PROPOSAL OF A NEOTYPE, ATCC 15381, FOR
VIBRIO MARINUS (RUSSELL 1891) FORD 1927
AND REQUEST FOR AN OPINION¹

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SUMMARY. Vibrio marinus (Russell 1891) Ford 1927 American Type Culture Collection strain 15381 is proposed as neotype strain (culture) of Vibrio marinus, a description of which is presented. A request for an Opinion on the proposal of strain ATCC 15381 as the neotype by the Judicial Commission of the International Committee on Bacteriological Nomenclature is made.

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The taxonomy, referring to identification and classification, of marine bacteria has not received the attention of bacteriologists such as is required for preparing precise and useful descriptions of bacteria isolated from the sea and the marine environment. Except for the early work of Russell (1891), Fischer (1894) and Benecke (1905), relatively little information about the systematics of marine bacteria has reached print. There is indication, however, that the increased interest in the world oceans as a source of food, minerals and latent energy, will suffuse the field of marine bacteriology. Investigation of the food chain operating in the world oceans requires some knowledge of life at the lowest levels of the pyramid.

The problems encountered in identification and classification of marine bacteria cannot be ascribed totally to lack of attention or interest, insomuch as the organisms isolated from the marine environment often demonstrate peculiar features of very low temperature optima, inorganic ion requirements or barophilic tendencies. To study these isolates satisfactorily and determine descriptions for purposes

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of comparison with data of other workers, rigid definition of media, methods and procedures is imperative. Therefore, in studying the commensal flora of marine animals, one of several projects currently underway, an effort was made to collect taxonomic data concerning the bacterial species which were isolated and examined in pure culture.

A group of isolates identified as Vibrio marinus (Russell 1891) Ford 1927 were obtained and have been described elsewhere (Colwell and Morita, 1964). These strains appear to represent a truly marine group since representative isolates have been collected at different times of the year in various areas of Puget Sound and off the Pacific Coast (Colwell, 1962).

The American Type Culture Collection strain 15381 (National Collection of Marine Bacteria 1144, Colwell and Morita MP-1) is herein proposed as the neotype strain of Vibrio marinus (Russell) Ford. This strain conforms to the description as given by Russell (1891) and Ford (1927).

American Type Culture strain 15382 (National Collection of Marine Bacteria 1143, Colwell PS 207) is placed as a forma specialis and designated as alternate neotype strain for the species.

Materials and Methods

Strain PS 207 was isolated from the skin of a Pacific cod caught off Port Orchard, Washington. Sampling conditions and procedures have been described elsewhere (Colwell, 1962). Strain MP-1 was isolated from a water sample raised from 1200 m depth in the north Pacific Ocean (Morita and Haight, 1964). The water (ca. 500 ml, temperature 3.24°C) was treated as described by Colwell and Morita, 1964, in isolating the organism.

The stock medium for maintaining the cultures was an artificial seawater broth of the following composition:

Proteose-peptone (Difco), 10g; yeast extract (Difco), 3g; artificial seawater ("Seven Seas Mix," Utility Chemical Co., Paterson, N.J.), 1000 ml; pH 7.2. Incubation temperature of all inoculated stocks and test media, 18°C.

Morphological, cultural, environmental and biochemical characteristics were determined as described by Colwell and Morita (1964). Requirement for seawater was established by streaking pure cultures on agar plates containing

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artificial seawater agar and on agar plates with medium of identical composition except that distilled water was used as diluent. The requirement for specific ions was determined by the use of several media which contained one or all of the following: NaCl, 2.4%; KCl, 0.07%; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.53%; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.70%. All test media subsequently were made up with the four inorganic salts listed above as the "synthetic seawater" diluent.

A standard inoculum was one drop from a Pasteur pipette (ca. 1/20cc) of a 24 hr. artificial seawater broth culture.

The tests and test procedures followed have been listed previously (Colwell and Morita, 1964).

