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# Effect of Geminated Rough Rice Media on Growth of Selected Probiotic Bacteria

[1]Nathanon Trachoo, [2]Chantelle Boudreaux, [1]Anuchita Moongngarm,
[3]Supachai Samappito and [3]Rumpai Gaensakoo
[1]Department of Food Technology and Nutrition, Faculty of Technology,
Foodborne Pathogens and Biofilm Research Laboratory, Mahasarakham University 44000 Thailand
[2]Department of International Health, Johns Hopkins University Bloomberg School of Public Health,
615 N. Wolfe Street, Baltimore, MD 21205 USA
[3]Department of Biotechnology, Faculty of Technology, Mahasarakham University 44000 Thailand

**Abstract:** Effect of germinated rice media on growth of probiotic bacteria including *Lactobacillus acidophilus*, *L. pentosus*, *L. plantarum* and *L. fermentum* were studied. Germinated rough rice powder was obtained by allowing rice grains to germinate in water with hulls at 80% relative humidity and 30°C for 48 h. The germinated rice grains were dried, dehulled and powdered with a laboratory hamper mill. The powder (5% w/w) was well-mixed with water to prepare the growth medium for the probiotic bacteria strains. This was compared with medium with regular rice powder. NaCl (2%w/w) was also added to study the bacteria growth. Chemical analysis including vitamins B (thiamin, niacin and pyridoxine), reducing sugar and total protein contents in rice, brown rice and germinated rough rice were determined. Germination of rice grains increased many nutrients. Vitamins B, reducing sugar and total protein contents of germinated rough rice were higher ($p<0.05$) than those of brown rice and rice, respectively. Addition of NaCl lowered the growth of probiotic bacteria. *L. plantarum* exhibited the highest ($p<0.05$) growth in medium containing germinated rough rice powder with and without NaCl compared to the other strains. The results from this present study suggested that germinated rough rice can be used as a supplement for *L. plantarum* in food fermentation.

**Key words:** Lactic acid bacteria, probiotic bacteria, germinated rough rice

## INTRODUCTION

In the human, intestinal bacteria lining the surface of the gut act as the first layer of defense against pathogenic bacteria or other harmful elements ingested with food (Isolauri *et al*., 2004). Increased awareness of the fundamental importance of these species has helped to drive research into probiotic bacteria, bacteria which can be ingested with the specific intent of increasing the health of the subject. Many species of lactic acid bacteria are considered to have great potential in improving the digestion and overall health of humans through various pathways, including both improved lactose digestion and immunomodulation (Trachoo and Boudreaux, 2006). Some probiotic are easily accessible and can be administered through various food products such as yoghurt or other milk products while some strains are not popular. Probiotics are traditionally included as either starter and non-starter cultures in fermented dairy products such as yogurt, cheeses and buttermilk. Growth and survival of probiotic strains in dairy products have been studied (Dave and Shah, 1998; Krasaekoopt *et al*., 2006). However the consumption of dairy products by Asian populations is limited. There are a number of fermented cereal based foods such as sour porridge, fermented flat breads and pancakes, which are common in Africa and Asia (Charalampopoulos *et al*., 2002). Cereal extracts from malt, wheat and barley were reported to protect *Lactobacillus acidophilus L. plantarum* and *L. reuteri* in acidic conditions (Charalampopoulos *et al*., 2003). Rice is a major crop in Asia. It would be economical and beneficial to use rice and rice products as media for probiotics. For these reasons, it is of interest to search for appropriate rice based media for probiotics. Recent studies reported increased levels of nutrients and bioactive compounds in germinated rice (Hagiwara *et al*., 2004; Oh *et al*., 2003; Tian *et al*., 2004). These compounds include proteins, amino acids, sugars, vitamins, gamma-oryzanol, gamma-aminobutyric acid (GABA), tocotrienols and tocopherols and other phytochemical substances. Some of these

