Prosthecomicrobium litoralum, a New Species from Marine Habitats

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Marine strains of multiply appendaged prosthecate bacteria have been isolated. All three strains included in this study have characteristics that are typical of the genus *Prosthecomicrobium*. The deoxyribonucleic acid base compositions of the strains tested range from 66 to 67 mol% guanine plus cytosine; these values are similar to the value reported for *Prosthecomicrobium enhydrum*. However, the new strains which we studied differ from *Prosthecomicrobium enhydrum* because of their marine origin, immotility, requirement for a minimum salinity for growth of 5‰, and lack of colony pigmentation. The marine strains have an absolute sodium ion requirement for growth, which is consistent with their being indigenous to marine environments. A new species, *Prosthecomicrobium litoralum*, is proposed.

Most prosthecate bacteria have been isolated from freshwater sources, although representatives of caulobacters and hyphomicrobia have been reported from marine habitats. Two species of the genus *Caulobacter*, *Caulobacter maris* and *Caulobacter halobacteroides*, are of marine origin (8), and one species of the *Hyphomicrobium* group, "*Hyphomonas neptunium*" (formerly designated *Hyphomicrobium neptunium*), has been isolated from seawater (4, 5). More recently, additional strains of "*Hyphomonas*" spp. have been isolated from Pacific hydrothermal vents. To our knowledge, marine strains of the prosthecate genera *Prosthecomicrobium* and *Ancalomicrobium* have not been reported previously. It is true that multiply appendaged bacteria resembling members of these genera have been obtained from brackish and marine samples (6, 7), but the appendages of these organisms are not bound by the cell walls of the organisms as prosthecae are; instead, they are composed entirely of protein and are, therefore, structurally and chemically analogous to pili (2, 3). Thus, these nonprosthecate bacteria are very different from *Prosthecomicrobium* and *Ancalomicrobium* (9). The objective of this study was to determine whether marine strains of multiply appendaged prosthecate bacteria could be isolated.

MATERIALS AND METHODS

The enrichment and isolation procedures which we used have been described previously in detail by Staley and Bauld (10) for *Planctomyces maris*, with

the following exception. A number of sample localities supported dense populations of *Ulva* spp., and in such cases enrichment cultures in media containing dilute peptone (final concentration, 0.01%, wt/vol) were established both with and without small *Ulva* fronds. The media used and the procedures adopted for all physiological investigations, light microscopy, and electron microscopy have been fully described by Bauld and Staley (1). Any variations in these methods are noted below.

RESULTS

Enrichment and isolation of strains. Three multiply appendaged bacteria were successfully enriched and axenically cultivated from littoral marine waters from the Oregon and Washington coastlines (Table 1). In two of the three samples, enrichment of the organisms was achieved when small pieces of *Ulva* fronds, which were found on rocks in the sampling areas, were included in the initial dilute peptone enrichment medium.

Morphology. The three strains used in this investigation had overall morphological features that were typical of the genus *Prosthecomicrobium*. Cells observed by phase-contrast microscopy appeared to be short to long rods about 1.0 µm diameter (Fig. 1). The most distinctive feature noticeable by phase-contrast microscopy was the irregular surfaces of the cells, which imparted somewhat fuzzy outlines to the cells. Occasional cells, particularly cells of the Tillamook Bay strain (strain 511-2B1), had one or more much longer prosthecae that could be readily recognized (Fig. 2). All three strains were gram negative.

Numerous individual prosthecae were readily apparent when whole cells of the three strains were examined by electron microscopy (Fig. 3).

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TABLE 1. Sources and selected characteristics of *Prosthecomicrobium litoralum* strains

Property	<i>Prosthecomicrobium litoralum</i> strain:		
	511-2B1	520-5A3	524-16 ^T
Field sample locale	Tillamook Bay, Oregon; enclosed bay between Garibaldi and Bay City on Highway 101	Richmond Beach, Puget Sound, north King County, Washington	Point Lowell, Camano Island State Park, Puget Sound Washington State Park
Sample description	Seawater near rocks, with <i>Ulva</i> added; 11.0°C; surface sample; 29 July 1973	Shoreline seawater; 15.0°C; depth, ca. 1 m; 14 August 1973	Shoreline seawater, <i>Ulva</i> from rocks included; 27 August 1973
Morphology	Short appendages (<1.0 µm); nonmotile	Short appendages (<1.0 µm); nonmotile	Short appendages (<1.0 µm); nonmotile
Optimum temp range (doubling time) ^a	24–30°C (11–13.5 h)	26–35°C (10–12 h)	26–35°C (10–12 h)
Shortest doubling time (temp) ^a	10 h (26°C)	10 h (34°C)	10 h (31°C)
Minimum growth temp ^{a,b}	5°C	1°C	5°C
High-temp response ^{a,c}	No growth at 38°C; slight growth at 34°C	Slight growth at 39°C	Slight growth at 39°C
Salinity optimum (range)	25‰ (5–45‰)	25‰ (10–35‰)	25‰ (5–35‰)

^a Determined by using static cultures in screw-capped tubes in a temperature gradient aluminum block. The cultures were hand shaken once daily. Medium B-SW of Bauld and Staley (1) was used, growth was measured by determining optimal densities at 560 nm.

