NOTES

Role of Carbon and Nitrogen Sources in Bacterial Growth and Sporulation

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A simple growth medium is reported, in which Bacillus megaterium forms heat-stable spores, heat-labile spores, or only vegetative cells by changing the carbon and nitrogen source. Studies carried out in such media could be very useful in elucidating biochemical events which lead to bacterial sporulation.

If suitable conditions exist during the lifecycle of sporeforming bacteria the bacteria pass through three distinctly different stages: the vegetative cell (VC), the heat-labile spore (HLS), and finally the mature heat-stable spore (HSS). Under favorable conditions, the spore again germinates to give rise to VC. The nature of the biochemical processes that trigger spore formation is as yet not well understood. Knowledge of these processes would be useful in the proper understanding of and in controlling sporulation. One approach towards such studies would be to develop different synthetic growth media that would support the formation of only one type of cell in the sporeforming bacteria; i.e., (i) a VC medium in which only vegetative growth is possible and the VC formed cannot be transformed into spores, (ii) an HLS medium in which the VC formed can be converted only into HLS, and (iii) an HSS medium in which all of the cells can develop into HSS. After developing such media, one can compare different metabolic pathways or look for new metabolic reactions in the cells growing in different types of media at different stages of growth. It is possible that the metabolic pattern of the cells growing in VC, HLS, and HSS media may be strikingly different. Such studies could give us an insight into the biochemical events that initiate sporulation as well as those which are necessary for spore formation.

This communication describes a synthetic medium, wherein by a simple change in the concentration of the carbon and nitrogen source

or by the use of different carbon and nitrogen sources, the medium gives either VC, HLS, or HSS. In these studies, Bacillus megaterium 753 (U.S. Department of Agriculture) was used.

<table>
<thead>
<tr>
<th>Carbon and nitrogen source</th>
<th>Cell counts/ml&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TVC</td>
</tr>
<tr>
<td>Ammonium glutamate (30 mM)</td>
<td>280</td>
</tr>
<tr>
<td>Ammonium glutamate (10 mM)</td>
<td>82</td>
</tr>
<tr>
<td>Glucose (10 mM), ammonium nitrate (1.2 mM), and ammonium chloride (1.0 mM)</td>
<td>120</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cell counts were made after 44 hr of growth, because in this organism sporulation is completed between 38 to 44 hr. Abbreviations: TVC, total viable counts; HLS, heat-labile spore; HSS, heat-stable spore.

<sup>b</sup> Expressed × 10<sup>4</sup>.

This organism was chosen because of its simple growth requirements. The cells were grown by the active culture technique of Halvorson (1). Total viable counts (TVC), HLS, and HSS counts...
were determined as described earlier (2). When vegetative cells were not transformed into spores, the TVC represent the VC count. The cells were grown in the basal medium containing the following components plus the carbon and nitrogen sources as indicated below (all of the concentrations are expressed in millimolar final concentrations): FeCl₃, 0.0036; MgCl₂, 0.04; MnCl₂, 0.1; Na₂SO₄, 0.48; CaCl₂, 0.75; and KH₂PO₄, 10. When 30 mM ammonium glutamate was added to the above basal medium, it gave only VC; i.e., in this medium VC failed to sporulate. When the concentration of ammonium glutamate was reduced to 10 mM in the above medium, more than 85% of the VC formed were transformed into HLS. When ammonium glutamate was replaced by 10 mM glucose, 1.2 mM NH₄NO₃, and 1.0 mM NH₄Cl in the above medium, more than 90% of the cells were converted into HSS (Table 1). It is apparent from the above results that glutamate in higher concentration completely represses the biochemical processes that are necessary for spore formation in B. megaterium 753, and at lesser concentration it allows the formation of heat-labile spores. Studies are in progress aimed at understanding the mode of action of glutamate in bacterial sporulation.

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I thank K. G. Gollakota for helpful discussion.

LITERATURE CITED

ERRATA

Production of Anticapsin by *Streptomyces griseoplanus*

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Volume 21, no. 6, page 1076, column 2, line 16 of the *Results*: Insert “(Fig. 2)” after “basal medium 3.”

Page 1077, column 1, line 4: Change “(Fig. 2)” to “(Fig. 3).”

Page 1077, column 2, line 5: Change “(Fig. 3)” to “(Fig. 4).”

Page 1078, column 2, line 16: Change “(Fig. 4)” to “(Fig. 5).”

Page 1079, column 1, line 4: Change “(Fig. 3)” to “(Fig. 4).”

Survey of Interferon Production and Sensitivity in Human Cell Lines

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Volume 22, no. 1, page 102, column 2, line 10: Change “4 × 10^5 hemagglutination units/cell” to “4 × 10^-5 hemagglutination units/cell.”

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Volume 22, no. 1, page 131, column 2, last line of Table 1: “120” should read “1,200,” “less than 1.0” should read “10,” and “110” should read “1,100.”