Dipicolinate-induced Germination of Bacillus stearothermophilus Spores

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Riemann and Ordal (6) first reported that calcium dipicolinate (Ca:DPA), a 1:1 chelate which forms in solutions containing calcium chloride and sodium dipicolinate (Na2DPA), can induce germination in aerobic and anaerobic bacteria. Since then, other investigators have also observed Ca:DPA-induced germination in spores of a number of mesophilic species (2, 3, 4, 5; H. Riemann, Ph.D. Thesis, Univ. of Copenhagen, Denmark, 1963).

This study concerns the effect of several variables on germination in a thermophilic organism, Bacillus stearothermophilus, in the presence of Na2DPA. Recently, we observed that Na2DPA, but not Ca:DPA, induced changes indicating germination in spores of B. stearothermophilus. Previously, Riemann had found that some strains of putrefactive anaerobe 3679 germinated in Na2DPA alone (5; Ph.D. Thesis, Univ. of Copenhagen, Denmark, 1963). Furthermore, he reported that small concentrations of CaCl2 depressed Na2DPA-induced germination, but that this inhibition was overcome when increased concentrations of CaCl2 were added.

Spores of a rough variant of B. stearothermophilus strain M (1) were produced by incubation for 5 days at 55°C on nutrient agar (Difco) plates containing 0.16 mM MnSO4 and 0.1 mM CaCl2. Surface growth was removed with distilled water, cleaned by treatment with lysozyme (0.5 mg/ml in 0.1 M KCl) for 45 min at room temperature, washed three times by centrifugation with distilled water, and stored at 4°C.

Na2DPA was prepared by dissolving the required amount of reagent grade dipicolinic acid (Aldrich Chemical Co., Milwaukee, Wis.) in 0.1 M sodium hydroxide. The pH value of germination solutions was adjusted with the appropriate amount of buffer solution prior to adding the spore suspension.

Germination was followed by measuring the changes in absorbancy at 600 nm and determining the per cent decrease of initial absorbancy (about 0.4) after incubation for 1 hr. Comparable evidence for germination was also obtained by determining the loss of heat resistance and by spore darkening under phase-contrast microscopy.

Germination of B. stearothermophilus spores occurred over a very narrow pH range, being optimum at 5.5 (Fig. 1). Since the acid pK2 values for DPA are 2.16 and 4.76 (7), the predominant

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Fig. 1. Effect of pH on dipicolinate-induced germination of B. stearothermophilus spores. Unheated spores were incubated in germination solution (4 mM Na2DPA) adjusted to the pH indicated. McIlvaine’s buffer solutions (60 mM citrate and 120 mM sodium phosphate) were used over a pH range of 4.0 to 6.0, and Sorenson’s sodium phosphate buffer (120 mM) was used over a pH range of 5.5 to 8.5. The per cent decrease in initial absorbancy at 600 nm was determined after incubation for 1 hr at 53°C. Data shown for pH 5.5 and 6.0 represent mean values of results from both citrate- and phosphate-buffered systems.
state (about 95%) of the molecule should be in the doubly ionized form at about 5.5, the pH range for optimum germination. Consequently, we regard dipicolinate anion as the effective inducer of germination in the system described here. In other preliminary studies, neither ethylendiaminetetraacetic acid, another chelating agent, nor L-alanine induced germination of B. stearothermophilus spores over a wide pH range.

Appreciable germination in the presence of Na$_2$DPA was detected over a temperature range of 30 to 60 C and was greatest between 40 and 50 C (Fig. 2). Slight germination also occurred at 20 and 70 C, but not at 80 C.

Table 1 shows the inhibitory effect of several divalent cations on dipicolinate-induced germination of B. stearothermophilus spores. Of the cations tested, cobalt was completely inhibitory, whereas magnesium, calcium, and manganese were also effective, although to slightly lesser degrees.

These results indicate that the unchelated ligand induced germination of B. stearothermophilus spores and that this germination was impeded by cations which were chelated by the dipicolinate molecule. The effectiveness of cation inhibition is dependent on the strength of the chelate formed since the order of inhibition (Co$^{++}$ > Mn$^{++}$ > Ca$^{++}$ > Mg$^{++}$) corresponds directly to magnitudes of stability constants for these cation-dipicolinate chelates (8; H. Riemann, Ph.D. Thesis, Univ. of Copenhagen, Denmark, 1963; H. P. Fleming, Ph.D. Thesis, Univ. of Illinois, Urbana, 1963; and M. Jaye, Ph.D. Thesis, Univ. of Illinois, Urbana, 1964).

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LITERATURE CITED


### Table 1. Divalent cation inhibition of dipicolinate-induced germination of B. stearothermophilus spores

<table>
<thead>
<tr>
<th>Cation$^b$</th>
<th>Initial absorbancy decrease</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>Mg$^{++}$</td>
<td>27</td>
<td>52</td>
</tr>
<tr>
<td>Ca$^{++}$</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td>Mn$^{++}$</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>Co$^{++}$</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* Unheated spores were incubated in germination solution [4 mm Na$_2$DPA and 120 mm Sorenson’s phosphate buffer (pH 5.5)]. The per cent decrease in initial absorbancy at 600 nm was determined after incubation for 1 hr at 50 C.

$^b$ As chlorides; 7 mm final concentration.

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![Graph](http://jb.asm.org/)

**Fig. 2.** Effect of temperature on dipicolinate-induced germination of B. stearothermophilus spores. Unheated spores were incubated in germination solution [4 mm Na$_2$DPA, and 120 mm Sorenson’s phosphate buffer (pH 5.5)] and the per cent decrease of initial absorbancy at 600 nm was determined after 1 hr at the temperatures indicated.