^b Based on a fivefold increase in absorbance at 560 nm after 18 days at the temperature specified.

^c Slight growth was defined as a threefold increase in absorbance at 560 nm within 7 days.

The prosthecae were usually short (i.e., less than 1.0 µm long) and tapered toward their tips. However, some appendages occasionally exceeded 2.0 µm in length, a characteristic which has been also reported for *Prosthecomicrobium pneumaticum*.

Motility was not observed for any of the marine isolates. Likewise, gas vacuoles were not observed.

Physiological properties. The general physiological properties of the isolates are summarized in Table 1. The isolates were heterotrophic and mesophilic, with optimum growth temperatures

ranging from ca. 25 to 35°C. All three strains grew slowly at refrigerator temperatures (ca. 4°C), suggesting that they may be able to grow throughout the year in the northern Pacific habitat in which they were found. The minimum doubling times of 10 h were relatively long compared with the 6.3 h reported for *Prosthecomicrobium enhydrum* (9).

FIG. 1. Phase-contrast photomicrograph of *Prosthecomicrobium litoralum* strain 524-16^T. Note the somewhat irregular outlines of the cells due to the numerous short prosthecae. Bar = 2.0 µm.

FIG. 2. Phase-contrast photomicrograph of *Prosthecomicrobium litoralum* strain 511-2B1. This strain typically produces longer prosthecae (arrow) in addition to shorter prosthecae. Bar = 2.0 µm.

FIG. 3. Negatively stained electron micrograph of *Prosthecomicrobium littoralum* strain 524-16^T. Bar = 1.0 μ m.

The range of salinity tolerance for growth was moderately wide, with an optimum salinity of 25‰. The greatest salinity range (5 to 40‰) was exhibited by strain 511-2B1, which was isolated from the waters of an enclosed marine embayment that is subjected to seasonal salinity variations. None of the strains grew in media devoid of sodium ions even though full sodium ion osmolarity of an artificial marine salts solution was compensated for with potassium chloride. Strains 520-5A3 and 524-16^T (type strain) showed some growth in the presence of 50 mM sodium chloride, and strain 511-2B1 grew slightly in the presence of 100 mM sodium chloride when salinity was corrected for with potassium chloride. Optimum growth for all strains occurred when the sodium chloride concentration was between 150 and 470 mM, irrespective of the potassium ion concentration.

Carbon source utilization. The utilization of various carbon sources by the three strains which we studied was tested (Table 2) and compared with the utilization of carbon sources by *Prosthecomicrobium enhydrium* and *Prosthecomicrobium pneumaticum* as reported by Staley (9). In contrast to the other two marine strains, strain 511-2B1 did not use maltose, rhamnose, or trehalose. Some differences in the utilization of sugar alcohols were also observed. With the exception of strain 511-2B1, the marine strains used all of the sugar alcohols tested. This pattern was similar to the pattern observed in *Prosthecomicrobium pneumaticum*, but differed markedly from the pattern observed in *Prosthecomicrobium enhydrium*, which did not use any of these compounds as carbon sources.

All three strains grown on plates containing medium B-SW supplemented with glucose (final concentration, 0.1%) as a carbon source showed slight agar digestion after incubation at 20 to

25°C for 30 to 60 days. Although we are not aware that this has been reported previously for other prosthecae bacteria, strains of *Ancalomicrobium* and *Caulobacter* are known to hydrolyze this polysaccharide.

DNA base composition. The deoxyribonucleic acid (DNA) base compositions of two of the isolates, strains 520-5A3 and 524-16^T, were determined by using the CsCl buoyant density procedure. Strain 520-5A3 had a guanine-and-cytosine content of 66 mol%, whereas the value for strain 524-16^T was 67 mol%.

DISCUSSION

Our results demonstrate that marine strains of *Prosthecomicrobium* do exist. The strains which we isolated have all of the major properties of the genus *Prosthecomicrobium*; all are prosthecae bacteria that contain the numerous short prosthecae which are characteristic of this genus. In addition, they resemble bacteria in the genus *Prosthecomicrobium* in their overall carbon source utilization patterns and other general physiological properties, such as optimum growth rate and temperature optimum. Furthermore, the range of their DNA base compositions (66 to 67 mol% guanine plus cytosine) is consistent with the values that have been obtained for the genus *Prosthecomicrobium*.

