Amylase Production by Thermomonospora curvata

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Thermomonospora curvata produces an extracellular inducible amylase which does not accumulate products repressive to cellulase production during growth on starch-cellulose ratios similar to those of compost.

During a study (8) by the U.S. Public Health Service on the open-window method of municipal solid-waste composting, the average starch and cellulose dry weights of the ground refuse at the beginning of the process were 4 and 49%, respectively. The starch was rapidly degraded to low levels by 28 days, whereas most of the cellulose degradation occurred only after the starch was utilized. This apparent inhibition of cellulose degradation by the presence of starch in compost is consistent with reports (7, 15) that starch as a carbohydrate source resulted in repression of cellulase production in bacteria and fungi. The effect that starch had on cellulase production prompted experiments on the ability of Thermomonospora curvata to degrade starch and on the effect of starch degradation products on its cellulytic potential. This thermophilic actinomycete was abundant in compost samples taken during a previous study (12) and was later identified (9); its identity has been confirmed by whole-cell hydrolysis patterns chromatographically determined by the method of Lechevalier (5). Its potential as a cellulose decomposer in composting has been described (10, 11).

Although Bergey’s Manual of Determinative Bacteriology (4) describes T. curvata as unable to hydrolyze starch, our compost isolate grew well on starch as the sole carbon source in a medium containing 0.1 M phosphate (pH 8), 0.2% (NH₄)₂SO₄, 0.01% MgSO₄·6H₂O, 0.1 mM CaCl₂, and 0.5–10 μg/ml concentrations of biotin and thiamine. The amylase activity that accumulated in the fluid of cultures shaken at 52°C was measured by using reaction mixtures of 2 ml of a 4% solution of soluble starch (Fisher Scientific Co.), 1 ml of 1.0 M acetate buffer (pH 6), and 1 ml of suitably diluted cell-free culture fluid. Reducing sugar accumulation during a 10-min incubation at 65°C was measured by the Bernfeld method (2). The following data are the means of at least two experiments.

The inducible nature of amylase production by T. curvata is illustrated in Table 1. High activity was observed during growth on carbohydrates with α-1,4-glucoside linkages, whereas little activity was observed during growth on β-1,4-linked carbohydrates or glucose, although dry cell weight reached 1 mg/ml or greater. Shaken cultures on suitable substrates rapidly accumulated extracellular amylase in proportion to soluble protein and dry cell weight during the first 2 or 3 days of growth (Fig. 1). In unshaken cultures, amylase activity was much lower, although accumulation of extracellular protein was comparable to that in shaken cultures. Fluids from the unshaken cultures had specific activity only about 6% that of shaken cultures, and total soluble reducing sugar (measured as glucose) remained below 16 μg/ml. Therefore, all further studies on amylase production were made with shaken cultures.

Although a literature search revealed no report on a highly purified amylase from a thermophilic actinomycete, a partially purified amylase from Thermomonospora vulgaris produced glucose and maltose from starch (1), and thermophilic bacteria such as Bacillus acidocaldarius (3) possess amylolytic patterns of glucose, maltose, and maltotriose. The presence of such soluble sugars has long been known to be repressive toward cellulase production in bacteria and fungi (6, 13). We have found T. curvata to be no exception in this regard, since glucose and maltose were effective repressors of cellulase production even at 100 to 500 μg/ml. Therefore, it was important to determine the effect of starch degradation on cellulase production. When the ratio of starch to cellulose (supplied as cotton fibers) was varied over a range encompassing that in raw compost, amylase activity peaked early and then declined rapidly. Varying the ratio of starch to cellulose had no apparent effect on cellulase production (Fig. 2), and soluble protein and reducing sugar accumulation were not appreciably altered. Chromatography of desalted samples by the method of Welker and Campbell (14) during early growth of cultures containing starch-cellulose mixtures failed to reveal reducing sugars with Rₐ values.
of glucose, maltose, and maltotriose, although spots having Rf values of larger oligosaccharides were apparent. In later samples, a single spot having the Rf of cellulobiose was apparent, as would be expected (11). The inability of starch to repress cellulase production in T. curvata is unusual; this inability may be due to an amylolytic pattern that does not accumulate extracellular concentrations of repressive sugars such as glucose and maltose. Purification of the amylase from T. curvata and determination of its products will allow evaluation of this hypothesis, and such work is currently in progress.

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**TABLE 1. Effect of carbon source on amylase production by T. curvata**

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Sp act (amylose units/mg of protein)</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>0</td>
</tr>
<tr>
<td>Cellulobiose</td>
<td>0.34 (2)</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>0.61 (6)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0</td>
</tr>
<tr>
<td>Starch</td>
<td>16.4 (3)</td>
</tr>
<tr>
<td>Glycogen</td>
<td>18.9 (3)</td>
</tr>
<tr>
<td>Amylose</td>
<td>13.9 (5)</td>
</tr>
<tr>
<td>Maltose</td>
<td>17.8 (3)</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>15.5 (3)</td>
</tr>
</tbody>
</table>

*C Cultures were grown at 52°C in mineral salts-vitamin medium with indicated carbon source for 6 days. Specific activities are the peak values observed during that period, and numbers in parentheses indicate day on which maximal enzyme activity occurred.

**FIG. 1. Growth (O), amylase production (○), and soluble protein accumulation (Δ) by T. curvata in mineral salts-vitamin medium with 0.5% maltose as carbon source in 100-ml shake cultures at 52°C.**

**FIG. 2. Amylase (Δ) and cellulase (○) production in cultures containing: (A) no starch; (B) 0.05% starch; (C) 0.1% starch; (D) 0.2% starch. All cultures contained 8 mg of chopped cotton fibers per ml as cellulose source in the mineral salts-vitamin medium.**

**LITERATURE CITED**


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