Phylogenetic characterization of marine bacterium strain 2-40, a degrader of complex polysaccharides

José M. González¹ and Ronald M. Weiner²

Author for correspondence: Ronald M. Weiner. Tel: +1 301 405 5446. Fax: +1 301 314 9489. e-mail: rw19@umail.umd.edu

¹ Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA
² Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA

The marine bacterium strain 2-40 was isolated from the salt marsh cord grass, Spartina alterniflora, in the Chesapeake Bay watershed, VA, USA. It is Gram-negative, requires sea salts and is a strict aerobe. It degrades numerous complex polysaccharides and synthesizes eumelanin. By 16S rDNA analysis, the isolate was shown to be a member of the γ-subclass of the Proteobacteria, related to Microbulbifer hydrolyticus and to a cellulolytic nitrogen-fixing bacterium.

Keywords: phylogeny, tyrosinase, 16S rDNA, complex polysaccharides, hydrolytic enzymes

In the salt marsh ecosystem, complex polysaccharides (CP) derived from plants are degraded by microorganisms, principally bacteria, once the plant detritus reaches the aquatic environment (Benner et al., 1986). Due to the high primary productivity and abundance of vascular-plant-derived lignocellulosic material, the degradation of these polymers is an important environmental process relating to the food web and carbon cycling. To learn more about the type of organisms involved in the degradation of CP, bacteria were isolated from decaying Spartina alterniflora (Andrykovitch & Marx, 1988).

One isolate stood out for its ability to quickly degrade agar and other CP: strain 2-40 (ATCC 43961) was isolated on 1% peptone-half-strength-seawater agar and other CP: strain 2-40 (ATCC 43961) was isolated from a sample of partially degraded Spartina alterniflora at the Chesapeake Bay salt marsh in Matthews County, VA, USA (19 p.p.t. salinity). Its enzyme systems have been proposed as a model for CP degradation (Weiner, 1998; Ensor et al., 1999), and its pleomorphic morphology (Weiner et al., 1998) and tyrosinase activity (Kelley et al., 1990) have been the subject of extensive investigations.

Strain 2-40 was cultured in marine broth 2216 (Difco) with 0.2% agar. The organism is Gram-negative, pleomorphic and has a single polar flagellum (Fig. 1).

Mean cell width and length are 0.5 and 1.5–3.0 µm, respectively. In stressed cultures, filaments and coils 20 µm long are formed.

The biochemical tests (Table 1) were done as described by Smibert & Krieg (1981), except that Instant Ocean (IO; Aquarium Systems) was used instead of sodium chloride at a final concentration of 3.5%. Strain 2-40 is catalase- and peroxidase-positive and nonfermentative. Growth requires sea-salt-based medium. It grows in the mineral medium of Niven (1977), although it grows better with organic nitrogen than with ammonium. When tyrosine or peptone are present, eumelanin is produced during late phases of growth. An ethanol-insoluble, anthrone-positive polymer accumulates when the organism is growing in glucose salts medium.

Strain 2-40 multiplies from 4 to 37 °C. The optimum pH for growth is 7.5, although it also grows at pH 4.5–10.0. The optimum sea salt concentration is 23–35 g l⁻¹, although it grows up to 100 g l⁻¹. At 60 g sea salt l⁻¹, cells become filamentous; at 80 g l⁻¹, the filamentous forms dominate and coiled cells appear; at 100 g l⁻¹, cells cling to the flask wall. Strain 2-40 does not grow below 10 g sea salt l⁻¹, and, consequently, does not grow on nutrient agar plates unless they are amended with salt.

The C+G content of the DNA, determined by thermal denaturation (Tm), is 46.7 mol %.

A number of polysaccharides were tested, as described previously (Ensor et al., 1999), for release of reducing sugars in medium inoculated with spent medium of
2-40. The following CP were positive: agar, agarose, alginic acid, arabin, carrageenan, carboxymethylcellulose, chitin, fucoidan, glycogen, laminaran, pectin, pullulan, sodium polygalacturonate, starch and xylan. The following compounds were negative after 2 d incubation at 30°C: cellulose, inulin, polygalacturonic acid and Sephadex.

Methods used for antibiotic sensitivity testing were described previously (Weiner et al., 1985). Strain 2-40 was resistant to bacitracin (0.04 U), erythromycin (5 µg), nitrofurantoin (300 µg), P Taxo (5 µg), penicillin G (10 U), polymyxin (300 U) and streptomycin (2 µg), and susceptible to ampicillin (10 µg), novobiocin (30 µg), sulfadiazine (300 µg) and tetracycline (30 µg).

