

Recognition of *Beneckea natriegens* (Payne et al.) Baumann et al. as a Member of the Genus *Vibrio*, as Previously Proposed by Webb and Payne

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On the basis of a study of the biochemical, cultural, molecular genetic, morphological, nutritional, and physiological characters of the type strain (ATCC 14048) of *Beneckea natriegens* (Payne et al.) Baumann et al., we support the proposal by Webb and Payne that this organism is a member of the genus *Vibrio*.

A controversy has surrounded the taxonomic validity of the genus *Beneckea* since it was initially proposed by Campbell (2) to accommodate peritrichous cells of the family *Achromobacteraceae* which attack chitin. Baumann et al. (1) extended the description of *Beneckea*, although the relationship of their results to those of Campbell is unclear (2). Essentially, *Beneckea* was originally described as comprising gram-negative, peritrichous, facultatively anaerobic, straight rods of marine origin which are capable of degrading chitin and fermenting glucose with the production of acid but no gas (2). The revised description of *Beneckea* by Baumann et al. (1) includes gram-negative, fermentative, sodium-requiring rods that are motile by means of single polar flagella. The overall deoxyribonucleic acid (DNA) base composition was given as 45 to 48 mol% guanine plus cytosine (G + C). However, Shewan and Véron (9) regard *Beneckea* as a genus incertae sedis and *B. natriegens* as a species of uncertain taxonomic position. Strains whose names bear the specific epithet "*natriegens*" acquired a checkered history for they have, at one time or another, been placed in the genera *Pseudomonas* (8), *Beneckea* (1), and *Vibrio* (10). On the basis of the phenotypic characters and DNA base composition of the type strain (ATCC 14048) of this species, we support the transfer of this organism to the genus *Vibrio*, as previously effected by Webb and Payne (10).

MATERIALS AND METHODS

Bacterial strain. A culture of the type strain of *Beneckea natriegens* obtained from the American Type Culture Collection, Rockville, Md., under the number 14048 (= strain 111 of P. Baumann) was used in this study. Bench cultures were maintained at room temperature, i.e., ca. 25°C, on marine 2216 agar slants (Difco), with subculturing every 4 weeks.

Phenotypic characterization. The strain was examined through a wide range of tests currently used in extensive numerical taxonomy studies and as described by Colwell and Weibe (3). These tests included

biochemical, cultural, nutritional, morphological, and physiological characters.

Determination of DNA base composition. The G+C content of purified DNA, prepared by the method of Marmur (6), was determined from the thermal denaturation temperature (T_m) (7) by using a Gilford 2400-S recording spectrophotometer at 260 nm programmed for temperature increases of 0.5°/min. The G+C content (in moles percent) was calculated from the T_m by the equation of DeLey (5).

Electron microscopy. Cells were grown in a medium containing 0.25 M NaCl, 3.8 mM KCl, 0.018 M $MgSO_4 \cdot 7H_2O$, 1% (wt/vol) nutrient broth (Difco), 0.5% (wt/vol) peptone (Difco), and 0.25% (wt/vol) yeast extract (Difco). After 3 h of incubation at 27°C, with aeration, a 0.2-ml sample of the cell suspension was negatively stained by using 2% (wt/vol) aqueous uranyl acetate. A second 0.2-ml sample was spread over an agar medium (as above, but gelled with 2% [wt/vol] agar). After 12 h of incubation at 27°C, the cells were washed off the surface of the agar with a glutaraldehyde solution (2% [vol/vol]; buffered with 0.2 M sodium cacodylate at pH 7.4 and containing the three salts mentioned above). The cells were subsequently negatively stained and examined by using a Hitachi HU11A transmission electron microscope.

RESULTS AND DISCUSSION

The phenotypic characters of *B. natriegens* ATCC 14048 determined in this study matched those of the description of strain 111 provided by Baumann and co-workers (1) insofar as the organism was a gram-negative, small, motile, facultatively anaerobic, rod-shaped bacterium that degraded starch, gelatin, and lipids but not sodium alginate or blood, grew on marine 2216 agar between 4 and 40°C, was unable to decarboxylate arginine, but utilized a wide range of carbon compounds, including DL-alanine, cellobiose, ethanol, D-(–)-galactose, glycine, L-(–)-histidine, D-(–)-mannitol, L-(–)-ornithine HCl, propanol, L-(–)-serine, sodium acetate, sodium butyrate, sodium caprylate, sodium citrate, sodium gluconate, sodium glutamate, sodium malate, sodium pelargonate, sodium propionate, so-

dium valerate, sucrose, L-(–)-threonine, and L-(–)-tyrosine, as sole sources of carbon and energy. In contrast to the description of *Beneckeia* by Campbell (2), peritrichous cells were not observed and chitin was not degraded.