However, our isolates are not identical to any known *Prosthecomicrobium* species. In contrast to previously described species of the genus, but consistent with their marine origin, our strains have an obligate growth requirement for sodium ions. Moreover, they cannot grow at salinity levels less than 5‰. They also differ in other respects from the previously described species of *Prosthecomicrobium*, *Prosthecomicrobium enhydrium* and *Prosthecomicrobium pneumaticum*. For example, *Prosthecomicrobium enhydrium* cells are motile by single polar to subpolar flagella, whereas the cells of the marine strains are nonmotile. In addition, *Prosthecomicrobium enhydrium* produces a yellow pigment, whereas the marine strains are nonpigmented. *Prosthecomicrobium pneumaticum* is immotile like the marine strains, but it produces gas vacuoles, which are not formed by any of the marine strains included in our investigation. Furthermore, the guanine-plus-cytosine contents of the marine strains are lower than those of *Prosthecomicrobium pneumaticum* (11).

Because of these major differences between the marine strains of *Prosthecomicrobium* and the currently accepted species, we conclude that a new species should be established in the genus. We propose to name this species *Prosthecomicrobium littoralum*; this new species is described below.

TABLE 2. Carbon source utilization by strains of *Prosthecomicrobium litoralum* and the type strains of *Prosthecomicrobium enhydrium* and *Prosthecomicrobium pneumaticum*^a

Carbon source ^b	Utilization by strain:				
	511-2B1	524-16 ^T	520-5A3	<i>P. enhydrium</i> ATCC 23634 ^T	<i>P. pneumaticum</i> ATCC 23633 ^T
Arabinose	+	+	+	+	+
Cellobiose	+	+	+	+	+
Fructose	+	+	+	+	+
Fucose	+	+	+	+	+
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+	+	+	+	+
Maltose	—	+	+	+	+
Mannose	+	+	+	+	+
Melibiose	—	—	—	—	+
Raffinose	—	—	—	—	—
Rhamnose	—	+	+	+	+
Ribose	+	+	+	+	+
Sucrose	+	+	+	NT	NT
Trehalose	—	+	+	NT	NT
Furanose	+	+	+	NT	NT
Xylose	+	+	+	+	+
Adonitol	—	+	+	—	+
Arabitol	+	+	+	—	—
Dulcitol	—	+	+	—	+
Erythritol	—	+	+	—	—
Ethanol	—	—	—	—	+
Glycerol	+	+	+	—	+
Inositol	+	ND	+	—	+
Mannitol	+	+	+	—	+
Sorbitol	—	+	+	—	+
Sodium acetate	+	+	+	NT	NT
Sodium benzoate	—	—	—	—	—
Sodium fumarate	—	—	—	+	—
Sodium glucuronate	+	—	—	NT	NT
Sodium β -hydroxybutyrate	+	+	+	NT	NT
Sodium lactate	+	+	+	NT	NT
Sodium malate	+	+	+	NT	NT
Sodium propionate	—	—	—	?	+
Sodium pyruvate	+	+	+	NT	NT
Sodium succinate	+	+	—	+	—
Gelatin	—	—	—	NT	NT
Glycine	—	—	—	NT	NT
Pectin	—	—	—	—	—
Urea	—	—	—	NT	NT
Agar ^d	+	+	+	NT	NT

^a See reference 9.^b The carbon sources were in 2% (wt/vol) solutions; the final concentration of carbon source in each experiment was 0.1%. See reference 1 for methodology.^c +, Carbon source utilized; —, carbon source not utilized; ?, utilization uncertain; ND, no data; NT, not tested.^d Colonies developed recessed areas when they were grown for 1 month or more on medium B-SW.

***Prosthecomicrobium litoralum* sp. nov.** *Prosthecomicrobium litoralum* (li.to.ra'lum. L. adj. *litoralis* of the seashore) cells are short to long rods (diameter, ca. 1.0 μ m) with numerous tapering prosthecae. Most prosthecae are short (i.e., less than 0.5 μ m long), although occasional prosthecae are longer. Gas vacuoles are not produced. Nonmotile. Colonies are white and raised initially, becoming cream colored and

umbonate after prolonged incubation.

Sodium ions are required for growth. The minimum salinity for growth is 5‰; the optimum salinity 25‰. The optimum temperature for growth is 27 to 34°C.

The guanine-plus-cytosine content of the DNA ranges from 66 to 67 mol%, as determined by buoyant density (two strains tested).

Description of the type strain. The type strain

of the species is strain 524-16. This strain has been deposited in the American Type Culture Collection as ATCC 35022 and has all of the properties described above for the species. Additional properties are given in Tables 1 and 2.

The observation that all of the strains of *Prosthecomicrobium litoralum* which we studied utilize agar may be of ecological significance. It suggests that these marine strains may degrade algal polysaccharide materials in littoral regions. In addition, *Prosthecomicrobium litoralum* can utilize numerous sugars and some sugar alcohols, which comprise the degradation products of polysaccharides. These nutritional features may explain why enrichment with *Ulva* fronds was so successful. *Prosthecomicrobium litoralum* may also use dissolved organic carbon excreted by marine phytoplankton.

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