Beta-Amylase Production by *Bacillus polymyxa* on a Corn Steep-Starch-Salts Medium

Dwight E. Hensley, Karl L. Smiley, Joyce A. Boundy, and Adolph A. Lagoda

Northern Regional Research Center, Agricultural Research, Science and Education Administration, United States Department of Agriculture, Peoria, Illinois 61604

Bacteria were found that are capable of producing good yields of β-amylase in unrefined media. The culture filtrates are free of α-amylase and isoamylase.

It is now well established that microorganisms, especially from the genus *Bacillus*, produce a β-amylase very similar to that found in higher plants such as barley and sweet potatoes. Like the β-amylase from higher plants, it is usually accompanied by α-amylase or isoamylase (1, 5, 7, 9). In addition to the problem of contaminating amylases in the crude culture filtrates, it has generally been found that commercial peptone or polypeptone is required for high yields of β-amylase (2, 7, 8). Satisfactory β-amylase yields were obtained from *Bacillus megaterium* in a medium containing 5% casein and 2% distiller’s residue as a nitrogen source (3). Although casein is less expensive than peptone, it is costly for large-scale fermentations. Recently Napier received a patent (E. J. Napier, U.S. patent 4,011,136, March, 1977) in which it is claimed that selected strains of *Bacillus circulans* can produce good yields of β-amylase with corn steep liquor as the principal nitrogen source. However, from the description in the patent, it is apparent some fractionation is necessary to obtain β-amylase free from other enzymes.

We have found some strains of *Bacillus polymyxa* that also produce good yields of β-amylase on a corn steep-starch medium. Crude culture filtrates of these organisms are apparently free of α-amylase and isoamylase as evidenced by the fact that starch digests, made with the filtrates, consist almost entirely of maltose and a β-limit dextrin.

Thirty strains of *B. polymyxa* provided by the Northern Regional Research Laboratory (NRRL) culture collection were tested. Of these, three showed definite evidence of a β-limit dextrin in digests of amylopectin by crude culture filtrates. Strain NRRL B-367 was chosen for further study because, under the conditions provided, it produced little mucoid polysaccharide characteristic of other strains and because it produced good yields of β-amylase on a corn steep liquor medium.

The β-amylase was produced in 20-liter stainless steel fermentors (10-liter working volume). The inoculum medium (500 ml in a 2,800-ml sterile Fernbach flask) and the preinoculum medium (50 ml in a sterile 300-ml Erlenmeyer flask) consisted of 0.3% beef extract, 0.5% peptone, and 0.5% glucose. The preinoculum was prepared by inoculating the sterile medium from a fresh slant of NRRL B-367 and incubating at 30°C for 24 h on a rotary shaker at 150 rpm. The entire contents of the preinoculum flask was added to the sterile inoculum medium, which was incubated under the same conditions.

The fermentor medium contained the following (in grams per liter): KCl, 1.0; MgCl₂·6H₂O, 0.2; NaH₂PO₄, 5.4; Na₂HPO₄·2H₂O, 7.0; CaCl₂·2H₂O, 0.25; FeSO₄·7H₂O, 0.005; MnSO₄·5H₂O, 0.001; and soluble (Linter) starch (Pfanstiehl), 20.0. The nitrogen sources tested with the above medium consisted of peptone (Difco Laboratories), Amber BYF-300 (Amber Laboratories), Vico D-300 (A. E. Staley), and corn steep liquor (CPC International), all at a 1% dry basis. The pH of the medium was adjusted to 6.8, and the medium was sterilized at 121°C for 25 min. The fermentor was inoculated and incubated for 48 h at 35°C. Agitation was maintained at 300 rpm, and air was admitted through a pipe sparger at 1.0 volume/volume/min.

After completion of a run, the cells were removed by centrifugation, and the cell-free liquor was used as an enzyme source. The enzyme activity was determined by reacting 2 ml of (0.2%) amylose or soluble starch substrate with 0.2 ml of suitably diluted enzyme at pH 6.5. After 1 h at 40°C, the reaction was stopped by boiling the sample in a water bath for 10 min. Reducing sugar was measured with an automatic analyzer instrument by reduction of alkaline ferricyanide reagent (4). Reducing sugar was calculated as maltose. A unit of activity is defined as that amount of β-amylase required to produce 1 μmol of maltose in 1 min under the above conditions.

The pattern of action of NRRL B-367 was studied by incubating the crude enzyme with...
0.5% amylpectin (waxy maize starch) for periods up to 3 days at pH 6.5 and 40°C. The hydrolytic products were characterized by their starch-iodine complexes (6), by gel permeation chromatography, and by high-pressure liquid chromatography. For these extended tests, toluene may be layered on reaction mixtures to prevent the growth of contaminants.

In the presence of 2% soluble starch, all the nitrogen sources gave comparable results. The yields of β-amylase were as follows (micromoles of maltose produced per minute per milliliter): corn steep, 3.01; Difco peptone, 3.06; Amber BYF-300, 3.30; and Vico D-300, 3.40.

Of interest for large-scale production of pure β-amylase is the ability of low-cost corn steep liquor to substitute for peptone, an ingredient found necessary by Griffin and Fogarty (2). Yields of β-amylase may be increased by raising the percentages of starch and corn steep liquor. The data in Table 1 show that enzyme yield depends on an adequate starch and nitrogen supply.

Persistence of a starch-iodine color in digests of amylepectin by crude culture filtrates suggests the presence of a β-limit dextrin. It also shows that both α-amylase and debranching enzymes are absent because the achroic point is not reached even with reaction times up to 72 h.

The presence of a β-limit dextrin is also suggested by chromatographing the amylepectin digests on a Waters μ-Bondagel E linear column. In Fig. 1, peak A represents the β-limit dextrin, and its elution time corresponds to a mean molecular weight of about 7,000. Peak B represents the low-molecular-weight sugars.

The composition of the low-molecular-weight fraction was determined by precipitation of the β-limit dextrin from the amylepectin digests with 80% methanol, removal of the alcohol from the alcohol-soluble fraction, and finally chromatography on a Waters μ-Bondapak-NH2 column. Maltose was the only sugar found (Fig. 2). The absence of small amounts of maltotriose and glucose, which are typically present in β-

### Table 1. Relationships of corn steep liquor and starch concentration of β-amylase yield by B. polymyxa NRRL B-367

<table>
<thead>
<tr>
<th>Soluble starch (Lindner) (%) dry basis</th>
<th>Corn steep (%) dry basis</th>
<th>β-Amylase (μmol/min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>2.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>4.05</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4.51</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>6.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inorganic salts omitted in this experiment.

<sup>b</sup> Incubation (72 h) at 30°C.
maltotriose formed when amyllose is digested with β-amylase.

This study shows that B. polymyxa NRRL B-367 forms a typical β-amylase in a commercially feasible medium. Neither α-amylase nor debranching enzymes appear to be present in the culture broth.

We are indebted to Lawrence K. Nakamura for supplying us with the microorganisms.

**LITERATURE CITED**