Extensive transmission microscopy of *B. natriegens* ATCC 14048 grown in liquid media and on various solid surfaces, including agar, glass, polycarbonate filters, and plastic and metal surfaces, have consistently shown that only single, sheathed polar flagella (Fig. 1) or occasionally two (Fig. 2), or more rarely three, polar flagella are found. It is interesting that the G+C ratio of 45.1 mol% was lower than the value, 46.4 mol%, recorded by Baumann (1); our value agrees more closely with the 45.3 mol% reported by Webb and Payne (10).

Since the characters of the type strain of *B. natriegens* do not match those of the original description of *Beneckeia* as provided by Campbell (2), it appears unlikely that ATCC 14048 is a member of the genus *Beneckeia*. Furthermore, we agree with Shewan and Véron (9) that the genus *Beneckeia* should be considered a genus incertae sedis.

The taxonomic position of *B. natriegens* can be clarified by including this organism in the genus *Vibrio*. In particular, the organism possesses the general characteristics of the genus *Vibrio*, namely, it is a short, gram-negative, fermentative rod, is motile by means of one, and occasionally two or three, polar flagella, produces catalase and oxidase, and is susceptible to the vibriostatic agent O/129. Its DNA base composition of 45.1 mol% G+C falls well within the range established for *Vibrio* spp., i.e., 40 to 50 mol% (4, 9). Thus, the inclusion of *B. natriegens* in the genus *Vibrio* appears to be appropriate. Furthermore, the distinction between *B. natriegens* and other vibrios (*V. cholerae*, *V. parahaemolyticus*, *V. anguillarum*, *V. fischeri*, and *V. costicola*), as measured by DNA base composition, arginine dihydrolase, growth at 5 and 37°C, starch hydrolysis, and the utilization of sodium citrate as the sole carbon source for energy and growth, warrants the recognition of this organism as constituting a separate species. Thus, we recommend that *Beneckeia natriegens* be regarded as a member of the genus *Vibrio*, as previously proposed by Webb and Payne (10).

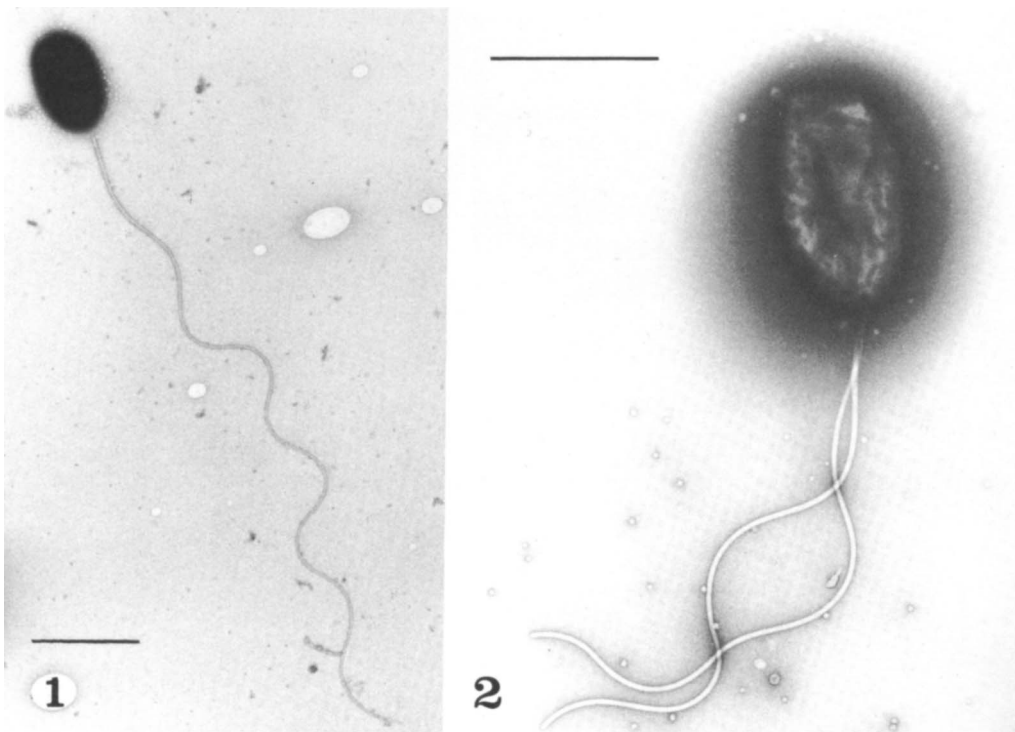


FIG. 1. ATCC 14048 cell, after 3 h of growth in liquid medium, stained with 2% (wt/vol) uranyl acetate. Bar equals 1 μ m.

FIG. 2. Negatively stained cell of ATCC 14048 showing two polar flagella. The culture was grown for 12 h on a solid medium. Bar equals 1 μ m.

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REPRINT REQUESTS

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