NOTES

Patterns of Spore Locations in Pairs of *Bacillus cereus* Sporangia

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The location patterns, relative to the cross wall, of terminal-to-subterminal *Bacillus cereus* spores were determined in pairs of sporangia. The presence of three types of patterns suggests that spores are randomly located, but medium-dependent variability of the frequency ratios of the patterns strongly suggests that nonrandom localization cannot be discounted.

Spores of certain strains of *Bacillus megaterium* are located at the old ends of the sporangia (6, 7, 9, 11) (the ages of cell ends being defined as described elsewhere [2, 4]). This nonrandom spore location pattern suggested the use of spores as topological markers in sporation deoxyribonucleic acid (DNA) strand segregation studies (A. Hitchins, J.

![Figure 1](https://www.asmscience.org/Content/Files/Content/Files/Journals/JB/1976/00125-011251976-125-366_367/125_366_367_FIG1.jpg)

**Fig. 1.** Phase-contrast photomicrographs of spore location patterns in pairs of sporangia of *B. cereus* and *B. megaterium*. (a, b, and c) *B. cereus* in YE medium (8.25 h) (d, e, and f) *B. cereus*, overnight culture in YEG medium. Spores are paracentral to subterminal. Paracentral spores were assigned to the half of the sporangium not containing the unidentified inclusions. (g, h, and i) *B. cereus* in R medium (7 h); spores are terminal to subterminal. (j) *B. megaterium* ATCC 19213, overnight culture in sucrose-salts medium. The pictographs corresponding to the various patterns are as follows: (SO x OS), panels c, f, i, and j; (SO x SO), panels a, d, and g; (OS x SO), panels b, e, and h. The scale bar represents 5 μm. Photomicrographs were taken through an oil immersion objective (phase contrast; Plan 100; numerical aperture, 1.3) on Plus-X Pan 35-mm film (Kodak). A green filter was used.
Theor. Biol., in press). However, before using *B. megaterium* spores as markers in segregation studies, it seemed logical to check the spore location pattern in *B. cereus* strain 2 since it is the only sporeformer in which the sporulation DNA segregation pattern has been examined. Kogoma and Yanagita found that "old" and "new" chromosomes segregate randomly into *B. cereus* spores (8).

A culture of *B. cereus* 2 was obtained from M. Kondo, Osaka University, Japan. Sporangia of the bacterium were produced in the various media listed in Table 1. Spore locations in over 2,000 pairs of sporangia were observed by phase-contrast microscopy as described previously (7). As expected from their polar developmental sites in *B. cereus* sporangia (5), the spores were generally located terminally or subterminally (Fig. 1). Consequently, it was possible to determine whether spores were associated with the halves of sporangia that are either distal or proximal to the cross wall separating neighboring sporangia. Three kinds of spore location patterns can be expected if the spores in pairs of sporangia are located randomly with respect to each other. The three expected patterns were always observed (Table 1 and Fig. 1) and they are represented as follows: (SO X OS), (SO X SO), and (OS X SO). The component symbols of these pictographs are: S, phase-dark or phase-bright spores; O, the half of the sporangium not associated with a spore; curved lines (parentheses), the halves of cross walls that split during cell separation to form the termini of single cells or chains; X, markedly idented crosswalls; I, crosswalls not markedly identified (see below).

Although all three patterns were present in all of the cultures examined, the frequency ratio of (SO X OS):(SO X SO):(OS X SO) forms was not always consistent with the 1:2:1 ratio expected for random spore locations. The inconsistency was especially noticeable under the conditions used by other workers (8) to study DNA segregation (Table 1), but the significance of this marked sporulation medium effect was not investigated.

The relative frequencies of the three possible spore location patterns remained constant throughout spore maturation from the time phase-dark forespores were detected until the phase-bright spore stage (Table 1, experiment 1). This observation, plus the report that spores do not relocate during their maturation (1), and the fact that no Brownian motion of intrasporangial spores was ever detected suggest that the variability of the relative frequencies of the three patterns was not due to movement of the spores within their sporangia. A temporally constant spore location pattern was also observed in *B. megaterium* (7).