**Corresponding Author:** Nathanon Trachoo, Department of Food Technology and Nutrition, Faculty of Technology, Foodborne Pathogens and Biofilm Research Laboratory, Mahasarakham University 44000 Thailand
Tel: +66-43-722-092 Fax: +66-43-722-092

compounds may promote the growth of probiotic bacteria. This study therefore investigated effects of germinated rice media on the growth of four species of probiotic bacteria, *L. acidophilus*, *L. pentosus*, *L. plantarum* and *L. fermentum*. Selected chemical analyses were also conducted to trace changes in the chemical composition of rice after germination.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions:** Four probiotic bacteria were used, *L. acidophilus*, *L. pentosus*, *L. plantarum* and *L. fermentum*. These were taken from the TISTR Culture Collection at Bangkok MIRCEN, Thailand Institute of Scientific and Technological Research. The bacteria were activated three times before use. Freeze dried cells were first allowed to incubate in Lactobacilli MRS broth (Criterion, USA) for 24 h, then streaked on an MRS agar plate for 24 h incubation and finally streaked onto an MRS slant for a final 24 h incubation period. *L. fermentum*, *L. plantarum* and *L. pentosus* were incubated at 30°C, while *L. acidophilus* was incubated at 42°C.

**Rice powders:** Kho Ko 6, the widely consumed rice variety in Thailand, was purchased from a local store in one batch. The rice grains were soaked in water for 48 h at 28°C with 6 h water refreshment and allowed to germinate in a chamber with 80% relative humidity and 30°C for 48 h. The germinated rice grains were dried in an oven at 45°C for 48 h, dehulled and powdered with a laboratory hammer mill (FT2-A, Armfield, Hampshire, England). Finally, germinated rough rice powder was sieved to 20 meshes. The moisture content of this germinated rough rice powder was less than 10%. White rice powder was obtained by powdering polished rice grains with a laboratory hammer mill and sieving to 20 meshes.

**Media preparation:** Deionized water was combined with 5% white rice or germinated rough rice powders. The media were placed in a shaking incubator at 100 rpm for 30 min at 40°C to mix, then filtered with grade 4 filter paper (Whatman, Maidstone, England) to remove undissolved particles. The resulting solutions were added with 0% or 2% NaCl. The mixtures were then autoclaved at 121°C for 12 min.

**Chemical analysis:** Vitamin B (thiamin, niacin and pyridoxine) contents were determined using the method according to Erbas *et al.* (2005). Briefly, the powders were mixed with n-hexane and water. The mixtures were then homogenized and centrifuged. The supernatant was filtered though a Whatman 42 (Kent, UK) paper filter and 0.45 μm membrane filter. Twenty microliters of each powder extract was injected in a high performance liquid chromatography equipped with a UV-Vis detector (Spectra-Physics SP8800, USA) which was adjusted to 254 nm in absorbance mode. An analytical column ($5C_{18}$, $150\times4.6$ $mm^2$) was used with an isocratic solvent system consisting of 95% of 50 mM $KH_2PO_4$ and 5% acetonitile. All chemicals were HPCL grade. Reducing sugar was determined by dinitrosalicylic method (Miller, 1959) and used glucose solutions as standards. Total proteins were determined by Kjeldalh method (AOAC, 1995), with the conversion factor of 5.95.

**Microbiological analysis:** Four milliliters of 0.1% peptone solution was added to the activated bacteria slant and vortexed for 3 to 4 sec to mix. The cell suspension (100 μL) was added to each medium. The numbers of each probiotic bacteria were determined by spread plating technique on MSR agar at 0, 2, 6 and 24 h. *L. fermentum*, *L. plantarum* and *L. pentosus* were incubated for 24 h at 30°C, while *L. acidophilus* was incubated for 24 h at 42°C. Three repetitions were conducted for each species.

**Data analysis:** This research was conducted in the Foodborne Pathogens and Biofilm Research Laboratory (FBRL), Department of Food Technology and Nutrition, Mahasarakham University, Thailand in winter 2005. The same frozen stock cultures and equipment were used in all replicates. Data was analyzed with SAS software (SAS Institute, Cary, NC) using PROC GLM. Significant differences between means were determined using Least Significant Difference (LSD) test. Significance was determined by least square means at $p = 0.05$. Experiments were repeated three times.

