**Lactobacillus acidophilus** Utilization of Sugars and Production of a Fermented Soybean Product

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Abstract

Eight strains of *Lactobacillus acidophilus*, obtained from the ARS Culture Collection, were tested for their ability to use monosaccharides [fructose, galactose, glucose and D(+)mannose], disaccharides (lactose, maltose and sucrose) and two oligosaccharides (raffinose and stachyose). As judged by titratable acidity and pH changes during growth, strain variation existed in the ability of the microorganisms to utilize the sugars. Strain NRRL B-1910 was a superior utilizer of raffinose and stachyose as measured by medium pH changes. Therefore, B-1910 was selected as inoculum for the successful production of a yoghurt-like soybean product, which has potential as a good protein food source.

Résumé

On a fait l'étude de huit lignées de *Lactobacillus acidophilus*, en provenance de la Collection de cultures ARS pour leur aptitude à utiliser les monosaccharides (fructose, galactose, glucose et D(+)mannose), les disaccharides (lactose, maltose et sucrose), et deux oligosaccharides (raffinose et stachyose). D'après les changements de l'acidité titrable et du pH avec la croissance, on a observé que l'aptitude à l'utilisation des sucres variait avec la lignée. Les changements du pH médium ont indiqué que la lignée NRRL B-1910 était supérieure aux autres pour l'utilisation du raffinose et du stachyose. En conséquence, on a choisi cette lignée comme inoculum pour la production réussie d'un yoghurt-a base de soja, lequel a un potentiel comme une bonne source de protéines alimentaires.

Introduction

*Lactobacillus acidophilus* ferments glucose by homofermentation into lactic acid (Breed et al., 1957). Mitra and Steinkraus (1974) have shown that *L. acidophilus* strain ATCC 4356 uses glucose and sucrose but not melibiose, raffinose and stachyose. Aside from generalizations about *L. acidophilus*, the literature contains little about various strains of the species using other specific sugars. One purpose of our study was to determine how eight strains of the microorganism used fructose, galactose, glucose, lactose, maltose, D(+)mannose, raffinose, stachyose and sucrose. A second purpose was to produce a yoghurt-like product from soybean using "preferred" strains of the lactobacilli. The *L. acidophilus* strain that utilized best the three primary soluble sugars in soybeans (sucrose 5.0%, stachyose 3.8% and raffinose 1.1%) (Smith and Circle, 1972) was chosen to ferment soybean milk. Also included were organoleptic evaluations of the fermented product.

Materials and Methods

**Cultures** Eight lyophilized strains of *L. acidophilus* were supplied by the ARS Culture Collection, maintained at the Northern Regional Research Laboratory. The lyophilized strains were grown in a medium consisting of liver extract, 10%; yeast extract, 0.5%; tryptone, 1.0%; K₂HPO₄, 0.2%; glucose, 0.5%; and unweighed particles of liver chips. The cultures were maintained on agar slants containing 2.0% agar; 4.5% A.P.T.¹ media (consisting of: Bacto-Yeast extract, 7.5 g; Bacto-tryptone, 12.5 g; Bacto-dextrose, 10.0 g; sodium citrate, 5.0 g; thiamine hydrochloride, 0.001 g; sodium chloride, 5.0 g; dipotassium phosphate, 5.0 g; manganese chloride, 0.14 g; magnesium sulfate, 0.8 g; ferrous sulfate, 0.04 g; sorbitan monooleate complex, 0.2 g) (Baltimore Biological, Cockeysville, Maryland); and 0.1% Tween 80. Stock culture transfers were made on a monthly basis to maintain viability.

**Inoculum** Inoculum was grown and standardized in a broth of 4.5% A.P.T. media and 0.1% Tween 80. The A.P.T.-Tween 80 broth was sterilized in 12 x 125 mm glass tubes by autoclaving at 121°C for 15 min. After cooling the sterile medium, specific strains were aseptically added and grown stationary for 24 hr at 37°C. Cells were then harvested in a Safety-Head centrifuge (Clay-Adams, Inc.) at about 3500 rpm for 10 min. Supernatant was discarded; the cells were suspended in sterile 0.1% peptone solution and centrifuged two additional times. After the final wash, cells were resuspended in peptone, and optical density was adjusted to 10-12% light transmission at 500 nm in a Bausch and Lomb Spectronic 20.

**Carbohydrate utilization** Test medium (50 ml) for carbohydrate utilization was sterilized in 250-ml Erlenmeyer flasks. The medium consisted of peptone, 1.0%; yeast extract, 1.0%; Tween 80, 0.1%; and the specific sugar selected, 0.5%. The sugar solutions were sterilized by Millipore filtration and, subsequently, added to the test medium. After specific sugar addition, standardized inoculum (2.0%) of each strain of the bacterium was added separately to the flasks and incubated (stationary) at 37°C.

At successive 12-hr incubation intervals (for a total of 48 hr), 10 ml of uniformly suspended *L. acidophilus* cultures were examined for growth and acid production. A Beckman Model G pH meter was used to measure pH changes. Readings were standardized with sterile test medium as controls. Culture samples (5.0 ml at each 12 hr) were titrated with 0.025 N NaOH with phenolphthalein as the indicator. Titratable acidity was determined by subtracting the amount of acid present in an uninoculated medium sample from that found in the test culture.

