International Food Research Journal 20(5): 2967-2971 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Short Technical Communication

Evaluation of brown rice and germinated brown rice as an alternative substrate for probiotic food formulation using *Lactobacillus* spp. isolated from goat milk

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Article history

Received: 13 July 2011
Received in revised form: 21 March 2013
Accepted: 28 March 2013

Keywords

Lactobacillus sp.
Germinated brown rice
Nutritive evaluation
Probiotic food

Abstract

The efficiency of brown rice and germinated brown rice as a substrate for probiotic food was investigated. *Lactobacillus* isolated from goat milk was used for fermenting the rice medium. Brown rice (BR) obtained by dehulling the paddy, was germinated for 48 h. The dried powders of BR and Germinated BR (GBR) were compared for the growth of *Lactobacillus* sp. and nutritive evaluation such as carbohydrates, protein, fat, fiber and ash. The studies showed that GBR has increased protein and fiber content as compared to BR. Thus, we propose the use of GBR as an alternative substrate in making probiotic food formulations.

Introduction

Consumers prefer healthy and safe foods like functional foods over conventional ones to improve overall health and well-being and reduce the risk of specific diseases (Juliano, 1993; Premsuda *et al.*, 2011). Over the last few years, there has been an increase in awareness of the beneficial effects of probiotics. Probiotics commonly stem from the category of lactic acid bacteria especially of genera *Lactobacillus* and *Bifidobacterium* convert sugars and carbohydrates into lactic acid a major end product of carbohydrate metabolism, generated from pyruvate by lactic acid dehydrogenase. The metabolites such as lactic acid, bacteriocins are considered to be important in their beneficial effects (Trachoo *et al*., 2006) such as promotion of intestinal lactose digestion; positive influence on intestinal and urogenital flora (antibiotics and radiation induced colitis, yeast infections and vaginitis in women); prevention and reduction of intestinal tract infections (*Candida enteritis*, *Helicobacter Pylori*); improvement of immune system; provides antagonistic environment for pathogens; blocking adhesion sites from pathogens etc.. (Bengmark, 1998; Holzapfel *et al.*, 1998; Holzapfel and Ulrich Schillinger, 2002; Ishibash and Shimamura, 1993; Wolfson, 1999).

The probiotic foods found in the market are milk-based, although in recent years other substrates like oats (Jaskari, 1998), barley, wheat (Ivan Salmerona, 2009), rice bran (Premsuda *et al*., 2011), millet (Vicki Lei, 2006) and maize (Helland *et al*., 2004) have been explored in new probiotic formulations. Amongst these substrates, rice is one of the most promising alternatives to milk due to its ability to support the growth of probiotic bacteria and their protective bile resistance effect (Charalampopoulos, 2002). Rice, rice bran and broths have been found to support the growth of probiotic bacteria. In many cases, the fermentation media were supplemented with growth enhancers like quercetin, gallic acid, (Yun *et al.*, 2004; Barka *et al*., 2004; Helland *et al*., 2004; Trachoo *et al.*, 2006; Premsuda *et al*., 2011; Supriya Yadav, 2011) casein, (Anatoly Bezkorovainy, 2001) sugars like glucose, lactose (Georgieva *et al*., 2009) to enhance sacchrification and improve fermentability (Helland *et al*., 2004; Yun *et al*., 2004; Trachoo *et al*., 2006; Premsuda *et al*., 2011). Brown rice contains compounds like tocopherols, tocotrienols, anthocyanins, polyphenols, g-oryzanol, enzymes, polyunsaturated fatty acids, resistant starch and antioxidants (Haggiwara *et al*., 2004; Parroda *et al*., 2006) and germinated brown rice contains bio-functional components like dietary fibers, phytic acids, vitamins and γ-aminobutyric acid (GABA) (Ohtsubo *et al*., 2005). The purpose of this work was to study the effect of germinated brown rice and brown rice as a substrate (without the addition of hydrolytic enzymes or growth promoters) for the

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growth of probiotic *Lactobacillus* spp., which could be used as probiotic dietary supplement with high GABA, protein and fibre content.

