Isolation and Identification of Obligate Thermophilic Sporeforming Bacilli from Ocean Basin Cores

J. W. BARTHOLOMEW AND GEORGE PAIK1

Department of Bacteriology, University of Southern California, Los Angeles, California

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ABSTRACT

BARTHOLOMEW, J. W. (University of Southern California, Los Angeles), AND GEORGE PAIK. Isolation and identification of obligate thermophilic sporeforming bacilli from ocean basin cores. J. Bacteriol. 92:635–638. 1966.—Obligate thermophilic sporeforming aerobic bacilli were isolated from 11 ocean basin cores taken from locations in a 150 mile long area off of the coast from Ensenada, Mexico, to Santa Catalina Island, and ranging as far out from shore as 160 miles. Isolated strains of bacilli were all identified as being identical, or closely related, to Bacillus stearothermophilus.

Thermophilic sporeforming bacilli have been isolated from ocean-bottom mud samples at depths of 50 to 500 meters (3) and from two deep ocean basin cores at a maximal depth of 1,378 meters (1). The present paper reports additional isolations of these organisms from a wide geographical area, and the identification of seven of the isolates.

MATERIALS AND METHODS

Cores, obtained by use of an Emery and Dietz (5) coring device, ranged from 60 to 175 cm in length. These represented penetrations of 90 to 250 cm into the bottom sediment. A sterile scalpel was used to cut away the outside of the cores. A second sterile scalpel was employed to remove aseptically specimens from the center of the cores. Specimens were preserved in sterile bottles at 5 C until used. Most platings were made within 24 hr after the samples were taken.

Seawater samples were taken at 5 and 500 meter depths with the use of J-Z bacteriological water-sampling equipment and rubber bottles, as described by Zobell (8). Platings were made on 1.0% Proteose Peptone, 0.3% Beef Extract, 1.5% agar, made up with filtered seawater. The samples were diluted 1:10 and 1:100 in sterile seawater. The plates were held at 65 C in an air incubator for 48 hr before counting and for 96 hr before isolating strains from colonies. To prevent drying out, the plates were sealed with plasticine. The counts reported represent the average obtained from four plates.

The media and methods of Gordon and Smith (6) were used to identify strains, except that seawater was substituted for distilled water in the formulations. Incubation temperatures of 55 C were used for all biological tests. Broth and agar slant cultures were incubated in water baths. Size measurements were obtained through the use of crystal violet-stained smears, an oil immersion objective, and a filar micrometer.

RESULTS

In basins from Ensenada, Mexico, to 15 miles south of Santa Catalina Island, and as far out from shore as 160 miles, cores were taken at depths ranging from 878 to 2,060 meters. None of the cores taken was beyond the continental slope. Thermophilic sporeforming bacilli were found in all the sample cores and, except in one, throughout the length of all cores (Table 1). The counts ranged from 5 to 900 organisms per gram of wet sediment material. Thirty isolates were made from colonies on various plates. All of these isolates grew at 65 C, but not at 28 C. All were gram-positive sporeforming bacilli, capable of aerobic growth.

Seven of these isolates were studied further to see if they could be identified as to species (Table 2). All generated spores that caused at least slight swelling of the sporangium, produced acid, but not gas, from glucose, hydrolyzed starch, and grew at 65 C but not at 37 C. These characteristics are sufficient for their tentative identification as Bacillus stearothermophilus as described in Bergey's Manual of Determinative Bacteriology. According to Gordon and Smith (6), the ability to grow at 65 C but not at 37 C is sufficient in itself to distinguish B. stearothermophilus from all other Bacillus species. On the basis of the

1 Present address: Unit 1, Laboratories and Pathology, Los Angeles County General Hospital, Los Angeles, Calif.
study of a large number of strains, Gordon and Smith (6) listed four organisms which possibly might be confused with B. stearothermophilus, i.e., B. circulans, B. subtilis, B. pumilus, and B. coagulans. However, a comparison of the characteristics of our isolates with the descriptions of these four organisms as given in Bergey’s Manual (Table 2) reveals that, on the basis of morphology, temperature range, starch hydrolysis, gelatin hydrolysis, production of nitrates from nitrates, citrate utilization, Voges-Proskauer tests, and inhibition of growth on soybean agar, all of our strains clearly could be differentiated from these four organisms.