**Fermentation of soybean milk** A schematic outline of the fermentation process is shown in Figure 1. Soybean milk was prepared along with subsequent denaturation as follows: Beeson soybeans were thoroughly washed and dehulled after soaking for 24 hr in distilled water at room temperature. The soaked beans were placed in distilled water and blended into a thick slurry in a Waring Blender at maximum rpm (high speed). The ratio of water to dry beans was 9:1. The slurry was filtered through several layers of cheesecloth into a 1000-ml Erlenmeyer flask. Portions of 50 ml raw soybean milk was then poured into 100-ml beakers, covered with cotton-filled gauze lids, and denatured by autoclaving at 121°C for 5 min; imme-

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Table 1. The utilization of various sugars by eight strains of Lactobacillus acidophilus evaluated by determining titratable acidity after 48 hr of incubation.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>629</th>
<th>1833</th>
<th>1858</th>
<th>1910</th>
<th>1911</th>
<th>1912</th>
<th>2092</th>
<th>2178</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>6.1</td>
<td>4.7</td>
<td>7.0</td>
<td>6.6</td>
<td>9.7</td>
<td>10.3</td>
<td>5.9</td>
<td>9.8</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.6</td>
<td>1.5</td>
<td>7.8</td>
<td>3.4</td>
<td>7.1</td>
<td>9.6</td>
<td>3.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.6</td>
<td>7.4</td>
<td>13.1</td>
<td>5.9</td>
<td>9.2</td>
<td>10.3</td>
<td>8.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.9</td>
<td>4.7</td>
<td>3.4</td>
<td>5.7</td>
<td>5.7</td>
<td>13.3</td>
<td>8.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Maltose</td>
<td>4.3</td>
<td>3.3</td>
<td>3.2</td>
<td>5.5</td>
<td>10.0</td>
<td>12.3</td>
<td>5.1</td>
<td>7.6</td>
</tr>
<tr>
<td>D (+) mannose</td>
<td>3.4</td>
<td>6.0</td>
<td>6.2</td>
<td>5.3</td>
<td>11.6</td>
<td>13.5</td>
<td>3.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.9</td>
<td>1.3</td>
<td>1.9</td>
<td>2.4</td>
<td>5.4</td>
<td>2.1</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Stachyose</td>
<td>1.2</td>
<td>1.4</td>
<td>1.7</td>
<td>3.5</td>
<td>1.9</td>
<td>2.7</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.0</td>
<td>4.4</td>
<td>13.4</td>
<td>8.5</td>
<td>3.6</td>
<td>5.2</td>
<td>8.9</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Titratable acidity = ml 0.025N NaOH required to neutralize 5.0-ml sample: average of duplicate experiments. Least significant difference is 1.11 for all strains; significance level is 0.05 for fructose and 0.01 for all other sugars.

...nitrogen—2092 and 2178 and two strains, 1910 and 1911...pigments. In addition, three strains, 1910, 1912, and 1911, used glycerol, whereas 2092 and 2178 utilized xanthan gum. In the next trial, 1910 and 1911, but not 2092 and 2178, utilized stachyose. However, data indicate that strain variation in rates of utilization of specific sugars is attributable to the varying levels of enzymes that convert sugars into glucose.

The two strains utilizing raffinose also utilized stachyose (Figure 3) with NRRL B-1910 being outstanding. NRRL B-1910 had not completed growth at the end of 48...
hr., whereas B-1911 reached a growth plateau after 12-hr incubation. Again, the presence of an alpha-galactosidase allowed B-1910 to utilize stachyose fully as it did.

Soybean yoghurt production Strain B-1910, which best utilized raffinose and stachyose, was selected for the soybean yoghurt production. Other experiments indicated that 5.0% inoculum, an incubation time of 24 hr at 37°C and addition of 0.5% gelatin to the medium would give the best product. Several temperatures (85°C, 100°C and 121°C) and times (15 and 5 min and 3 sec) were used in denaturing soybean milk before deciding upon 121°C for 5 min. Denaturing soy proteins allows for a greater water-holding capacity by the yoghurt and therefore less syneresis. When soybean milk was not denatured, much syneresis occurred. High temperature also destroys the lipoxigenase systems that partially contribute to rancidity. Incubation at 37°C minimized syneresis and resulted in a smooth curd.

A distinct advantage in choosing the strain that ferments both stachyose and raffinose lies in the fact that the human gastrointestinal tract does not possess alpha-galactosidase (Gitzelman and Auricchio, 1965). When a food containing these sugars is ingested, they pass undigested to the lower gut where various bacteria evolve undesirable methane, carbon dioxide and hydrogen gasses (Gall, 1968; Richards et al., 1968; and Steggerda et al., 1966).

Along with a reduction of undesirable flatulence factors, fermenting soybean milk reduces objectionable flavors associated with soy products (Wang et al., 1974). The NRRL taste panel determined that the grassy/beany flavor of soybean was reduced considerably in the soybean milk fermented yoghurt (Table 2). The grassy/beany flavor of soybean products is a common complaint of Westerners. The soybean yoghurt with 3.0% sucrose added was judged more desirable than soybean milk or soybean yoghurt. The taste panel's only complaint was that a rancid "off-flavor" existed in the yoghurt itself and if this flavor could be eliminated, the yoghurt would be acceptable as a substitute for traditional foods. For example, dairy yoghurt has been a long standing tradition in Iranian diets, and soybean yoghurt would be an inexpensive, substitute protein source for that country. The American Soybean Association has served as liaison between USDA and the Iranian Government in the development of an acceptable soybean yoghurt.
product. Iranian taste preferences for an extremely tart-flavored yoghurt was taken into consideration during the development of our fermented soybean product.

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References


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