Materials and Methods

Isolation and identification of Lactobacillus spp. from goat milk

The isolation of lactic acid bacteria from goat milk (procured from Local market, in Thanjavur) was done by using de Man Rogosa and Sharpe (MRS) agar [HiMedia, Mumbai]. Briefly, about 1 ml of goat milk was mixed in MRS broth (HiMedia, Mumbai), appropriately diluted and plated onto MRS agar. The plates were then incubated at 37°C for 24 h. Every colony was examined microscopically for Gram reaction, cell and colony morphology. Few basic biochemical tests including catalase reaction, indole test, Voges Proskaeur, methyl red test, and citrate were carried out and subsequently the promising colonies were picked and subcultured in MRS broth (Al-Allaf *et al.*, 2009).

Evaluation of antimicrobial activity of Lactobacillus spp.

For detecting antagonism against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, we performed the disc diffusion method of Hamadan and Mikolajcik with slight modifications (Hamadan *et al.*, 1974; Apella, *et al*., 1992). Sterile filter discs (10 mm) were dipped in to the culture broth of *Lactobacillus* spp. incubated for 42 h and placed on solidified nutrient agar seeded with 12 to 14 h cultures of test microorganisms and incubated at 37ºC for 14 to 16 h. Discs dipped in un-inoculated MRS broth served as control. Zones of inhibition were then measured. The antibiotic activity tests were done in triplicates and the mean values were recorded.

Preparation of rice media

White Ponni paddy was procured from Soil and Water Management Research Institute, TNAU, Kattuthotam. The paddy was dehulled as brown rice. The rice grains were soaked in water for 24 h at room temperature and allowed to germinate in a container at room temperature for 24 h. The rice grains with highest germination percentage were selected and dried in an oven at 40ºC for 24 h and powdered using Udy Cyclone pulverizer equipped with a 0.8-mm sieve. The moisture content of the powder substrates was greater than 70% as analyzed by MJ33 Mettler Toledo Infrared Moisture Analyzer. After cooling, the whole rice flour was stored in sealed bags at –30°C.

Proximal nutritional analysis for BR and GBR

Determination of total carbohydrate by Anthrone method

Briefly, 100 mg of samples containing carbohydrates were taken and hydrolyzed into simple sugars with 2.5 N HCl. The mixture was heated for 3 h in boiling water bath where glucose is dehydrated to hydroxymethyl furfural that forms green color with anthrone reagent having an absorption maximum at 630 nm. The spectral data was recorded using a double beam spectrophotometer (Shimadzu UV 1601) (Hedge *et al*., 1962).

Estimation of protein by Lowry's method

About 100 mg of sample was taken and alkaline copper solution was added. The protein binds to copper in alkaline medium and produces Cu^{2+}. In the second step, Folin-Ceocaliteau reagent was added and incubated in the dark for 30 min, where Cu^{2+} catalyses oxidation of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum blue. This reaction produces strong blue color. The readings were taken at 660 nm using a double beam spectrophotometer (Shimadzu UV 1601) (Lowry's *et al*., 1951).

Determination of crude fat content by Soxhlet method

About 10 g of sample was taken in a porous thimble and placed in the Soxhlet apparatus. The crude fat was extracted with hexane which was recycled again. This extends the contact time between the solvent and the sample and allows it time to dissolve all of the fat contained in the sample. The percentage oil content of the sample was calculated using the formula [(B-A)/W] ×100, where W is weight of sample taken, A is weight of empty flask and B is weight of flask + oil (Min and Steenson, 1998).

