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# *Clostridium oceanicum*, sp. n., a Sporeforming Anaerobe Isolated from Marine Sediments

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Fourteen strains of a terminal-spored anaerobe were isolated from marine sediments obtained off the Atlantic and Pacific coasts of tropical South America. These strains are proteolytic, lecithinolytic, only slightly saccharolytic, often form cells with two spores, and appear unlike any described species of terminal-spored, proteolytic anaerobe. The name *Clostridium oceanicum* is suggested. The type strain (no. 25647) is deposited in the American Type Culture Collection.

In the course of an investigation into the clostridial flora of marine sediments (4), strains were isolated which did not belong to any recognized species. Fourteen of these strains were uniform enough in morphological and cultural characteristics to form a well-defined group and, because they did not appear to belong to any species hitherto described, seemed to deserve further study for possible species designation.

## MATERIALS AND METHODS

Eight strains (VPI Anaerobe Laboratory accession no. 2223, 2225, 2226, 2228, 2230, 3017, 3023, and 3035) were isolated from sediment samples from the Pacific Ocean, taken off the coasts of Peru and Ecuador on cruise 15 of the R/V Anton Bruun in March and April 1966. Six strains (VPI 3072, 3082, 3087, 3088, 3094, and 3187) were isolated from sediment samples taken in the Caribbean and off the northeast coast of Brazil on cruise 14 of the R/V Atlantis II of the Woods Hole Oceanographic Institution during November and December 1964.

The cultural reactions and metabolic characteristics of these strains were studied by the methods of Moore et al. (2) and those of Smith and Holdeman (5). The production of chitinase was investigated by the use of Brain Heart Infusion-agar containing ball-milled crab chitin sterilized by autoclaving, and the production of elastase by the use of the same medium containing elastin particles. Both of these media were surface-inoculated, incubated anaerobically for about 10 days, and examined for zones of clearing around the colonies. Collagenase was determined by suspending collagen fibers in sterile culture filtrates, incubating overnight at 37 C. A few drops of chloroform were added to prevent microbial growth, for the collagen fibers could not be sterilized by heat or chemical agents. The disappearance of the collagen fibers was taken as evidence for the production of collagenase by the bacteria concerned. This method of determining collagenase has been found to serve satisfactorily for demonstrating this enzyme in culture filtrates of *Clostridium histolyticum*, *C. perfringens*, *C. limosum*, and *Bacteroides melaninogenicus*. The production of deoxyribonuclease and ribonuclease was determined by the modifications of the method suggested by Jeffries et al. (1).

## RESULTS

All strains were gram-positive rods (0.5 to 1.0 μm in diameter and 2 to 11 μm long), were motile by peritrichous flagella, and formed oval spores, mostly in the terminal but occasionally in the subterminal position. The guanine plus cytosine content of the DNA of two strains was found to be 27 and 28%. Some strains were not motile at 37 C although they were so at 25 C. Spores were formed in both ends of some cells of all strains. The proportion of apparently double-spored cells varied from about 1 to 95%, depending on the medium, temperature, and length of incubation. It was considered likely that this appearance of two spores in a single cell might be an artifact resulting from two daughter cells sporulating before they had separated. Cells were stained in wet mount with 0.01% crystal violet in an effort to demonstrate transverse septa without success. Electron micrographs showed no evidence of incipient cell division or cross-wall formation in cells with two developing spores (Fig. 1).

These organisms were moderately fastidious anaerobes. Surface colonies could not be regularly attained with some strains on such media as egg yolk-agar. Individual colonies could be obtained, however, by "sloppy streaking," i.e., streaking freshly poured egg yolk-agar just before it solidified. No difficulty was encountered in growing any strain in prereduced media when

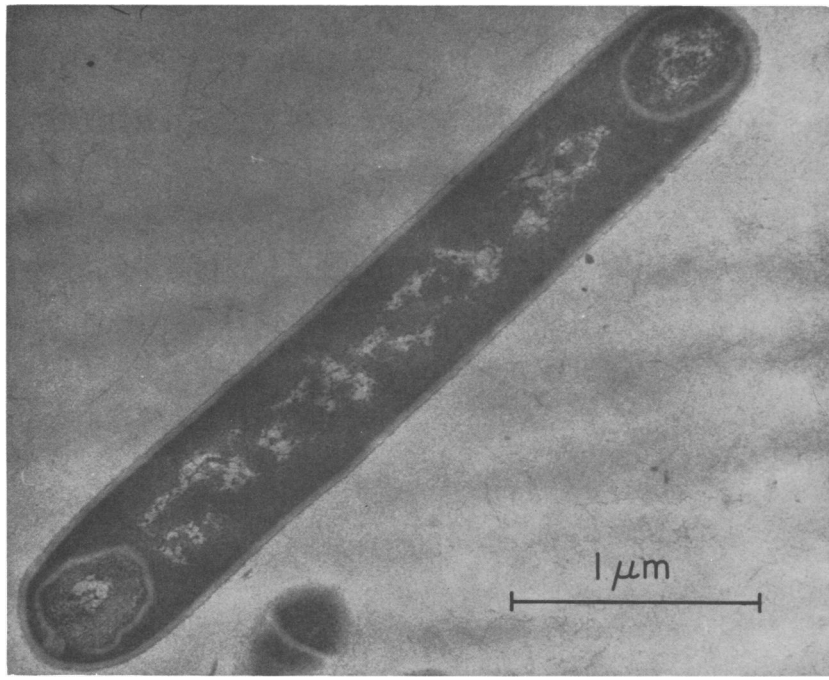


FIG. 1. *Electron photomicrograph of a sporulating cell of C. oceanicum.*

TABLE 1. *Variation in cultural characteristics of strains of Clostridium oceanicum*