The medium-dependent departures of the relative frequency ratios of the three forms from the 1:2:1 ratio expected for randomly located

### Table 1. Frequency of spore location patterns of sporangial pairs of *B. cereus*

<table>
<thead>
<tr>
<th>Expt</th>
<th>Medium*</th>
<th>Time (h)*</th>
<th>Frequency ratios of spore location patterns†</th>
<th>Chi-square statistic‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>YE</td>
<td>5</td>
<td>(SO) 2.1 X (OS) 1.4 (SO) 1.98</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>YE</td>
<td>&gt;12</td>
<td>(SO) 1.6 X (OS) 1.3 (SO) 8.28</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>YE</td>
<td>&gt;12</td>
<td>(SO) 1.6 X (OS) 1.3 (SO) 4.01</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>YEG</td>
<td>&gt;12</td>
<td>(SO) 3.1 X (OS) 2.0 (SO) 6.91</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>YES</td>
<td>6</td>
<td>(SO) 2.0 X (OS) 2.2 (SO) 20.08</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>GGGS</td>
<td>7.75</td>
<td>(SO) 3.4 X (OS) 4.7 (SO) 42.20</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>7</td>
<td>(SO) 5.4 X (OS) 9.1 (SO) 69.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>(SO) 3.6 X (OS) 4.6 (SO) 25.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5</td>
<td>(SO) 0.9 X (OS) 0.2 (SO) 39.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(SO) 1.1 X (OS) 0.4 (SO) 15.34</td>
<td></td>
</tr>
</tbody>
</table>

*YE medium contains the salts mixture of Slepecky and Foster (10) and yeast extract (0.1%, wt/vol). YE medium was supplemented with sucrose (0.1%, wt/vol) to make YES medium or with glucose (0.1%, wt/vol) to make YEG medium. GGGS medium is a glucose-glutamate-glycine salts medium (3) modified (8) by the addition of thymine (0.0025 mg/ml) and 2'-deoxyadenosine (0.25 mg/ml). R medium is a replacement sporulation medium (3). Cultures (50 ml or less) were incubated at 30 C with a shaker speed of 200 rpm. The inocula were spores (heated at 70 C, 30 min) (for most media), pregerminated spores (for GGGS medium), or cells grown in GGGS medium (for R medium).

†Time elapsed since the end of logarithmic growth or since transferral of cells to R medium.

‡The frequency of the (SO X OS) pattern was arbitrarily set at unity. Sporangia occurred mainly as singlets and doublets. Clumps of sporangia were not analyzed. Samples were examined with a Zeiss Universal phase-contrast microscope. Over 100 pairs of sporangia were examined in each sample. Data are corrected to one decimal place.

§The value of the chi-square statistic (2 degrees of freedom) for a probability of 0.1 is 4.61.

¶In experiment 1, the percentages of phase-dark spores were 0, 50, 76, 15, 1, and 2 and those of phase-bright spores were 0, 52, 82, 98, and 98 at 4, 5, 6, 7.25, 8.25, and 9.25 h, respectively.
spores suggest that the real pattern of spore location may be nonrandom. Departures from a nonrandom pattern may only be apparent since the genealogical relationship of the members of sporangial pairs could not be established because of the infrequency of L-form (putatively younger) cross walls. In this context, it is important that the three sporangial pairs with L-form cross walls observed in this study all exhibited the (SO I OS) pattern typical of B. megaterium (Fig. 1j).

The possible nonrandom location of spores in sporangia of B. cereus could be tested if the ages of the sporangial ends can be determined by a selective tagging procedure. However, since B. megaterium spores are nonrandomly located in their sporangia, it is simpler to use this bacterium to correlate the sporulation DNA segregation pattern with the spore location pattern.

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LITERATURE CITED