Determination of fiber and ash content

Con. sulphuric acid (10 mL, 1.25%) was added to 2 g of defatted sample (residue after extraction of oil) and filtered. The residue was treated with sodium hydroxide (10 mL, 1.25%) and filtered. The filtrate was dried at 105°C for 2 h and weighed to obtain fiber content of the sample. It was then burned at 550°C to yield ash content in a muffle furnace. The fiber and ash content were calculated as follows: (Henneberg and Stohmann, 1864)

$$\% \text{ Fiber content} = [(B-A)/W] \times 100$$

$$\% \text{ Ash content} = [(B-A)-(C-A)/W] \times 100$$

where, W = Weight of sample taken, A = Weight of empty crucible, B = Weight of crucible + insoluble residue and C = Weight of crucible + ash.

Fermentation of rice media by Lactobacillus spp.

About 10 g of BR and GBR powdered rice samples were taken and Lactobacillus spp. at a concentration of 10^9 CFU/ml was mixed thoroughly. The samples were allowed to ferment for 48 h at 37°C. After 48 h, the samples were freeze dried using Syclon-10N lyophilizer.

Results

Gram positive and catalase-negative isolates with characteristic cell arrangements were considered as lactic acid bacteria (Girum Tadesse *et al*., 2007). In the present study *Lactobacillus* spp. isolated from goat milk was identified as Gram positive non-motile rods, under microscopic examination. The colonies were milky white, smooth and round. The organisms were also confirmed by the following bio-chemical tests that showed positive for citrate; negative for indole, methyl red, catalase and Voges-Proskaeur tests.

According to the present study the *Lactobacillus* spp showed the broadest range of inhibitory action against *S. aureus* and *P. aeuroginosa* and least activity against *K. pneumoniae* (Figure 1). The primary antimicrobial effect exerted by the lactic acid bacteria was due to the production of lactic acid and reduction of pH (Daeschel, 1989; Vandenburg, 1993; Rossland, 2005). In addition, lactic acid bacteria produces various antimicrobial compounds, which can be classified as low-molecular mass (LMM) compounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds and high molecular-mass (HMM) compounds like bacteriocin (Jay *et al.*, 1982). Production of antimicrobial substances against pathogens has been proposed as one of the mechanisms by which they improve health and treat diseases caused by food pathogens (Bengmark, 1998).

In this study, BR and GBR powders were used as food substrates for *Lactobacillus* spp. The resulting mixture was brown in color and had a fruity aroma after fermentation. Germination causes many changes in the nutritional composition of plant seed like sugars, proteins and free amino acids (Palmiano and Juliano, 1972). Chemical analysis (Figure 2) showed significant increase in levels of protein (6.9 %) and fibre (5%) in GBR as compared to BR (Hisswey *et al*., 2002). The fermentation of rice media by lactic acid bacteria is supported by carbohydrates, minerals, vitamins and bio-functional compounds present in GBR and BR (Helland *et al.*, 2004; Trachoo *et al.*, 2006). The results from this study suggested that BR and GBR can be used as a supplement for *Lactobacillus* spp. in food fermentation.

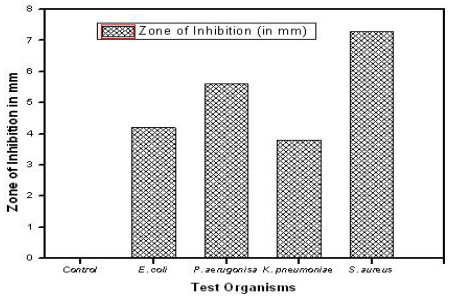

Figure 1. Antimicrobial activity of *Lactobacillus* spp.