## RESULTS AND DISCUSSION

In this experiment, rice and germinated rough rice powders were used with 0 and 2% NaCl as growth media for *L. acidophilus*, *L. pentosus*. *L. plantarum* and *L. fermentum*. Five percent of each rice powder was mixed with water in a shaker at 100 rpm for 30 min at 40°C. This was to release soluble substances into the aqueous phase. The resulting mixture was brownish white in color. NaCl was added to simulate environments in many fermented cereal and meat products which contain 2-10% NaCl.

Chemical analysis revealed significant increased ($p<0.05$) levels of some vitamin B contents of germinated rough rice as compared to brown rice and white rice (Table 1). Germinated rough rice was obtained by allowing

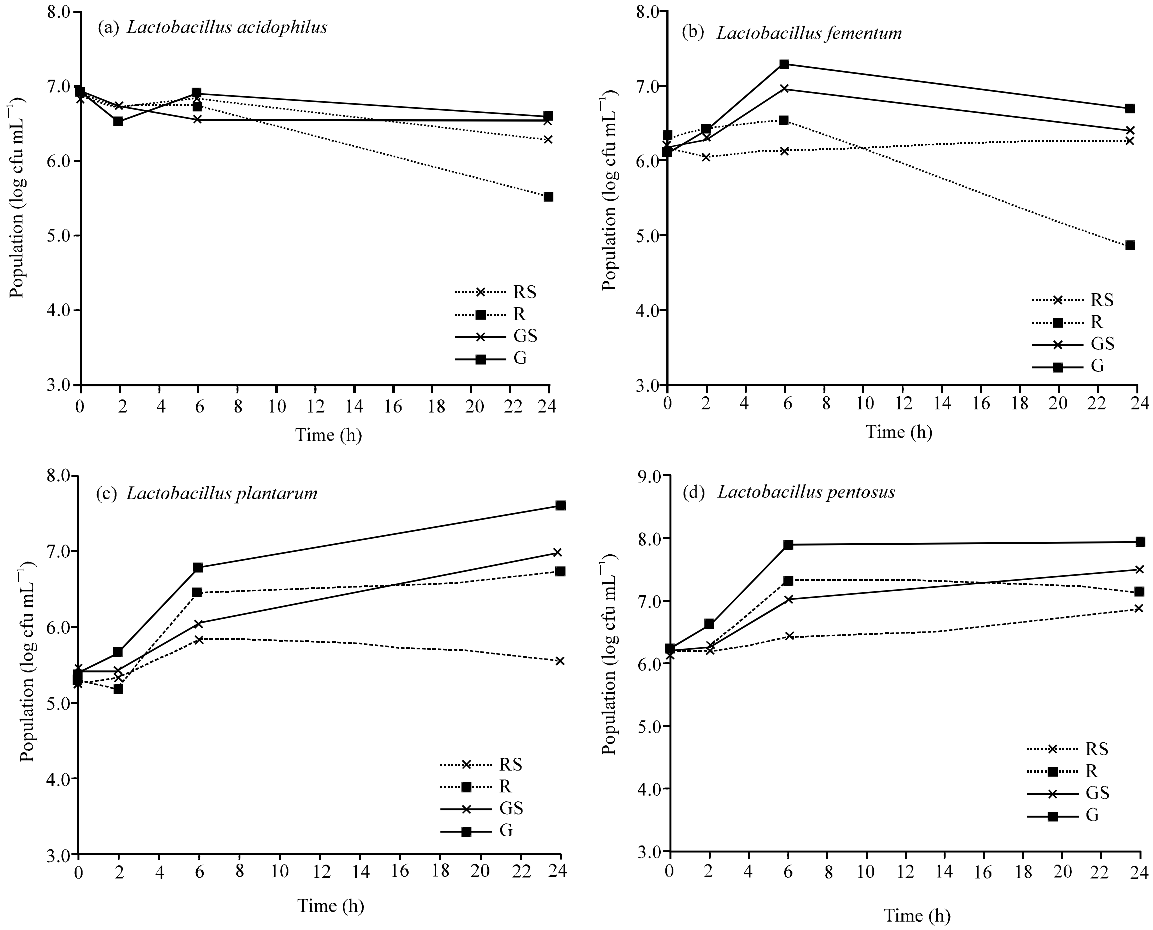


Fig. 1: Growth of four probiotic bacteria 1: (*Lactobacillus acidophilus*), 2: (*L. fermentum*), 3: (*L. plantarum*) and 4: (*L. pentosus*) in media containing 5% rice powder and 2% NaCl (RS), 5% rice powder (R), 5% germinated rough rice powder and 2% NaCl (GS) and 5% germinated rough rice powder (G)