The homogeneity of the group of strains was studied by carrying out a numerical analysis using the computer method of Colwell and Liston (1961). A set of 123 coded features was obtained for each strain and from the coded data. The triangular matrix of similarity values was calculated by computer (Sneath, 1957). The computer output was further treated according to the methods of Liston, Weibe and Colwell (1963) and Quadling and Colwell (1963) for assessment of homogeneity and analysis of variance within the group.

Results

Sixteen isolates were included in the study of Vibrio marinus and as a group, the strains were homogeneous, with a high mean similarity index, $\bar{X}S = 81\%$, determined by numerical analysis of the coded features.

All strains were Gram-negative, slightly curved rods, short to medium length ($0.6 \mu \times 1.0 \mu$), with rounded ends and occurring as singles and pairs. "Round bodies" or rounded forms were often present in wet mount preparations. Temperature appears to play a significant role in the formation of these round body forms.

Colonies on seawater agar were grayish, opaque, raised (2-5 mm diam.) with an entire edge and a smooth surface. No diffusible pigments were observed, either in daylight or under ultraviolet. In seawater broth, the appearance of the growth was uniform with an even, slight turbidity and no pellicle. Fifteen of the sixteen strains studied were actively motile.

The organism designated as PS-207 (Colwell, 1962) was

able to grow at temperatures from 20 °C to 30°C, 30°C being the maximum (Burton and Morita, 1963; Morita and Burton, 1963). Temperature characteristics of the other strains showed a maximum growth temperature of 24°C.

All of the sixteen strains were seawater-requiring, that is, no growth was obtained on media without added seawater. A requirement for Na^+ is strongly indicated by the inability of the organisms to grow in media containing various combinations of 0.01 M KCl, 0.026 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.028 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.1% trypticase. Furthermore, a replacement of NaCl with the osmotic equivalent of sucrose did not support growth.

Additional features are listed in Tables 1 and 2.

Discussion

Very few descriptions of marine species of bacteria are available in the literature which can be used for identification and classification purposes. ZoBell and Upham (1944) have published a list of sixty "new species" but the descriptions are scanty and the media and methods used are not reproducible. The more recent descriptions of bacterial isolates from the marine environment have relied on "aged seawater" as the test media (Kriss, 1959; Shewan, 1963). For those strains of marine bacteria which are capable of growth on a defined medium, it should be possible to present a full and complete description for reference by other workers in marine bacteriology.

The particular group of microorganisms hereindescribed demonstrate a detectable requirement for the Na^+ ion and grow only at low temperatures, 0 °C to 30°C, which is not surprising in view of the saline composition of seawater and its temperature range of -1.5°C to 27.4 °C.

In 1891, Russell described a bacterial species which he had isolated from samples taken in the Bay of Naples. The species, Spirillum marinum (Russell, 1891) was described by him as follows:

"Spirillum marinum.

Dieser Bacillus wurde zwar sowohl im Schlamm wie im Wasser gefunden, war aber durchaus kein häufiger Bewohner irgend einer Localität. Mikroskopisch steht er den

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Table 1. Selected characteristics descriptive of the species
Vibrio marinus.

Characteristic ^a	Result ^b
No. of strains examined	16 (100)
Salt requirement (seawater) c. 0.5 M	16 (100)
Temperature relations (growth) c. 0°C - 20°C	16 (100)
Oxidase positive	16 (100)
Catalase positive	16 (100)
Glucose fermented, without production of gas	16 (100)
Acid produced from maltose	14 (87.5)
Starch hydrolyzed	5 (31.5)
Chitin digested	16 (100)
Gluconate oxidized to ketogluconate	0 (0.0)
Cellulose decomposed	0 (0.0)
Growth on calcium lactate medium	0 (0.0)
Growth on Simmons' citrate medium	0 (0.0)
Visible, diffusible pigment produced	0 (0.0)
Litmus milk acid	1 (6.3)
Gelatin liquefied	14 (87.5)
Casein hydrolyzed	16 (100)
Growth on L-proline-synthetic seawater medium	16 (100)
Indole produced	0 (100)
Methyl red positive	1 (6.3)
Voges-Proskauer positive	1 (6.3)

^a Test media prepared with synthetic seasalts.
See materials and methods.

