviations

X biomass as logarithm of colony forming units per millilitre/(log CFU/mL)
t time/h
X_{max} maximum biomass/(log CFU/mL)
X_0 initial biomass/(log CFU/mL)
v_{max} maximum growth rate/(log CFU/(mL·h))
λ_X growth lag phase/h
S total reducing sugars concentration/(g/L)
S_0 initial total reducing sugars concentration/(g/L)
$Y_{X/S}$ yield coefficient for biomass formation on sugar/[(log CFU/mL)/(g/L)]
m_S maintenance coefficient/[(g/L)/(log CFU/(mL·h)]

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