TRACE ELEMENT REQUIREMENTS OF \textit{BACILLUS SUBTILIS} FOR MYCOBACILLIN FORMATION

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In studies on the nutritional requirements of \textit{Bacillus subtilis} during mycobacillin formation in complex media, it was observed that the addition of a mixture of salts (KH$_2$PO$_4$, MgSO$_4$, FeSO$_4$, and MnSO$_4$) to a casein hydrolyzate-glucose medium greatly enhanced mycobacillin formation (Majumdar and Bose, 1959). Reports by a number of workers (Jansen and Hirschman, 1944; Hendlin, 1949) indicate that elements (K, Fe, Mg, Mn, etc.) possess a special role in the formation of the two antibacterial antibiotics bacitracin and subtilin by \textit{B. subtilis} in a defined medium. The present communication deals with a study to assess the individual role played by trace elements in the biosynthesis of mycobacillin.

\textbf{MATERIALS AND METHODS}

A strain of \textit{B. subtilis}, known to produce an antifungal antibiotic, mycobacillin, was used throughout the study (Majumdar and Bose, 1958). The effect of trace elements on mycobacillin production was studied in the following medium: glucose, 10.0 g; glutamic acid, 5.0 g; KH$_2$PO$_4$, 1.0 g; KCl, 0.5 g; MgSO$_4$·7H$_2$O, 0.2 g; FeSO$_4$·7H$_2$O, 0.01 g; MnSO$_4$·4H$_2$O, 0.01 g; distilled and demineralized water, 1 L (pH adjusted to 7.0). In practice, the nutrient under test was first omitted from and then added to the basal medium in graded doses in separate flasks to determine optimal concentrations. In each experiment the composition of the basal medium was so altered as to include an optimal amount of an element from the previous study. In these experiments, however, a purified basal medium was used. The chief source of trace element impurities lies in sugar, amino acid, and the salts, e.g., KH$_2$PO$_4$, KCl, and MgSO$_4$ of the medium. Only analytical grade reagents were used after a special purification process which was as follows:

The necessary amounts of glucose, glutamic acid, and KH$_2$PO$_4$ were each dissolved separately in 200 ml of glass distilled and demineralized water, and the resulting solution was shaken twice with a mixture of 0.1 g of 8-hydroxyquinoline and 5 ml of chloroform in a separating funnel first at pH 7.2 and then at pH 5.2. After each extraction, the solution was washed 3 times with 5 ml and then once with 10 ml of chloroform to free the medium from traces of 8-hydroxyquinoline. Another source of contamination is glassware; clean pyrex glass was used throughout the experiments. The ingredients so purified were incorporated in the media along with other nutrients. The glucose solution was, however, added to the medium after separate sterilization. The medium was dispensed in 50 ml volumes in 250-ml Erlenmeyer flasks and inoculated with 0.2 ml of cell suspension. For the preparation of this inoculum, the culture was previously grown in 50 ml of the defined medium in 250-ml flasks for 2 days at 30 °C. The growth after harvesting was washed 3 times with sterile water and then resuspended in 50 ml of water. The medium so inoculated was incubated at 27 °C for 6 days. After fermentation, the potency of the culture filtrate was determined by paper disc method against the sensitive organism \textit{G. Brus (Aspergillus niger)}. The results are expressed in terms of bacillomycin units per ml, being calculated from the standard curve drawn between the zone of inhibition in mm as ordinate and concentration of bacillomycin as abscissa (0.1 mg of a standard preparation of bacillomycin in 1 ml of 0.75 per cent NaHCO$_3$ solution produces 17.5 mm as zone of inhibition and this has been taken as a unit of activity) (Majumdar and Bose, 1955).

As a measure of cell growth, the dry weights of pellets were employed. Drying of cells was carried out at 60 °C for 24 hr (Feeney et al., 1948).

\textbf{RESULTS}

\textit{Effect of trace elements on mycobacillin formation.}

The results indicating the effect of trace elements Fe, Mn, Cu, Zn, Co, and Ni on mycobacillin formation are shown in tables 1 to 3.
TABLE 1
Effect of iron and manganese on mycobacillin production (Fe added as FeSO₄·7H₂O and Mn as MnSO₄·4H₂O)

<table>
<thead>
<tr>
<th>Conc of Fe or Mn (µg/ml)</th>
<th>Growth (µg/L)</th>
<th>Activity (units/ml)</th>
<th>Conc of Fe or Mn (µg/ml)</th>
<th>Growth (µg/L)</th>
<th>Activity (units/ml)</th>
</tr>
</thead>
<tbody>
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<td>0.0</td>
<td>0.0</td>
<td>470</td>
<td>0.0</td>
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<tr>
<td>0.025</td>
<td>870</td>
<td>0.85</td>
<td>0.025</td>
<td>1000</td>
<td>0.57</td>
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<tr>
<td>0.05</td>
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<td>1.19</td>
<td>0.05</td>
<td>1184</td>
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<tr>
<td>0.125</td>
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<td>1.75</td>
<td>0.125</td>
<td>1560</td>
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<tr>
<td>1.25</td>
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<td>2.34</td>
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<td>1540</td>
<td>2.34</td>
</tr>
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<td>2.34</td>
</tr>
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<td>2.0</td>
<td>5.0</td>
<td>1520</td>
<td>2.34</td>
</tr>
</tbody>
</table>

TABLE 2
Effect of copper and zinc on mycobacillin production (Cu added as CuSO₄·5H₂O and Zn as ZnSO₄·7H₂O)

<table>
<thead>
<tr>
<th>Conc of Cu or Zn (µg/ml)</th>
<th>Growth (µg/L)</th>
<th>Activity (units/ml)</th>
<th>Conc of Cu or Zn (µg/ml)</th>
<th>Growth (µg/L)</th>
<th>Activity (units/ml)</th>
</tr>
</thead>
<tbody>
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<tr>
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<tr>
<td>1.25</td>
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<td>1.75</td>
<td>1.25</td>
<td>1490</td>
<td>2.0</td>
</tr>
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</table>

It appears from the results that trace elements Fe, Mn, and Cu are required in amounts per ml of 0.5, 1.25, and 0.05 µg, respectively, for maximum production of mycobacillin, whereas trace elements like Zn, Co, and Ni seem to be without effect. The optimal concentrations for growth were, however, different.

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SUMMARY

Studies on the trace element requirements of Bacillus subtilis during mycobacillin formation show that the elements Fe, Mn, and Cu are required in amounts per ml of 0.5, 1.25, and 0.05 µg, respectively, whereas trace elements Zn, Co, and Ni seem to be without effect.

REFERENCES


HENDLIN, D. 1949 The nutritional requirements of a bacitracin-producing strain of Bacillus subtilis. Arch. Biochem., 24, 435-446.