^b Number of strains; figures in parentheses are percentages of the total number of strains positive for the characteristic listed.

Table 2. Additional characteristics scored for the species Vibrio marinus.

Characteristic ^a	Result ^b
No. of strains examined	16 (100)
Growth on skim milk agar	0 (0.0)
Growth on sodium caseinate agar	16 (100)
Nitrate reduced to nitrite	15 (93.8)
Nitrite reduced	1 (6.3)
Hydrogen sulfide produced	1 (6.3)
L-phenylalanine converted to phenylpyruvic acid	0 (0.0)
L-phenylalanine converted to melanins	0 (0.0)
Growth on vitamin-free casamino acids	5 (31.3)
Growth on L-phenylalanine + salts agar	1 (6.3)
Growth on DL-alanine + salts agar	5 (31.3)
Growth on B-alanine + salts agar	0 (0.0)
Growth on L-arginine + salts agar	0 (0.0)
Growth on L-lysine + salts agar	0 (0.0)
Growth on sodium formate agar	0 (0.0)
Growth on Koser's citrate medium	4 (25.0)
Growth on Sabouraud maltose agar	0 (0.0)
Growth on Sabouraud dextrose agar	0 (0.0)
Growth on ethanol agar	16 (100)
Ethanol converted to acetic acid	0 (0.0)
Fucose oxidized	0 (0.0)
Agar digested	0 (0.0)
Galactose acid	7 (43.8)
Glucose gas	0 (0.0)
Growth in glucose + iodoacetate medium	4 (25.0)
Glucose + iodoacetate → acid	0 (0.0)
Aesculin hydrolyzed	1 (6.3)

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Table 2. (continued)

Characteristic ^a		Result ^b
Sensitive to:		
2, 4 diamino-6, 7 diisopropylpteridine (0/129)		11 (68.8)
Penicillin - high concentration disc		0 (0.0)
Dihydrostreptomycin	10 µg	0 (0.0)
Chloromycetin	30 µg	16 (100)
Erythromycin	15 µg	15 (93.8)
Novobiocin	30 µg	16 (100)
Terramycin	30 µg	16 (100)
Tetracyclin	30 µg	15 (93.8)
Aureomycin	30 µg	14 (87.5)
Kanamycin	30 µg	14 (87.5)
Polymyxin B	300 units	13 (81.3)

^a Test media prepared with synthetic seasalts.
See Materials and Methods.

^b Number of strains: figures in parentheses are percentages
of the total number of strains positive for the character-
istic listed.

gebogenen, vibrioenartigen Bacterienformen nahe. Er ist klein und in jungen Culturen gewöhnlich zu Zweien vereinigt. Gewöhnlich ist er mehr oder minder gebogen, ähnlich wie das Choleraspirillum, doch kommen auch gerade Formen vor, aber wenn mehrere Individuen zu einem Filament vereinigt sind, so gewinnt das Ganze einen dem Spirillum ähnlichen Habitus. Das Spirillum marinum besitzt zwei Bewegungsformen, eine rotirende und eine fortschreitende, so dass es sich sehr rasch korkzieherartig über das Gesichtsfeld hinweg bewegt. Eine Sporenentwicklung wurde nicht beobachtet. Mit Löffler'schen Blau färbt sich das Spirillum leicht und nimmt in Fuchsin einen sehr dunkeln Ton an.

Aussehen der Culturen.

