**Pseudomonas creosotensis** sp. n., a Creosote-tolerant Marine Bacterium

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**Abstract**

O'NEILL, Thomas B. (U.S. Naval Civil Engineering Laboratory, Port Hueneme, Calif.), Richard W. DRISKO, and Harry Hochman. *Pseudomonas creosotensis*, sp. n., a creosote-tolerant marine bacterium. Appl. Microbiol. 9:472–474, 1961.—In a study of the marine biological environment in which creosoted pilings are located, a previously unreported species of bacteria was isolated. This species was detected on creosoted piling from 11 widely differing locations and was the predominant species of bacteria found on these pilings. The new organism was identified as a gram-negative rod belonging to the genus *Pseudomonas* and has been named *Pseudomonas creosotensis*. It has been completely described by the standard morphological and biochemical tests.

The destruction of wooden piling by marine wood-boring organisms has long been a source of great concern to the U.S. Navy. Pressure treatment of piling with creosote has been the most successful preservative system thus far developed. However, even when this system is used, early failure of piling occurs in some harbors. During the course of investigations of the marine biological environment of Hueneme Harbor, a previously unreported bacterial species was discovered which has an unusually high tolerance to creosote. Subsequent investigations revealed that this organism has a wide geographical distribution, and the present paper describes its isolation and characterization.

**Materials and Methods**

One-quarter-inch cubes of southern yellow pine were subjected to 29 in. of vacuum for 1 hr and then steeped in varying concentrations (Table 1) of coal tar creosote in xylene for 4 days. Untreated cubes were used as controls. Several cubes from each creosote concentration were grouped, and each group was placed in a wide-mouth bottle held in a wooden sampling rack. The rack was wrapped in paper, sterilized in an autoclave, and, after cooling, submerged in Port Hueneme Harbor where the paper wrapping was removed. The sampling rack was suspended approximately 5 ft above the mud line in water approximately 18 ft deep at high tide and 8 ft deep at low tide. Port Hueneme Harbor is a landlocked harbor with a perimeter of 12,800 ft. The average water temperature during the period of sampling was 18.5°C.

After 8 days of immersion, sterile glass stoppers were placed in the bottles, and the rack was removed from the water. The elaborate sterilizing and wrapping procedure was used to lessen the possibility of contamination by nonmarine organisms. At the laboratory the cubes were aseptically removed and placed on sea water nutrient agar. The interval between collection and plating was less than 1/2 hr. The agar plates were incubated at both 20 and 36°C. Isolation and growth of pure cultures were later performed by standard streaking and dilution methods.

To determine if existing creosoted piling possesses a characteristic microflora, trained personnel removed splinters aseptically from creosoted piling in various parts of the world and submitted them to the Naval Civil Engineering Laboratory (NCEL) for examination. A maximum of 3 days elapsed between collecting and plating the specimens, except for the sample from Guam which was plated 6 days after collection. Specimens were collected from Boston Naval Shipyard, Charlestown, Mass.; Norfolk Naval Shipyard, Portsmouth, Va.; Key West Naval Station, Key West, Fla.; Coco Solo Naval Station, Colon, Panama Canal Zone; U.S. Naval Station, San Diego, Calif.; Puget Sound Naval Shipyard, Bremerton, Wash.; Cook Inlet, Anchorage, Alaska¹; Pearl Harbor, Hawaii; and U.S. Naval Ship Repair Facility, Apra, Guam.

A single form of bacterium predominated in all of the samples investigated. The following description is of the bacteria isolated from Port Hueneme Harbor, and, unless otherwise noted, the bacteria from other

<table>
<thead>
<tr>
<th>Per cent by volume of creosote*</th>
<th>Average uptake of solution per cube</th>
<th>Average retention of creosote</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.116</td>
<td>5.7</td>
</tr>
<tr>
<td>40</td>
<td>0.128</td>
<td>12.6</td>
</tr>
<tr>
<td>60</td>
<td>0.135</td>
<td>19.9</td>
</tr>
<tr>
<td>80</td>
<td>0.161</td>
<td>31.7</td>
</tr>
<tr>
<td>100</td>
<td>0.160</td>
<td>40.8</td>
</tr>
</tbody>
</table>

* The solvent, when required, was xylene.

¹ Mr. LaMar Hubbs of the Bureau of State Services, Arctic Health Research Center, Anchorage, Alaska, supplied the sample from Anchorage.
locations display similar characteristics of taxonomical significance.

The morphological and biochemical tests used in this study are identical to those utilized by ZoBell and Upham (1944). All media were prepared with aged\(^2\) or synthetic sea water\(^3\) instead of distilled water, unless otherwise noted.

