Selection of Probiotic Lactic Acid Bacteria Isolated from Fermented Plant Beverages

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Abstract: Screening for probiotic bacteria from non-human sources were performed in this study. Three hundred and twenty-seven strains of Lactic Acid Bacteria (LAB) were isolated from 90 samples of Fermented Plant Beverages (FPBs) and pickles collected from around Thailand. Potentially useful probiotic properties were investigated in vitro in parallel with a commercial probiotic strain of Lactobacillus casei, R obtained from a dairy probiotic product in Thailand. An isolate SS2, selected from a fermented star fruit beverage, survived in the human biological barriers (0.15 and 0.30% bile salt, pH values between 3-8, presence or absence of oxygen), resistance to some antibiotics in general use and showed other benefits to the host (antibacterial activity, utilizations of protein and starch). The isolate SS2 had a higher specific growth rate and better inhibitory properties against food borne pathogenic bacteria and spoilage organisms than the commercial probiotic R strain. It also grew well in MRS and the SPY2 medium that is free from animal-derived ingredients. (r=0.8). The isolate SS2 was therefore considered to be a potentially useful probiotic LAB for a non dairy product such as FPBs and was provisionally identified as a strain of Lactobacillus plantarum.

Key words: Probiotic lactic acid bacteria, biological barriers, antibiotic resistance, antibacterial activity, non-human origin, fermented plant beverages

INTRODUCTION

Probiotics are known as live microbial feed supplements that provide many benefits to the host by improving its microbial intestinal balance (Ganner and Schaffma, 1998). In general, the best probiotic strains to give to humans are those that originated from the human intestine as they may be assumed to be the true probiotics (Surawicz 1998; Marteau and Rambaud, 1993). Most probiotic microbes are LAB such as Lactobacillus casei, L. plantarum and Streptococcus lactis (Sindhu and Khetarpaul, 2001). Probiotic LAB are normally used as starter cultures for fermented dairy products like yogurt. In recent years, consumers who are lactose intolerant and worry about the cholesterol content in milk as well as vegetarians increasingly demand for non-dairy-based probiotics. In Thailand, fermented plant beverages are considered to be non-alcoholic and people who consume these FPBs believe that plant beverages are healthy due to their high nutritional value and presence of bioactive compounds derived from the plant substrates used and also during the fermentation processes (Kantachote and Charemjarittrakul, 2007). However, scaling up the beverage production for commercial use can have a problem with a high contamination by yeasts. To restrict this, starter cultures have been advocated (Prachayakij et al., 2007). Consequently, the aims of this study were to isolate and select an appropriate probiotic LAB from fermented plant products to use as a starter culture and restrict yeast growth for the production of FPBs.

MATERIALS AND METHODS

Selection and isolation of probiotic LAB: LAB strains were isolated from 90 samples of fermented plant beverages and pickles collected from different areas in Thailand and they were used to investigate their probiotics during year 2005-2006. The selection was conducted to investigate probiotic properties of LAB in order steps as following provided while any strains which showed a good performance for each probiotic property were again selected for a next step. Possible biological barriers were investigated as described by Conway et al. (1987), such as survival in 0.15 and 0.30% (w/v) bile salt and also tolerance to pH values of 2, 3, 4, 5, 8 and 9. Briefly, LAB strains were grown in MRS (de Man Rogosa...
RESULTS AND DISCUSSION

One probiotic lactic acid bacterium, isolate SS2, was selected from 327 strains of LAB isolated from samples of FPBs and pickles. The isolate SS2 was provisionally identified as Lactobacillus plantarum (data not shown) and it was isolated from a fermented star fruit (Averrhoa carambola) beverage from Si-Sa-Ket province in the Northeast part of Thailand. As shown in Table 1, the selected strain SS2 and a commercial strain R were able to grow with both 0.15 and 0.30% bile salt in a modified MRS medium. Both strains had survival rates of more than 90% at pH 3 and 4 after a 3 h incubation time. It was surprising that more than 50% of the isolate SS2 survived at pH 8, but the R strain died. However, neither grew at pH 2 or 9. These tests for screening probiotic bacteria are appropriate because normally they must transit through the acidic condition of the stomach and bile in the intestine (Lee and Salminen, 1995). In addition, oxygen, one of the human physiological barriers, has a strong negative effect on the survival of possible probiotics bacteria in commercial products (Shah et al., 1995). Fortunately, isolate SS2 and the commercial strain showed no significant differences in their growth in the presence