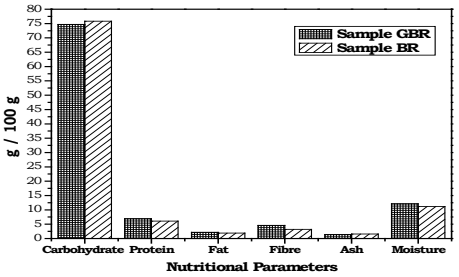

Figure 2. Nutritive evaluation of BR and GBR

Conclusion

Interest abounds in the formulation of probiotics for the diet conscious, by incorporating into foods other than fermented dairy products due to high fat and sugar content of yoghurt. Whole grain foods are highly appreciated for their nutritional value. This study supports the use of brown rice as an excellent substrate which contains the essential nutrients to support the growth of lactobacilli and can directly be used as substrates for fermentation of probiotic bacteria (Barka *et al.*, 2004; Trachoo *et al.*, 2006). However, the edibility and shelf life of the probiotic food formulation needs further study.

Acknowledgements

The work presented here was carried out in IICPT, Thanjavur. The authors thank Soil and Water Management Research Institute, TNAU, Kattuthotam for providing the paddy samples. We are grateful to Dr. Alagusundaram, Director IICPT, Thanjavur. We thank D. Selvakumar, Group Head, BTG and Prasanna S. Bakshi, DEBEL for their stimulating discussion during paper writing.

References

Al-Allaf, M. A. H., Al-Rawi, A. M. M. and Al-Mola, A. T. 2009. Antimicrobial activity of lactic acid bacteria isolated from minced beef Meat against some pathogenic bacteria. Iraqi Journal of Veterinary Sciences 23(1):115-117.

Anatoly, B. 2001. Probiotics: determinants of survival and growth in the gut. American Journal of Clinical Nutrition 73(2): 399-405.

Apella, M. C., Gonzalez, S. N., Nader, M. E., Romero, N. and Oliver, G. 1992. *In vitro* studies on the inhibition of the growth of Shigella sonnei by *Lactobacillus casei* and *Lactobacillus acidophilus*. Journal of Applied Bacteriology 73: 480-483

Barka, M. K., Mustafa, S., Kharidah, M., Suraini, A. A. and Abdul, M. Y. 2004. A nutritious medida (sudanese cereal thin porridge) prepared by fermenting malted brown rice flour with *Bifidobacterium longum* BB 536. Malaysian Journal of Nutrition 10(2): 183-193.

Bengmark, S. 1998. Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 42: 2-7.

Charalampopoulos, D., Wang, R., Pandiella, S. S. and Webb, C. 2002. Application of cereals and cereal components in functional foods: A review. International Journal of Food Microbiology 79: 131–141.

Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technology 43(1):164- 167.

El-Hissewy, A. A., Laila, F. R. and Hanaa, A.D. 2002. Effect of degree of milling on the chemical composition and nutritional value of the milled rice. Egypt Journal of Agricultural Research 80(1): 341-353.

Georgieva, R., Koleva, P., Nikolova, D., Yankov, D. and Danova S. Growth Parameters Of Probiotic Strain Lactobacillus plantarum, Isolated From Traditional White Cheese Biotechnol. & Biotechnol. Eq. 23/2009/ Se Xi Anniversary Scientific Conference Special Edition/On-Line 120 Years Of Academic Education In Biology 861-865.

Girum, T., Eden, E. and Mogessie, A. 2007. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. Internet Journal of Food Safety V (5): 13-20.

Hagiwara, H., Seki, T. and Ariga, T. 2004. The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. Biosciences, Biotechnology and Biochemistry 68: 444–447.

Hamadan, J. and Mikolajcik, E.M. 1974. Acidolin: an antibiotic produced by *Lactobacillus acidophilus*. Journal of antibiotics 27: 631-636.

Hedge, J.E. and Hofreiter, B.T. 1962. In: Carbohydrate Chemistry, 17 Whistler R.L. and Be Miller, J.N. (Ed). Academic Press, New York.

Helland, M.H., Wicklund, T. and Narvhus, J.A. 2004. Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. International Journal of Food Microbiology 91(3): 305-13.

Henneberg, W. and Stohmann, F. 1864. Begriindung einer rationellen Fiitterung der Wiederk~iuer. Vol. Ii. Schwetsehtke u. Sohn, Braunschweig, p. 324.

