Sporulation and Heat Resistance of *Bacillus stearothermophilus* Spores Produced in Chemically Defined Media\(^1\)

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The effect of amino acids on sporulation is discussed. Heat-resistant spores were produced in a chemically defined medium.

Campbell and Williams (2) developed and used a chemically defined medium for the study of spore germination and outgrowth but not for sporulation of thermophilic aerobic sporeformers. Sporulation for the rough variant of strain NCA 1518 of *Bacillus stearothermophilus* in a chemically defined medium was studied by Tandon and Gollakota (5). In our studies we explored the use of Campbell and Williams' medium as a sporulation medium for the smooth variant of *B. stearothermophilus* strain NCA 1518 by using each of the amino acids in Campbell and Williams' "complete" medium singly and some amino acids in selected combinations. A 10-ppm amount of MnSO\(_4\) was added to their medium to enhance sporulation. Spores were produced on an agar medium by using agar washed in water (15x), ethyl alcohol (2x), and in acetone (2x). The microorganism was grown on the "complete" medium to prevent transferring with the inoculum nutrients which were not a part of the composition of the chemically defined media. The petri plates were incubated for 48 hr at 55 C.

The amount of sporulation was determined by staining smears and by plate counts before and after boiling the suspension for 5 min. Dextrose tryptone agar (Difco) was used as the plating medium with the plates incubated for 48 hr at 55 C. After it was determined that this strain sporulated with only single sources of amino acids present, spores were produced with lysine used as the only organic nitrogen source in aerated cultures (1-liter Erlenmeyer flasks with a magnetic stirring bar) to test the heat resistance of the spores.

The heat resistance of the spores produced in the lysine broth was determined by heating the spores in sealed Pyrex tubes in an oil bath at 119 C; the survivors were determined in Trypticase soy agar. All plates were incubated at 55 C for 24 hr.

The data in Table 1 show that the per cent of sporulation was low when the "complete" medium of Campbell and Williams (2) was used (plate count, 27.0%; and direct count, 12.5%). The "complete" medium had 108.9 mg of amino acids present per 100 ml; yet, when individual amino acids were used by themselves (histidine, 4.5 mg/100 ml; tryptophan, 6.0 mg/100 ml; methionine, 6.0 mg/100 ml; and lysine, 19.5 mg/100 ml), there were more spores produced even though there was a lower concentration of total amino acids than in the "complete" medium. When arginine, glutamic acid, and valine were combined with histidine, less sporulation occurred (1.9, 5.6, and 0.0%, respectively) than when histidine was used by itself (37.2%). Yet, the total amount of amino acids was greater in the combinations than when histidine was used by itself. These data show that the combination of amino acids present in a sporulation medium can influence the degree of sporulation. These data also suggest that growth can occur without the amino acids, isoleucine, leucine, valine, methionine, histidine, and arginine which O'Brien and Campbell (4) listed as essential for growth.

Spores produced in lysine broth had heat resistance comparable with the resistance of spores produced in ordinary media. The mean D value at 119 C was 9.6 with a range of 6.5 to 15.2. All spore crops were examined for purity to make sure that no contamination had occurred in handling the cultures.

The data in this study suggest that this

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table 1. effect of single and combined amino acids on sporulation using the medium of campbell and williams (1953)

<table>
<thead>
<tr>
<th>amino acid</th>
<th>amino acid concn (mg/100 ml)</th>
<th>media ph</th>
<th>total plate count*</th>
<th>spore count*</th>
<th>per cent sporulation (plate count)</th>
<th>per cent sporulation (direct count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>complete medium</td>
<td>108.9</td>
<td></td>
<td>186 700</td>
<td>64 700</td>
<td>27.0</td>
<td>12.5</td>
</tr>
<tr>
<td>no amino acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>no nh₄cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>no amino acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cystine</td>
<td>4.8</td>
<td>7.15</td>
<td>1 400</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>valine</td>
<td>14.4</td>
<td>7.30</td>
<td>272 000</td>
<td>532</td>
<td>0.2</td>
<td>1.0</td>
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<tr>
<td>isoleucine</td>
<td>14.0</td>
<td>7.25</td>
<td>7 794 400</td>
<td>459 000</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>arginine</td>
<td>10.5</td>
<td>7.25</td>
<td>2 833 800</td>
<td>168 000</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>19.2</td>
<td>7.25</td>
<td>703 800</td>
<td>13 400</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>glutamic acid</td>
<td>10.0</td>
<td>7.05</td>
<td>519 000</td>
<td>54 900</td>
<td>10.6</td>
<td>7.8</td>
</tr>
<tr>
<td>histidine</td>
<td>4.5</td>
<td>7.20</td>
<td>11 878 800</td>
<td>4 399 300</td>
<td>37.2</td>
<td>41.6</td>
</tr>
<tr>
<td>tryptophan</td>
<td>6.0</td>
<td>7.25</td>
<td>14 403 800</td>
<td>7 109 300</td>
<td>38.6</td>
<td>59.1</td>
</tr>
<tr>
<td>methionine</td>
<td>6.0</td>
<td>7.5</td>
<td>13 378 800</td>
<td>7 109 300</td>
<td>53.1</td>
<td>73.9</td>
</tr>
<tr>
<td>lysine</td>
<td>19.5</td>
<td>7.25</td>
<td>9 578 800</td>
<td>4 721 800</td>
<td>49.3</td>
<td>89.7</td>
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<tr>
<td>arginine and histidine</td>
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<td>7.20</td>
<td>1 788 000</td>
<td>28 600</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>glutamic acid and histidine</td>
<td>14.5</td>
<td>7.10</td>
<td>650 700</td>
<td>36 200</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>valine and histidine</td>
<td>18.9</td>
<td>7.25</td>
<td>12 000</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
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</tbody>
</table>

* mean counts of duplicate plates incubated at 55°c for 48 hr. samples heated in boiling water bath for 5 min.

strain is converting single amino acids into other amino acids during growth and sporulation. bernlohr (1) found, for example, that a great deal of amino acid oxidation and interconversion occurs during sporulation. the data also show that the amino acid requirements for this strain may have changed from those found by campbell and williams (2), possibly as a result of mutation and selection because we grew the organism on the chemically defined medium. by selection we have obtained cultures of this strain which will grow and sporulate without any amino acids but with ammonium chloride as the only nitrogen source in the sporulation medium. our findings are also at variance with tandon and gollakota (5) because they used a medium containing arginine, histidine, leucine, isoleucine, methionine, and valine. they did not attempt, however, to determine the influence of single or combined amino acids upon sporulation, and they worked with the rough variant. it would appear, however, that the nutritional requirement for sporulation of a strain may be influenced by the variant form, as we suggested previously for vegetative growth (3). also, by selection, one can vary the amino requirements for growth and sporulation of a single variant form.

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