| Strain | Cellobiose fermentation | Soluble starch fermentation | Ribose fermentation |
|---|---|---|---|
| 2223 | + | − | + |
| 2225 | + | + | + |
| 2226 | + | + | − |
| 2228 | + | − | − |
| 2230 | + | + | − |
| 3017 | + | + | − |
| 3023 | + | + | − |
| 3035 | − | − | − |
| 3072 | + | + | − |
| 3082 | + | + | − |
| 3087 | + | + | − |
| 3088 | + | − | − |
| 3094 | + | + | − |
| 3187 | − | + | − |

nitrogen was used as the filling gas. When carbon dioxide was used as the filling gas, the *p*H of the medium often dropped close to the lowest level tolerated by these strains and growth was sparse.

Colonies on horse blood-agar, after 3 days of incubation, were 2 to 6 mm in diameter, irregular with an undulate edge, raised, glossy, translucent, gray, and surrounded by a zone of complete hemolysis.

Growth was most rapid at 30 to 37 C; growth at lower temperatures, to 5 C, was slower. No strain grew at 45 C. Of six strains tested, none grew in a medium containing 8% NaCl at room temperature; all grew in a medium containing 4% NaCl. All six strains grew at *p*H 8.6, 8.0, 7.5, 7.0, and 6.5; only one strain grew at *p*H 6.0.

All strains fermented glucose, fructose, galactose, mannose, and maltose. No strain fermented sorbose, xylose, arabinose, rhamnose, sucrose, lactose, raffinose, glycogen, erythritol, inulin, adonitol, glycerol, mannitol, dulcitol, sorbitol, salicin, esculin, amygdalin, inositol, or cellulose. All strains were proteolytic, liquefying gelatin, digesting cooked meat or the casein in milk medium, or both, and producing ammonia from cooked meat medium. All strains produced both deoxyribonuclease and ribonuclease and all strains produced lecithinase on egg yolk-agar although two strains did so weakly. No strain produced indole, hydrogen sulfide, elastase, chitinase, collagenase, nor lipase on egg yolk-agar. No strain reduced nitrate, sulfate, or sulfite. Cultural characteristics on strains which varied are shown in Table 1.

The major fermentation products from peptone-yeast extract medium included acetic, isobutyric, butyric, isovaleric, and isocaproic acids, with smaller amounts of valeric acid.

TABLE 2. *Distinguishing cultural characteristics of proteolytic, terminal-spored Clostridia*

| Characteristic | *C. oceanicum* | *C. cadaveris*[a] | *C. putrificum*[a] | *C. lentoputrescens*[a] | *C. capilovale*[b] |
|---|---|---|---|---|---|
| Spore shape | Oval | Oval | Oval | Spherical | Oval |
| Indole production | − | + | − | + | − |
| Lecithinase production | + | − | − | − | − |
| Hydrogen sulfide production | − | + | + | + | + |
| Nitrate reduction | − | − | − | − | + |
| Glucose fermentation | + | + | + | − | + |
| Mannose fermentation | + | − | − | − | + |
| Maltose fermentation | + | − | − | − | − |

[a] Tested under the same conditions as *C. oceanicum*.

[b] As reported by Breed et al. (Bergey's Manual of Determinative Bacteriology, 7th. ed.) and Prevot et al. (3).

Glucose in the same medium depressed acid production; in the presence of glucose, only butyric and lactic acids were produced in appreciable amounts, with smaller amounts of acetic acid. Most strains, in addition to these acids, also formed a small amount of alcohol, usually a combination of ethanol and propanol although occasionally isobutanol and butanol.

The species which these organisms resemble most closely are *C. cadaveris*, *C. capitovale*, *C. putrificum*, and *C. lentoputrescens*. The data in Table 2 show some of the differences in cultural characteristics existing among these organisms. It is apparent that the marine organisms are quite distinct from the other species. It is suggested that the name *C. oceanicum* be used to designate them, in view of their occurrence in marine sediments. One strain, 3082, has been deposited in the American Type Culture Collection as no. 25647.

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LITERATURE CITED

1. Jeffries, C. D., D. F. Holtman, and D. G. Gucse. 1957. Rapid method for determining the activity of microorganisms on nucleic acids. J. Bacteriol. 73:590–591.
2. Moore, W. E. C., E. Cato, and L. Holdeman. 1966. Fermentation patterns of some *Clostridium* species. Int. J. Syst. Bacteriol. 16:383–415.
3. Prevot, A. R., A. Turpin, and P. Kaiser. 1967. Les bacteries anaerobies. Dunod, Paris.
4. Smith, L. DS. 1968. The clostridial flora of marine sediments from a productive and from a non-productive area. Canadian J. Microbiol. 14:1301–1304.
5. Smith, L. DS., and L. Holdeman, 1968. The pathogenic anaerobic bacteria. Charles C Thomas, Publisher, Springfield, Ill.