Proposal of Vibrio mediterranei sp. nov.: A New Marine Member of the Genus Vibrio

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Vibrio strains isolated from plankton, sediments, and seawater in two coastal areas south of Valencia, Spain, were genotypically and phenotypically different from other Vibrio species. The guanine-plus-cytosine content of the strains was 43 mol%. Deoxyribonucleic acid-deoxyribonucleic acid hybridization studies revealed that the strains were genetically homogeneous and exhibited very low levels of genetic relatedness to Vibrio parahaemolyticus ATCC 17802T (T = type strain) (0%), Vibrio harveyi NCMB 1280T (11%), Vibrio vulnificus NCMB 2046T (7%), Vibrio nereis NCMB 1897T (0%), Vibrio natriengens NCMB (9%), and Vibrio splendidus NCMB 1T (0%). Strain CECT 621 of the Colección Española de Cultivos Tipo was designated the type strain of the proposed new species Vibrio mediterranei.

The genus Vibrio accounts for a major group of marine and estuarine bacteria. This genus has received much attention in recent years; there has been an increase from 5 species in Bergey’s Manual of Determinative Bacteriology, 8th ed. (12), to 29 currently recognized species (2–6, 8, 11, 13, 14).

In a preliminary study of marine Vibrio strains from water, sediments, plankton, and crabs in two coastal areas of Valencia, Spain, 10 phena were detected at 80% numerical taxonomy similarity levels by using the simple matching coefficient and the unweighted pair group method with arithmetic average (10). The phena included Vibrio alginolyticus, Vibrio harveyi, and Vibrio campbellii, but some other phena required further characterization by genetic approaches for specific assignment. Phenon 10, which included four strains with different origins, was phenotypically and genotypically distinct from all reference strains of Vibrio included in the study and also from all species described previously.

In this paper we present a genetic and phenotypic characterization of the strains belonging to cluster 10 (10) and describe Vibrio mediterranei sp. nov. as a new marine member of the genus Vibrio.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. They were maintained on marine agar slants (Difco Laboratories, Detroit, Mich.) at room temperature with bimonthly transfers and were also lyophilized.

Phenotypic characters. The minimal characteristics recommended for genus and specific assignment were determined as described previously (1). Photomicrographs of cells negatively stained with 1% (wt/vol) phosphotungstic acid in water (pH 7.0) were taken with a JEOL model 100-S electron microscope.

DNA isolation and purification. The deoxyribonucleic acids (DNAs) of the strains listed in Table 1 were isolated from cultures grown on solid medium (seawater-yeast extract agar in five or six Roux flasks) by using the method of Marmur as described by Johnson (7), with the following modifications.

In the first deproteinization, aqueous saturated phenol was used instead of a perchlorate treatment. This was followed by overnight dialysis against 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7). Ribonuclease treatment was carried out for 1 h; this was followed by the addition of 100 µg of predigested pronase (Sigma Chemical Co., St. Louis, Mo.) per ml to the DNA solution, which was then incubated for 2 h at 37°C and overnight at 28°C.

The purity of the DNA was checked by using the ratio of absorbance at 260 nm to absorbance at 280 nm and the ratio of absorbance at 260 nm to absorbance at 230 nm, which should have been more than 1.80 and 2.20, respectively.

DNAs were stored as solutions in 0.1× SSC over chloroform at 4°C and were precipitated with ethanol before use. The guanine-plus-cytosine (G+C) contents of the DNAs of strains 50 and 104 were determined from the midpoint values of the thermal denaturation profiles, which were obtained by using a Gilford model 250 spectophotometer at 260 nm. The instrument was programmed for temperature increases of 1°C/min. The G+C content was obtained from the thermal denaturation temperature (Tm) by using the Tm of Escherichia coli strain B DNA (G+C content, 51 mol%) as a control and the equation of Owen and Hill (9).

DNA was labeled with tritiated deoxycytidine triphosphate (catalog no. TRK.621; Amersham International plc, Amersham, Buckinghamshire, United Kingdom) by using an Amersham model N.5000 nick translation kit according to the instructions of the supplier. The reaction was stopped by adding 200 mM ethylenediaminetetraacetic acid, and the reaction mixture was passed through a 10-cm Sephadex G-50 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden). The first 20 fractions were collected (approximately 200 µl per fraction) and monitored for total and trichloroacetic acid-precipitable radioactivity. The first two radioactive fractions were selected for hybridization experiments.