Holzapfel, W. H. and Schillinger, U. 2002. Introduction to pre- and Probiotics. Food Research International 35 (2-3): 109-116

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. and Huis, I. 1998. Overview of gut flora and probiotics. International Journal of Food Microbiology 41: 85-101.

Ishibashi, N. and Shimamura, S. 1993. Bifidobacteria: Research and development in Japan. Food Technology 47: 126–135.

Ivan, S., Pablo, F., Dimitris, C and Severino S. P. 2009. Volatile compounds produced by the probiotic strain *Lactobacillus plantarum* NCIMB 8826 in cereal-based substrates. Food Chemistry 117(2): 265-271.

Jaskari, J., Kontula, P., Siitonen, A., Jousimies, S. H., Mattila, S. T. and Poutanen, K. 1998. Oat beta-glucan and xylan hydrolysates as selective substrates for Bifidobacterium and Lactobacillus strains. Applied Microbiology Biotechnology 49(2):175-81.

Jay, J.M. 1982. Antimicrobial properties of diacetyl. Applied and Environmental Microbiology 44: 525-532.

Juliano, B.O.: Rice in Human Nutrition, FAO, Rome, Italy (1993) pp. 1–2.

Lowry, O. H., Rosebrough, N. J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1): 265-275.

Min, D.B. and Steenson, D.F. 1998. Crude fat analysis. In. Food Analysis, S.S. Nielsen (Ed). (pp. 201-215). Aspens Publisher, Inc., Gaithersburg, MD.

Ohtsubo, K., Suzuki, K., Yasui, Y. and Kasumi, T. 2005. Bio-Functional Components in the Processed Pre-Germinated Brown Rice by a Twin-Screw Extruder. Journal Food Composition and Analysis 18: 303-316.

Palmiano, E.P. and Juliano, B.O. 1972. Biochemical changes in the rice grain during germination. Plant Physiology 49: 751-756.

Parrado, J., Miramontes, E., Jover, M., Gutierrez, J.F., Collantes, L. and Bautista, J. 2006. Preparation of a rice bran enzymatic extract with potential use as functional food. Food Chemistry 98: 742–748.

Premsuda, S., Pablo, F., José, A.V. and Severino, S. P. 2011. Fermentability of Rice by Human *Lactobacillus plantarum.* Food Technology Biotechnology 49 (1): 128–132.

Rossland, E., Langsrud, T., Granum, P.E. and Sorhaug, T. 2005. Production of antimicrobial metabolites by strains of *Lactobacillus* or *Lactococcus* co-cultured with *Bacillus cereus* in milk. International Journal of Food Microbiology 98(2):193-200.

Supriya, Y., Snehal, G., Smita, N. and Vaishali, A. 2011. Effect of supplementation of micronutrients and phytochemicals to fructooligosaccharides on growth response of probiotics and E. coli. Biofactors 37(1):

58–64.

Trachoo, N., Boudreaux, C., Moongngarm, A., Samappito, S. and Gaensakoo, R. 2006. Effect of germinated rough rice media on growth of selected probiotic bacteria. Pakistan Journal of Biological Sciences 9: 2657–2661.

Vandenbergh, P.A. 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. FEMS Microbiology Reviews 12: 221-238.

Vicki, L., Henrik, F. and Kim, F. M. 2006. Spontaneously fermented millet product as a natural probiotic treatment for diarrhoea in young children: An intervention study in Northern Ghana. International Journal of Food Microbiology 110(3): 246-253.

Wolfson, D. 1999. Turning Pro: The myriad benefits of probiotics. Health & Nutrition Breakthroughs 8: 12-14.

Yun, J.S., Wee, Y.J., Kim, J.N. and Ryu, H.W. 2004. Fermentative production of DL-lactic acid from amylase-treated rice and wheat brans hydrolyzate by a novel lactic acid bacterium, *Lactobacillus* sp. Biotechnology Letters 26: 1613–1616.