Note

Screening for Bacteria Producing Sucrose Phosphorylase and Characterization of the Enzymes

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Received May 12, 1995

Two microbial strains, No. 165 and No. 168, were isolated from soil as sucrose phosphorylase producers and identified as Leuconostoc mesenteroides subsp. mesenteroides and subsp. dextranicum, respectively. The sucrose phosphorylases were purified, characterized, and compared with the enzymes of L. mesenteroides AKU1102 and ATCC12291. As for the catalytic properties, these enzymes were close to each other, while as for the enzyme molecules, the No. 165 enzyme (M: 58,000) was slightly different from the other (M: 54,000), though their N-terminal amino acid sequences were almost the same.

Key words: sucrose phosphorylase; Leuconostoc mesenteroides; glucosyltransferase; N-terminal amino acid sequence; molecular diversity

Sucrose phosphorylase (SPase) [EC 2.4.1.7] catalyzes the phosphorolysis of sucrose to α-D-glucose-1-phosphate (G-1-P) and D-fructose and its reverse reaction. The enzyme is a glucosyltransferase that can transfer the glycosyl group of sucrose and G-1-P to P, and several sugars including D-fructose. The enzyme from Leuconostoc mesenteroides ATCC 12291 was reported to use various sugar alcohols and phenolic compounds as glucosyl acceptors. SPase, exclusively from L. mesenteroides, is used practically in the enzymatic assay of P, and feasibly in the enzymatic glycosylation of various compounds.

Few microorganisms are known to produce SPase, and this might be related to the fact that so far no simple direct screening test is available for such strains. The enzymes from L. mesenteroides and Pseudomonas saccharophila were purified and characterized, and recently, glucosyltransferase A of Streptococcus mutans was identified as an SPase. Among these enzymes, some differences in molecular weight and substrate specificity are observed. Although the physiological roles of SPase are little known, if the enzyme is not essential in cell-life, the enzyme might be allowed to accumulate many mutations and to be metamorphosed. From the point of enzyme evolution, we are interested in the structural and functional diversity of SPases and in molecular relationships with other disaccharide phosphorolases. For such comparative studies and also practical uses, we searched for new SPase-producers. This paper describes the screening of SPase-producers and the characterization of their enzymes.

To screen sucrose-utilizable microorganisms, two kinds of media described by Doudoroff were used: PS medium consisted of 0.5 g of sucrose, 0.22 g of KH₂PO₄, 0.61 g of Na₂HPO₄, 0.1 g of NH₄Cl, 0.01 g of yeast extract, 0.05 g of MgSO₄·7H₂O, 5 mg of ferric ammonium citrate, and 0.5 mg of CaCl₂ in 100 ml of tap water, pH 7.0, and LM medium consisted of 1 g of sucrose, 1 g of yeast extract, 1 g of peptone, 0.5 g of K₂HPO₄, 0.04 g of MgSO₄·7H₂O, 0.02 g of MnCl₂, 4H₂O, 2 mg of FeSO₄·7H₂O, 1 mg of thiamin·HCl, and 5 mg of L-ascorbic acid in 100 ml of tap water, pH 7.0. All cultivations were done at 30°C. From 30 samples of sugar-contaminated soil, approximately 700 strains were isolated on the PS and LM plates, and they were examined for the SPase activity as follows. The ceols grown in 2 ml of the PS or LM broth for 12 h with shaking were harvested, washed, and tolenuenized in 0.05 M phosphate buffer, pH 6.5, containing 5% tolenuene. A portion of the tolenuenized cells was incubated with sucrose at 37°C for 2 h and G-1-P produced was detected by paper chromatography.

Two bacterial strains, called No. 165 and No. 168, were obtained that grew in the LM medium and produced much G-1-P. The production of fructose as well as G-1-P was confirmed by HPLC analysis using a Shodex Ionpack KS-802 column (Showa Denko, Tokyo). These two strains were identified according to Bergey's Manual of Systematic Bacteriology. Both strains are Gram-positive cocci, usually occurring in pairs and chains, facultative anaerobes, ferment glucose to produce D-(-)-lactic acid and ethanol, and produce characteristic dextran slime from sucrose. No. 165 produces acid from arabinose, while No. 168 does not. Therefore, No. 165 was identified as L. mesenteroides subsp. mesenteroides and No. 168 was L. mesenteroides subsp. dextranicum.

In addition, we searched for SPase-positive strains in our bacterial collections, and the most active strain, L. mesenteroides AKU1102, was selected. Three strains, No. 165, No. 168, and AKU1102, were somewhat different in a few properties such as milk coagulation and vitamin requirements.

SPase activity was assayed by the measurement of P liberated from G-1-P. A reaction mixture containing 40 nm Tris-maleate buffer, pH 6.5, 50 mm fructose, 10 nm G-1-P, and enzyme was incubated at 30°C for 5 min, and after the addition of an equal volume of 16% trichloroacetic acid, P, in the mixture was measured by the Takahashi method. One unit of enzyme was defined as the amount of enzyme that produced 1 μmol of P, per min under these conditions. The SPase activities of both isolates were about 0.2 unit/ml OD₆₅₀ of culture when grown overnight in the LM medium and that of AKU1102 was half as much.

When these strains were cultured statically in the LM medium, the SPase activity of each culture increased with the growth but rapidly declined after the maximal growth. The addition of CaCO₃ (0.5%) to prevent the pH-lowering of the culture had no effect on the growth and the enzyme activity. Addition of sucrose increased the growth and enzyme production. Thus, each strain was cultured in 10 liters of LM medium containing 2% sucrose for 8 to 10 h and used for the enzyme purification. The three SPases were purified by the same procedures involving ultrasonic disruption, ammonium sulfate fractionation (50-90%), and column chroma.

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Abbreviations: SPase, sucrose phosphorylase; G-1-P, glucose-1-phosphate; HPLC, high pressure liquid chromatography; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.
Table Purification of Sucrose Phosphorylase from *L. mesenteroides* AKU1102

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein (mg)</th>
<th>Activity (unit)</th>
<th>Specific activity (unit/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell free extract</td>
<td>3420</td>
<td>2610</td>
<td>0.8</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ fraction</td>
<td>1380</td>
<td>1680</td>
<td>1.2</td>
<td>64</td>
</tr>
<tr>
<td>Butyl-Toyopearl</td>
<td>394</td>
<td>1650</td>
<td>4.2</td>
<td>63</td>
</tr>
<tr>
<td>DEAE-Toyopearl</td>
<td>106</td>
<td>1400</td>
<td>13.2</td>
<td>54</td>
</tr>
<tr>
<td>Superose 12</td>
<td>44</td>
<td>1370</td>
<td>31.1</td>
<td>52</td>
</tr>
<tr>
<td>Mono Q</td>
<td>19.4</td>
<td>726</td>
<td>37.4</td>
<td>28</td>
</tr>
</tbody>
</table>

* Measured by the method of Lowry et al.115

Fig. 1. SDS Polyacrylamide Gel Electrophoresis of Purified Sucrose Phosphorylases.

The purified enzymes from strains AKU1102 (lane 2), No. 165 (lane 3), and AKU1102 (lane 4) were electrophoresed using a 10% gel as described by Laemmli121 and stained with Coomassie brilliant blue.

Fig. 2. Comparison of N-Terminal Amino Acid Sequences of Sucrose Phosphorylases from Isolates No. 165 and No. 168, *L. mesenteroides* AKU1102, and ATCC 12291.129 S. mutans,131 and *A. vitii*.132

Asterisks indicate identical amino acids with those of the No. 165 enzyme.

References
Leucomostor Sucrose Phosphorylases