temperature for 7 days before use. Six to eight replicates were made from each of two sets of nine master plates with floc and velveteen, and were incubated at 20 C for 7 days. Results were assessed both qualitatively and quantitatively.

Replicates made with floc showed no colony spreading and merging due to excess moisture. However, small colonies were progressively squashed and merged with those close to them. Larger colonies were distorted in shape and sometimes replicated as groups of small, discrete colonies. Small colonies were lost. Cell material was spattered, producing colonies absent from the master plates. Fibers were shed onto the agar surfaces.

Shape, flexibility, and arrangement of the fibers appeared to account for the effects observed. Inocula were often found on fiber shafts instead of tips. When deposited, this material could smear, giving a squashed-colony effect. Colonies close together, when smeared, could then merge. Groups of fibers, behaving similarly, could account for the distortion and polka-dot reproduction of larger colonies. Flexed fibers carrying inocula, on springing back to their original shapes, could spray cells onto replicate surfaces.

Colonies might not be replicated for several reasons. Fibers could fail to contact the colonies. Examination of master-plate surfaces after impression showed fiber marks far apart and irregularly spaced. In contrast, pile marks were very close together and regularly spaced. Cell material might be picked up on shaft or tip of a fiber and not be deposited from either region because of a random lesser or greater flexion of the fiber. Shed fibers could result in the loss or misplacement of colonies.

In its present form, floc is decidedly inferior to velveteen. An improved material requires fibers which stand erect, are more resistant to deformation, and are more closely and regularly spaced.

BASE COMPOSITION OF DEOXYRIBONUCLEIC ACID OF MARINE AND NONMARINE VIBRIOS DEDUCED FROM BUOYANT-DENSITY MEASUREMENTS IN CESIUM CHLORIDE

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Identification and classification of bacteria isolated from the marine environment often present difficulties, since few species of marine bacteria have been adequately characterized and documented. A limited number of characteristics were reported or, in bacteriological testing procedures, insufficiently defined media were used, as, for example, "natural seawater" (ZoBell and Upham, Bull. Scripps Inst. Oceanog. Univ. Calif. **5**:239, 1944). Hence, much of the earlier taxonomic work on the marine bacteria is either vague or insufficiently precise for diagnosis of fresh isolates, particularly below the generic level of classification.

The use of high-speed computers in the identification of marine bacteria has been investigated by several workers (Shewan, Symp. Marine Microbiol., p. 499, 1960; Colwell and Liston J. Bacteriol. **82**:1, 1961). The correlation of Adansonian analysis data with those obtained from base composition determinations of purified deoxyribonucleic acid (DNA) isolated from microorganisms, by use of buoyant-density measurements (Schildkraut, Marmur, and Doty, J. Mol. Biol. **4**:430, 1960) and thermal denaturation temperatures of the DNA, i.e., helix-to-coil transition (Marmur and Doty, J. Mol. Biol. **5**:109, 1962), has been studied (Colwell and Mandel, J. Bacteriol. **87**:1412, 1964). A definite order of taxonomic clusters was obtained by these methods of analysis.

In the course of taxonomic investigation of bacteria isolated from seawater, seamuds, and marine animals and plants (Colwell, J. Appl. Bacteriol. **25**:147, 1963), strains of bacteria sharing features of the genus *Vibrio* were frequently isolated (Colwell and Gochnauer, Bacteriol. Proc., p. 40, 1963). A group of psychrophilic

marine bacteria, i.e., strains unable to grow at temperatures above 20 C and requiring seawater for growth, were isolated from seawater samples raised from a depth of 1,200 m off the coast of Oregon. These microorganisms were identified as *V. marinus* (Colwell and Morita, J. Bacteriol. **88**:831). The marine *Vibrio* strains demonstrated many of the features common to *V. cholerae* and *V. metschnikovii*, suggesting generic relationships at the least. We present results of base composition determinations made on DNA from five marine *Vibrio* strains and from *V. metschnikovii*, *V. cholerae*, and *Pseudomonas atlantica*. The marine vibrios, in previous analyses by computer, formed a taxonomic group with high similarity values (Colwell, Morita, and Gochnauer, Bacteriol. Proc., p. 37, 1964).

The marine vibrios were grown in and harvested from an artificial seawater medium consisting of 0.3% yeast extract, 1.0% Proteose Peptone, 2.4% NaCl, 0.07% KCl, 0.70% $MgSO_4 \cdot 7H_2O$, and 0.53% $MgCl_2 \cdot H_2O$ in distilled water at pH 7.0 to 7.2. Highly polymerized DNA in the native configuration was prepared by the method of Marmur (J. Mol. Biol. **3**:208, 1961). Base compositions were determined by measuring buoyant density in cesium chloride (Meselson, Stahl, and Vinograd, Proc. Natl. Acad. Sci. U.S. **43**:581, 1957). Details concerning procedures used have been reported (Colwell and Mandel, J. Bacteriol. **87**:1412, 1964).

Results of the buoyant-density measurements and base composition calculations, expressed as moles per cent guanine + cytosine (G+C content, are given in Table 1. The range of G+C content for the marine vibrios was about 39 to 48%. The *V. marinus* strain values were 40 and 42% G+C.

Certain points should be noted. First, the marine and the nonmarine vibrio G+C values fall into a single group; no significant separation of marine and nonmarine strains was noted. Saunders, Campbell, and Postgate (J. Bacteriol. **87**:1073, 1964) arrived at a similar conclusion in their study of sulfate-reducing bacteria of freshwater and saltwater origin. *Vibrio* sp. 329 is an agar-digesting strain; *Vibrio* sp. 822 and 859 are both chitin-digesting strains. *P. atlantica*, an agar- and alginate-utilizing organism with low overall similarity to the *Vibrio* strains (Colwell and Gochnauer, Bacteriol. Proc., p. 40, 1963), gave higher G+C DNA content, signficantly higher for generic differentiation at the least. The separation of these strains on the basis of both phenetic and genetic data casts strong doubt on classification schema which would group organisms on the basis of single features such as chitin digestion (*Beneckea*) and agar digestion (*Agar bacterium*), as presently listed in *Bergey's Manual* (Breed, Murray, and Smith, *Bergey's Manual of Determinative Bacteriology*, 7th ed., The Williams & Wilkins Co., Baltimore, 1957).

TABLE 1. *Marine and nonmarine vibrio DNA base composition from buoyant density in CsCl*

Organism	Density	G + C %
	g/cm³	
Vibrio sp. 329	1.698	39
V. marinus MP-1	1.699*	40
V. marinus 207	1.701	42
Vibrio sp. 822	1.702	43
V. metschnikovii ATCC 7708	1.703†	43
Vibrio sp. 859	1.707	48
V. cholerae 20 A10	1.708‡	49
Pseudomonas atlantica	1.714	55

* Thermal transition (T_m C) = 86.0 (Colwell, Citarella, and Ryman, *in preparation*).

† Thermal transition (T_m C) = 88.6 (Colwell and Mandel, J. Bacteriol. **87**:1412, 1964), nonmarine sp.

‡ Thermal transition (T_m C) = 89 (Schildkraut, Marmur, and Doty, 1962); chemical analysis = 43.3% G + C (Belozersky and Spirin, *In* E. Chargaff and J. N. Davidson [ed.], *The Nucleic Acids*, vol. 3, Academic Press, Inc., New York, 1960); nonmarine sp.