Strain 2-40 synthesizes a true tyrosinase, forming melanin from L-tyrosine. This enzyme also acts on L-DOPA, D-tyrosine, p-cresol, catechol, 2,6-dimethoxyphenol, syringaldazine, guaiacol and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) (Kelley et al., 1990; Solano & Sanchez-Amat, 1999).

For fatty acid methyl ester (FAME) analysis, 2-40 was grown at 27°C and harvested during the late exponential phase of growth. The FAME analysis was done using the MIDI system (Microbial ID). The following major fatty acids were detected: 16:0, 37%; 14:0, 15%; 12:1 3-OH, 6%; 10:0 3-OH, 11%; and 10:0, 5%. The major fatty acids whose levels were not quantified due to poor resolution of the chromatography system were: 18:1 ω7c, 18:1 ω9t and 18:1 ω12t. A search of the MIDI database revealed that the closest relative to 2-40 according to the fatty acid profile was Marinobacterium georgiense KW-40 with the following major fatty acids: 18:0, 1%; 16:0,

![Fig. 1. Transmission electron micrograph of strain 2-40, grown in 0.2% glucose minimal medium to exponential phase and stained with uranyl acetate. Note the single polar flagellum. Bar, 1 µm.](image-url)

**Table 1. Phenotypic comparison of selected traits of marine bacterium strain 2-40 with those of its closest relative based on 16S rDNA analysis, Microbulbifer hydrolyticus**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Strain 2-40</th>
<th><em>Microbulbifer hydrolyticus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Pleomorphic rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Cell length</td>
<td>1.0–20 µm</td>
<td>1.1–1.7 µm</td>
</tr>
<tr>
<td>Cell arrangement</td>
<td>Single, chains</td>
<td>Single, short chains</td>
</tr>
<tr>
<td>Flagellum</td>
<td>Single, polar</td>
<td>None</td>
</tr>
<tr>
<td>Surface nodular-like structures</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth temperature range</td>
<td>4–37 °C</td>
<td>10–41 °C</td>
</tr>
<tr>
<td>Growth pH range</td>
<td>4.5–10.0</td>
<td>6.5–8.5</td>
</tr>
<tr>
<td>Requirement for &gt;1% salt for growth</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Synthesis of eumelanin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth factor requirement</td>
<td>B cofactors stimulatory</td>
<td>–</td>
</tr>
<tr>
<td>Growth on monosaccharides</td>
<td>+</td>
<td>Limited number</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Agar</td>
<td>+</td>
<td>–</td>
</tr>
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</table>
Carbonoelasticus, pseudomonads, Oceanospirillum, Marinobacterium, Marinomonas and members of the family Halomonadaceae (Fig. 2).

Strain 2-40 is one of two marine isolates, the other being Marinomonas mediterranea MMB-1, which are reported to express polyphenol oxidase activity. Marinomonas mediterranea and strain 2-40 are, however, phylogenetically distant. Polyphenol oxidase characterizes properties with fungal laccases, enzymes known to be involved in lignin degradation (Solano & Sanchez-Amat, 1999). Therefore, the polyphenol oxidases of the two bacteria may be involved in the degradation of lignin in the aquatic environment.

It is significant that the 16S rDNA sequence of 2-40 is most closely affiliated with the 16S rDNA sequences of Microbulbifer hydrolyticus and a symbiont of shipworms. Several bacterial isolates were obtained in association with six different strains of shipworm and possibly other species of teredinids. The symbionts were proposed to be the same species of bacteria based on physiological and metabolic characteristics (Waterbury et al., 1983) and this was later confirmed by sequence analysis of the 16S rDNA (Distel et al., 1991). Although there is not yet enough data to ascertain the taxonomy of the shipworm isolate, it may belong to the genus Microbulbifer. Thus, Microbulbifer hydrolyticus, 2-40 and the shipworm symbionts form a cluster of marine bacteria with strong and extensive ability to degrade CP and other polymers of plant and animal origin. Both strain 2-40 and Microbulbifer hydrolyticus also express vesicular or nodular-like structures on the outer membrane under certain growth conditions. It is possible that, as new taxa are found that cluster with strain 2-40, this bacterium and the shipworm symbionts could be assigned to two new species of the genus Microbulbifer or of a closely related new genus.

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References