Diese Species scheint auf gewöhnlicher Gelatine üppiger zu wachsen als auf Meerwassergelatine. Auf Platten erscheinen die Colonieen zuerst als runde granulirte Massen, oft mit radiärer Streifung. Wenn die Colonie die Gelatine zu verflüssigen beginnt, so gewinnt sie ein rauheres Aussehen und in der flachen mit flüssiger Gelatine gefüllten Schale schwimmen flockige Massen herum.

In gewöhnlichen Gelatinestichculturen wächst der Bacillus Zuerst äusserst rasch, indem er die Gelatine in eine trübe Flüssigkeit verwandelt und eine dünne, halbdurchsichtige Membran darauf bildet. In Meerwassergelatinestichculturen ist das Wachsthum beschränkter, es beginnt ziemlich im Impfstich und gewinnt seine grösste Mächtigkeit an der Oberfläche, wo es zunächst eine flache Vertiefung in der Gelatine bildet, gefüllt mit einer trüben Flüssigkeit, auf deren Oberfläche die Colonie als dicke Haut schwimmt. Alte Culturen zeigen eine dünne trübe Flüssigkeitsschicht über der unveränderten Hauptmasse der Gelatine.

Auf Agar wächst Spirillum marinum einige Zeit sehr üppig und bildet einen feuchten, flüssigen, weisslichen Ueberzug, ähnlich wie Eiter. Derselbe sammelt sich am Boden des Rohres und wird im Laufe der Zeit wässerig.

Auf kartoffelculturen ist das Wachsthum dieses Spirillum höchst charakteristisch. In 24 Stunden erscheint ein röthlichbrauner, scharf conturirter Belag und ändert die Kartoffel in der Nähe des Impfstiches ihre Farbe. Diese zoogloea nimmt stetig zu, bildet eine dicke wachsartige Masse, welche später fast die ganze Kartoffel bedeckt, dabei

bleibt sie weich und dringt absolut nicht in den Nährboden ein, obgleich die Kartoffel nach und nach eine dunkel graugrüne Farbe annimmt.

In Meerwasserbouillon ist das Wachstum üppig und ruft einen reichlichen feinen Bodensatz hervor, während die darüber stehende Flüssigkeit stark getrübt erscheint. An der Oberfläche bildet sich eine weisse glatte Membran. Gewöhnliche Bouillon wird weinger stark getrübt" (pp. 198-199).

Although Russell comments on the fact that the organism as described by him was able to grow in nutrient gelatin, potato and "agar" without seawater, the requirement for Na^+ or NaCl cannot be discounted in view of the probable sodium content of these materials, at least in the case of the gelatin and potato media, sufficiently high to support growth of seawater-requiring strains.

The group of organisms described in this study and in earlier published work (Colwell and Morita, 1964) has been identified as Vibrio marinus, according to the emended description of Ford (1927). The species description has been further emended to include the features listed in this study.

Other data which are given for the species include the following. Observation of flagella stains, using light microscopy, and electron micrographs show that ATCC strains 15381 and 15382 possess a single polar flagellum. Base composition analysis of the deoxyribonucleic acid, determined for both strains, indicate over-all base composition of 40 and 42 moles percent guanine + cytosine for strains 15381 and 15382, respectively.

It should be pointed out that ATCC strain 15382 (PS 207) differs from ATCC strain 15381 (MP-1) in certain characteristics. The strain, PS 207, demonstrates the ability to hydrolyze aesculin, produce positive methyl red and Voges-Proskauer tests, reduce nitrites and produce hydrogen sulfide from cysteine. In addition, this strain possesses a slightly higher maximum temperature for growth (30°C). Therefore, strain ATCC 15382 (PS 207) is placed as a forma specialis of Vibrio marinus (Russell) Ford and is designated as the alternate neotype strain, representing a special form of the species.

American type Culture Collection strain 15381 (MP-1, NCMB 1144) is designated as the neotype strain for the

species Vibrio marinus (Russell) Ford as emended and extended by Colwell and Morita (1964).

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