**Experimental Results**

Bacteria were isolated from all of the wooden cubes examined, both creosoted and uncreosoted. Seven different species were associated with the uncreosoted wood, but only three different species of bacteria were associated with any one creosoted sample. However, a single species was common to all wood samples, both cubes and splinters. It possesses the following characteristics:

**Morphological Characteristics**

Rod forms, 0.4 to 1.5 by 0.7 to 7.5 \(\mu\), occurring most frequently as single cells, with short chains common. Motility by a single polar flagellum (Fig. 1), no spores, gram negative.

\(^2\) Aged sea water is sea water filtered through an ultrafine Millipore filter (Millipore Filter Corporation, Watertown, Mass.) and stored in a brown bottle for several weeks before use.

\(^3\) Synthetic sea water was prepared according to Lyman and Fleming (1940).

**Physiological Characteristics**

Growth in various media:

- **Gelatin plate:** colonies circular, 2 mm in diameter, convex, moist, colorless.
- **Gelatin stab:** slow stratiform liquefaction, beaded growth along stab, no pigment formed.
- **Agar plate:** subsurface colonies spindle shaped, approximately 0.3 by 1.0 mm; surface colonies irregular, spreading, translucent, smooth, moist, colorless.
- **Agar slant:** good growth, glistening, filiform, unpigmented although frequently becoming yellow or fluorescent with age.
- **Sea water broth:** heavy turbidity, heavy viscous sediment, membranous at surface.
- **Fresh water (distilled) nutrient broth:** slight growth.
- **Limulus milk** (prepared with distilled water): no growth.

- **Potato slant:** slow but heavy growth, filiform, cream colored to light yellow.
- **Indole formation from tryptophan:** positive.
- **Nitrate reduction:** positive.
- **Carbohydrate reactions:** produces acid but no gas from lactose, sucrose, glucose, cellobiose, maltose, mannitol, glycerol, and xylose. Does not ferment cellulose. The amount of acid formed during the fermentation of lactose by bacteria from Bremerton, Panama, San Diego, and Hawaii is very slight and the bacteria from Boston and Alaska do not ferment lactose. Glycerol is not fermented by the bacteria from Boston, Hawaii, and Alaska. Xylose is not fermented by the Boston form. The specimen from Guam forms acid and gas in glucose, sucrose, maltose, and mannitol.
- **Starch hydrolysis:** positive.
- **Hydrogen sulfide formation:** positive.
- **Oxygen relationships:** aerobic, facultative.
- **Ammonia production:** ammonia produced from peptone but not from urea.
- **Casein digestion:** negative.
- **Salt relationships:** grows in 12\% sodium chloride (i.e., sea water nutrient broth to which 12\% by weight NaCl was added) but not in 18\%. Forms from Virginia and Hawaii grow in a 6\% NaCl solution but not in a 12\% solution.
- **Temperature relationships:** optimum approximately 30 to 35 C, although specimens from Boston, Bremerton, and Alaska grow best at 20 to 25 C. No growth at 4 C.
- **Creosote tolerance:** Freshly collected Port Hueneme organisms grow in sea water nutrient broth containing 1\% creosote, i.e., 10,000 ppm, but not in broth containing 10\% creosote.

The organism is apparently a previously undescribed species of the genus *Pseudomonas*. Because of its tolerance for creosote, a most distinguishing characteristic, the species name of *creosotensis* is proposed.
DISCUSSION

The present status of knowledge concerning marine bacteria is comparable to that in 1944 when ZoBell and Upham stated, "Although the factors which influence the distribution and activities of marine bacteria have been extensively studied, very little is known concerning the character of these organisms themselves. . . . The few marine bacteria which have been studied sufficiently to warrant the application of genera and species names have, for the most part, been described in obscure publications that are not generally available."

Fourteen years later, Wood (1958) stated, "There has been considerable study of micro-organisms in the soil and, to some extent, in fresh water but those in the sea have received far too little attention when we consider the vast potentiality of the sea both as an adjunct to man's survival and as the place of deposition of the major parts of the world's sedimentary rocks and the minerals and ores contained therein."

The fifth edition of _Bergey's Manual of Determinative Bacteriology_ (Bergey et al., 1939) lists 86 marine bacteria; 19 years later, as a result of the reclassification of previously described species and the discovery of new species, the seventh edition (Breed, Murray, and Smith, 1957) has only 117 marine bacteria listed.

The entire concept of speciation in bacteria, as in other taxa, is subject to considerable question. Wood's (1953) conclusions on speciation of bacteria are especially pertinent and are supported by the present study. He frequently refers to the ability of most bacteria to become adapted to a wide range of physiological environments. In the present study, the organisms from different locations demonstrate varied optima of temperatures and salt tolerances and exhibited slightly different fermentative abilities. Many of these differences became manifest after prolonged periods in the laboratory during which time many subcultures were made.

ACKNOWLEDGMENT

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LITERATURE CITED