Hybridization of DNAs. The competitive nitrocellulose filter method was used for DNA hybridization (7). Most experiments were performed five times (the exceptions are indicated in Table 3). The hybridization mixtures contained 20% formamide (Sigma). The hybridization temperature used was Tm minus 25°C (optimal temperature). Nonspecific adsorption of radioactive DNA was evaluated by using membrane-bound salmon sperm DNA in noncompetitive adsorption experiments.

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TABLE 1. Bacterial strains used and sources of new isolates

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Origin</th>
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<tbody>
<tr>
<td>V. harveyi</td>
<td>NCMB 1280T</td>
<td></td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>ATCC 17802T</td>
<td></td>
</tr>
<tr>
<td>V. vulnificus</td>
<td>NCMB 2046T</td>
<td></td>
</tr>
<tr>
<td>V. splendidus</td>
<td>NCMB 1T</td>
<td></td>
</tr>
<tr>
<td>V. nereis</td>
<td>NCMB 1897T</td>
<td></td>
</tr>
<tr>
<td>V. natriengens</td>
<td>NCMB</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>CECT 101b</td>
<td></td>
</tr>
<tr>
<td>V. mediterranei</td>
<td>50T</td>
<td>Sea sediment</td>
</tr>
<tr>
<td>V. mediterranei</td>
<td>84</td>
<td>Seawater</td>
</tr>
<tr>
<td>V. mediterranei</td>
<td>85</td>
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</tr>
<tr>
<td>V. mediterranei</td>
<td>104</td>
<td>Sea plankton</td>
</tr>
</tbody>
</table>

* This strain, which was originally received as Vibrio pelagius NCMB 1900T, was identified as V. natriengens on the basis of its physiological characteristics.

b CECT, Coleccion Española de Cultivos Tipo.

RESULTS AND DISCUSSION

Features typical of the genus Vibrio were exhibited by the four strains of cluster 10. However, these strains were unlike previously described species in the genus. Therefore, we propose the new species described below.

**Vibrio mediterranei** sp. nov. Vibrio mediterranei (me.dि.ter-ra' ne. i. L. gen. n. mediterranei of the Mediterranean Sea) cells are gram-negative rods that are 1 to 2 by 0.5 μm and are motile with a polar flagellum when they are grown on liquid medium (Fig. 1). Non-sporforming. Chemoorganotrophic, with both oxidative and fermentative metabolism. Reduces nitrate to nitrite. Oxidase and catalase positive. No growth in the absence of NaCl in the culture medium.

Colonies on marine agar are circular, translucent, and not pigmented and do not swarm. The thiosulfate-citrate-bile-sucrose agar colonies are yellow (sucrose positive). No growth occurs at 40°C or in the presence of 10% NaCl.

Voges-Proskauer negative. Susceptible to 2,4-diamino-6,7-diisopropylpteridine (vibriostatic agent 0/129). Starch, esculin, DNA, and Tween 80 are hydrolyzed. No hydrolysis of gelatin, urea, or human blood. Arginine dihydrolase and ornithine decarboxylase negative; lysine decarboxylase and indole positive.

Acid but no gas is produced from D-glucose, D-fructose, D-ribose, maltose, mannitol, sucrose, D-mannose, D-galactose, D-cellobiose, D-trehalose, D-sorbitol, and D-ribose, but not from L-arabinose, myo-inositol, D-xyllose, dulcitol, or L-rhamnose.

Sole carbon and energy sources include sucrose, D-cellobiose, and putrescine, but not D-gluconate or gamma-aminoobutyrate. Other carbon and energy sources are L-alanine, L-serine, L-asparagine, glycine, L-glutamine, L-proline, L-threonine, L-glutamate, L-aspartate, malate, lactate, D-sorbitol, D-galactose, lactose, myo-inositol, and salin.

Additional characteristics which vary among strains are shown in Table 2.

Isolated from marine water, sediments, and plankton of the coastal area of Valencia, Spain.

The type strain is strain 50T (= CECT 621).

The genetic relationships between V. mediterranei and

![FIG. 1. Electron micrograph of a negatively stained V. mediterranei strain 50T cell. Bar = 1 μm.](image-url)
some other selected species are shown in Table 3. The DNA homology data confirm results obtained by using numerical taxonomy for these strains, which clustered apart from the other wild and type strains of *Vibrio* species at a similarity level of 86% (10).

*V. mediterranei* is readily distinguished from *Vibrio* species with similar G+C contents by the arginine dihydrolase, Voges-Proskauer and gelatin tests (Table 4). Arginine dihydrolase-negative species can be differentiated as indicated in Table 5.

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LITERATURE CITED