Table 1: Vitamin[1], reducing sugar[2] and protein[3] contents in powders of rice, brown rice and germinated rough rice

| Components | Rice | Brown rice | Germinated rough rice |
|---|---|---|---|
| Thiamin (mg/100 g) | $0.77^{c}$ | $2.62^{b}$ | $4.25^{a}$ |
| Niacin (mg/100 g) | $0.93^{c}$ | $13.90^{b}$ | $24.04^{a}$ |
| Pyridoxine (mg/100 g) | $2.88^{c}$ | $22.92^{b}$ | $55.83^{a}$ |
| Reducing sugars[1] (mg/100 g) | ND | $212.23^{b}$ | $407.00^{a}$ |
| Total proteins[2] (g/100 g) | ND | $7.91^{b}$ | $8.63^{a}$ |

[1]Determined by using HPLC method (Erbas *et al.*, 2005). [2]Determined by dinitrosalicylic method and used glucose as standard. [3]Determined by Kjeldalh method, conversion factor; 5.95. ND: Not Determined

rough rice to germinate with hulls in water. The water was refreshed everyday to reduce deteriorations from microorganisms (Feng *et al.*, 2004). This is different from germinated brown rice which germinates without hull. Germination causes many changes in nutritional composition of plant seeds sugars, proteins and free amino acids (Palmiano and Juliano, 1972). Total protein content as determined by the Kjeldalh method was also increased to 8.63% in germinated rough rice (Table 1). Increased total soluble proteins and some amino acids in brown rice after germination has also been reported (Tian *et al.*, 2004). After germination, reducing sugar content was also increased (Table 1). This is due to the break down of starch into glucose (Mayer and Poljakoff-Mayber, 1975). The increase of these nutrients in germinated rough rice medium should thus be beneficial for the probiotic bacteria. The growth of probiotic bacteria in media containing germinated rough rice powder was greater ($p<0.05$) than in media containing rice powder. Information on nutrient requirements for lactobacilli is limited. Only strains important to food fermentations have been studied. The probiotic bacteria used in this study are lactic acid bacteria which are considered fastidious microorganisms demanding various essential amino acids and vitamins for growth (Salminen and Wright, 1993). *L. plantarum* requires at least three vitamins and six amino acids for growth (Costilow and Fabian, 1953a; Costilow and Fabian, 1953b). A recent study also reported vitamins B requirement of *L. plantarum*, an important lactic acid bacteria in many fermented vegetable products (Ruiz-Barba and Jimenez-Diaz, 1995). *L. plantarum* exhibited the highest ($p<0.05$) growth in medium containing germinated rough rice powder with and without NaCl (Fig. 1c) compared to the other strains. This indicates that this medium was more desirable for *L. plantarum* than the others. *L. acidophilus* showed the lowest ($p<0.05$) growth in this medium (Fig. 1a). The present of NaCl in media significantly lowered ($p<0.05$) the growth of *L. acidophilus*, *L. pentosus*. *L. plantarum* and *L. fermentum* (Fig. 1a-d). This may be due to the fact that the addition of NaCl in media caused undesirable water activity level and osmotic pressure (Doyle *et al.*, 1997) for bacterial growth. Although slowing down the fermentation, addition of NaCl or sodium chloride is essential in many fermented foods because it prevents the growth of potential spoilaging microorganisms and withdraws juice from vegetable thus making a more favorable environment for development of the desired bacteria. Sodium ions also play important role in texture development of the final product (Battcock and Azam-Ali, 1998).

Germination of rice grains increased vitamins B, reducing sugar and total protein contents. The results from this study suggested that germinated rough rice can be used as a supplement for *L. plantarum* in food fermentation.

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