None of the four seawater samples taken at 5 and 500 meters yielded thermophilic bacilli on direct platings of 1.0 ml of seawater. Thus, the organisms found in the cores were not due to contamination during the retrieval of the cores from the ocean depths.

**DISCUSSION**

The results show a distribution of thermophilic bacilli in the ocean basins of the continental slope from Ensenada, Mexico, to Santa Catalina Island. Their presence in cores from areas beyond the continental slope is not yet determined. The presence of obligate thermophilic bacteria in an environment having a constant temperature of about 4 °C is difficult to explain. Other such alien bacteria are known to be present in deep ocean cores, e.g., obligate aerobic mesophiles, which will not grow in the anaerobic environment and at the temperatures found in deep cores, and bacteria, which will grow at 1 atm but not at the in situ pressures of such cores (9). Terrestrial run-off and airborne dust could explain the presence of such alien organisms in approximately the top 10 cm of the cores (4), but such sources are inadequate to explain the occurrence of these organisms at 175 cm core depth. One possible explanation could be that they are living fossils, deposited at the same time as the sediments. For example, using organic carbon for dating, Emery (4) reported that the surface 3 to 13 cm sediment of the Santa Catalina basin is about 1,970 years old. Such an age of the surface sediment is difficult to explain. At 13 to 25 cm core depth the age is 2,840 years, and at 406 to 419 cm core depth the sediment is 18,400 years old. If we extrapolate this data for our sample depth of 150 cm (Table 1), we obtain an age of 7,800 years. If, for the sake of conservatism, we subtract the surprising age of the surface sediment, we obtain a minimal age of about 5,800 years for the sediment at the 150 cm core depth. It is possible that “alien” sporeforming bacteria may have persisted in such sediments for these long periods of time. However, the burden of proof would fall on anyone who attempted to make such a statement, since this would imply bacterial spore ages of many thousands of years.

<table>
<thead>
<tr>
<th>Location of core site</th>
<th>Core no.</th>
<th>Depth (meters)</th>
<th>Distance from top of core</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 cm</td>
<td>30 cm</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Diego Trough 25 miles west of San Diego</td>
<td>1</td>
<td>1,218</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1,170</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,190</td>
<td>125</td>
</tr>
<tr>
<td>West Cortez Basin 120 miles from mainland, southwest of San Diego</td>
<td>4</td>
<td>1,196</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1,876</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1,762</td>
<td>110</td>
</tr>
<tr>
<td>Santa Catalina Basin 15 miles south of Santa Catalina Island</td>
<td>7</td>
<td>878</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1,316</td>
<td>630</td>
</tr>
<tr>
<td>Valero Basin, 160 miles from mainland, southwest of Ensenada, Mexico</td>
<td>9</td>
<td>1,874</td>
<td>130</td>
</tr>
<tr>
<td>East Cortez Basin 80 miles west of Descanso Pt., Mexico</td>
<td>10</td>
<td>1,832</td>
<td>95</td>
</tr>
<tr>
<td>San Clemente Basin 40 miles from mainland southwest of San Diego</td>
<td>11</td>
<td>2,060</td>
<td>0</td>
</tr>
</tbody>
</table>
The identification of all seven strains as *B. stearothermophilus* is not surprising in view of the isolation methods used. The plates were held for 96 hr at 65 C before isolation of strains. The isolates were subjected to similar treatments between transplants. Therefore, only organisms capable of growing aerobically at 65 C were selected. Such a procedure would automatically exclude such organisms as *B. coagulans*, and all other facultative thermophilic sporeforming organisms (6). It is of interest that only sporeforming organisms were isolated by the procedures used.

The use of seawater media in these experiments represented a respect for the environmental source from which these organisms were isolated. However, all strains could be grown on ordinary nutrient agar. This would indicate that these organisms were not highly adapted to their marine environment, since true marine forms require seawater for growth (7, 10).

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**

3. EGOROVA, A. A. 1938. Thermophilic bacteria in
1. The cultural requirements of heterotrophic aerobes. J. Marine Res. 4:42–75.