<table>
<thead>
<tr>
<th>Strain</th>
<th>0.15 and 0.30% bile salt</th>
<th>Acid-base tolerance</th>
<th>Nutrient digested</th>
<th>Oxygen effect</th>
<th>Cobalamin free medium</th>
<th>Antibiotic susceptibility test</th>
<th>SPY2/Animal ingredient free medium and MRS</th>
<th>Generation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS2</td>
<td>Growth at pH 3, 4, 5, 8</td>
<td>Protein, starch</td>
<td>NS</td>
<td>Growth</td>
<td>Resistance to VC, BC, GT, KC, ST, NF, PB</td>
<td>Growth r &gt; 0.8, p &lt; 0.05</td>
<td>51 min</td>
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<tr>
<td>R</td>
<td>Growth at pH 3, 4, 5</td>
<td>Protein</td>
<td>NS</td>
<td>Growth</td>
<td>Resistance to VC, BC, GT, KC, ST, NF, PB</td>
<td>Growth r &gt; 0.8, p &lt; 0.05</td>
<td>71 min</td>
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</tbody>
</table>

- Protein and starch utilizations were determined in 3% milk agar plates and 0.2% starch agar plates, respectively. | NS: No significant difference (p > 0.05) between growth with or without oxygen present. | VC: Vancomycin; BC: Bacitracin; GT: Gentamicin; KC: Kanamycin; ST: Streptomycin; NF: Norfloxacin; PB: Polymyxin B; other tested antibiotics were ampicillin (10 mcg), cephalothin (30 mcg), cefazolin (30 mcg), chloramphenicol (30 mcg), erythromycin (15 mcg), penicillin G (10 mcg), cefoperazone (75 mcg), tetracycline (30 mcg)

<table>
<thead>
<tr>
<th>Condition</th>
<th>S. aureus ATCC 25923</th>
<th>B. cereus ATCC 11778</th>
<th>S. sonnei ATCC 25922</th>
<th>S. flexneri ATCC 35</th>
<th>P. vulgaris ATCC 9141</th>
<th>P. rettgeri ATCC 15482</th>
<th>E. cloacae ATCC 13863</th>
<th>E. aerogenes ATCC 13046</th>
<th>E. coli ATCC 25922</th>
<th>C. cl. H7 ATCC 13046</th>
<th>S. pyogenes ATCC 19615</th>
<th>S. pseud intermedia ATCC 19615</th>
<th>V. parahaemolyticus ATCC 19615</th>
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<tbody>
<tr>
<td>1</td>
<td>SS2</td>
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1: Production of organic acids and H₂O₂. 2: No control of organic acid production but a limitation of H₂O₂ production. 3: Control of organic acids and H₂O₂ production, +++: >10 mm inhibition zone; ++: 7-10 mm inhibition zone; +: 5-7 mm inhibition zone and -: No inhibition zone.
or absence of oxygen. In addition, both strains achieved an OD_{600} \text{nm} > 2.00 in a cobalamin deficient medium so neither had a growth requirement for vitamin B_{12}. Hence, the isolate SS2 should not need to compete with the human body for cobalamin. This is especially important for vegetarians whose vitamin B_{12} levels are normally low because of its absence in their foods (Herbert, 1973). Utilization of protein and starch was observed in the milk and starch agar plates by the isolate SS2 but the R strain utilized only protein. This meant that the selected strain may provide more benefit to human digestion than the commercial strain.

The isolate SS2 had a higher antibacterial activity against 13 strains of food borne pathogenic bacteria and spoilage organisms than that found with the R strain in all conditions tested (Table 2). The antagonist activity of probiotics has long been used to preserve foods. The antimicrobial substances produced by probiotics bacteria act to improve the composition and the activity of the normal microbiota in the intestine (Ouwehand and Vesterlund, 2004). In addition, the antibiotic susceptibility tests showed that the SS2 and R strains were resistant to 7 of the antibiotics used, vancomycin (30 mcg), bacitracin (10 mcg), gentamicin (10 mcg), kanamycin (30 mcg), streptomycin (10 mcg), norfloxacin (10 mcg) and polymyxin (300 mcg). Results of a clinical trial indicated the advantages of administering both antibiotics and a probiotic strain to patients with recurrent Clostridium difficile infections (McFarland et al., 1994). Besides, coadministration may benefit patients whose normal intestinal microbiota has become unbalanced or greatly reduced in numbers due to the administration of various antimicrobial agents (Salminen et al., 1998). Both SS2 and R strains grew well in the MR5 and SPY2 broths with a Pearson's correlation level r > 0.8. That means both strains could grow well in media containing either animal or plant ingredients. The isolate SS2 had a generation time of 51 min in SPY2 medium, whereas the R strain took 71 min for one generation. This could indicate that SS2 could respond more quickly than R to any potential antagonist activity.

We conclude that the selected strain, L. plantarum SS2, could be developed as an effective starter culture for use as a probiotic in a fermented plant beverage produced especially for vegetarians or others preferring to use a fermented plant product free from animal products.

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REFERENCES


