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Deoxyribonucleic Acid Base Composition of Geographically Diverse Strains of *Leucothrix mucor*

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Leucothrix mucor is a colorless organism related to the blue-green algae (R. Harold and R. Y. Stanier, Bacteriol. Rev. **19**:49, 1955; E. G. Pringsheim, Bacteriol. Rev. **21**:69, 1957), which is widespread in the marine environment, occurring primarily as an epiphyte on algae (T. D. Brock, Limnol. Oceanog., *in press*). This organism is recognized and taxonomically defined by a set of morphological properties which include: (i) nonmotile colorless filaments (2 to 4 μ in diameter) of varying lengths, each filament composed of many cells; (ii) under certain conditions cells of the filaments round up and separate into individual cells called gonidia which exhibit gliding motility; (iii) production by gonidia of holdfast material, permitting attachment to surfaces or to other gonidia; and (iv) mutual attraction of gonidia at high gonidial density, leading to the formation of characteristic rosettes. Gonidial formation occurs predominantly under conditions where growth is slow, such as in media of low organic nutrient concentration (E. G. Pringsheim, Bacteriol. Rev. **21**:69, 1957) or at low temperatures (T. D. Brock, *unpublished data*). Another morphological feature found in all isolates is knot production (T. D. Brock, Science **144**:870, 1964), the knots forming best under conditions promoting rapid and prolific growth. Because of the distinctive morphology of *L. mucor* and because of its occurrence as an algal epiphyte, it is relatively easy to recognize the organism directly in natural collections. A fairly extensive survey of marine habitats (T. D. Brock, Limnol. Oceanog., *in press*) has revealed that *L. mucor* is found predominantly in temperate oceanic waters in regions where there is good aeration due to tidal current or wave action. Isolates have thus been obtained from geographically diverse areas, and these isolates have proved remarkably similar in morphological and physiological properties. All isolates, for instance, show similar nutrition, salt requirements, *p*H tolerance, and temperature tolerance, and the main difference seen so far is in the relative degree of conversion of filaments to gonidia.

It was therefore of interest to study the deoxyribonucleic acid (DNA) base composition of these strains, since this characteristic has proved useful in uncovering differences between otherwise closely related bacteria (J. Marmur et al., Ann. Rev. Microbiol. **17**:329, 1963). In addition to the strains isolated by the senior author, isolates were obtained from other people who had worked with *L. mucor*. Unfortunately, the strains of E. G. Pringsheim are no longer available (personal communication to T. D. Brock).

All strains were grown in shaken flasks in the following medium: NaCl, 11.7 g; $MgCl_2 \cdot 6H_2O$, 5.35 g; Na_2SO_4, 2 g; $CaCl_2 \cdot 2H_2O$, 0.75 g; KCl, 0.35 g; tris(hydroxymethyl)aminomethane, 0.5 g; Na_2HPO_4, 0.05 g; monosodium glutamate, 10 g; water, 1,000 ml; *p*H 7.6. Each strain was grown in 1 liter of medium dispensed in four 500-ml flasks for 2 days at 25 C; the cells were centrifuged and washed twice in the above medium without monosodium glutamate, and the final pellet was frozen at −70 C until needed. Between 3 and 6 g (wet weight) of cells were obtained per liter of culture medium, depending on the strain. The pellet was suspended in 50 ml of saline-ethylenediaminetetraacetic acid, and 10 to 15 mg of crystalline lysozyme was added. After 1 hr at 37 C, lysis was complete and the suspension was highly viscous. The DNA isolation and purification then followed the procedure of J. Marmur (J. Mol. Biol. **3**:208, 1961), with elimination of the final isopropanol precipitation. The buoyant densities of the DNA preparations were determined by CsCl density gradient centrifugation according to the method of C. L. Schildkraut, J. Marmur, and P. Doty (J. Mol. Biol. **4**:430, 1962), with *Bacillus subtilis* bacteriophage SP8 DNA as a density reference (1.742 g/ml).

The buoyant densities and the calculated guanine plus cytosine (GC) contents of the DNA

TABLE 1. *Buoyant density in CsCl of DNA of strains of Leucothrix mucor and estimated guanine plus cytosine contents*

Strain of *L. mucor*	Source	Algal associate	Buoyant density*	GC content (moles per cent)
			g/ml	
1	T. D. Brock, Puget Sound, Wash., 1963	Monostroma	1.7085	49.5
2	R. Lewin, strain 7, Woods Hole, Mass., 1959	Callithamnion	1.708	49.0
3	T. D. Brock, Puget Sound, Wash., 1964	*Callophyllis haenophylla*	1.708	49.0
4	T. D. Brock, Puget Sound, Wash., 1964	*Odonthallia flocosa*	1.708	49.0
5	R. Harold and R. Y. Stanier, Calif., 1955	Ulva	1.708	49.0
6	I. Anderson, strain I 11, Narragansett Bay, R. I.	None, isolated from sea water	1.707	48.0
7	T. D. Brock, Long Island Sound, 1965	Polysiphonia	1.7075	48.5
8	T. D. Brock, Long Island Sound, 1965	Unidentified red alga	1.708	49.0
9	T. D. Brock, Cape Reykjanes, Iceland, 1965	Unidentified red alga	1.7075	48.5
10	T. D. Brock, Cape Reykjanes, Iceland, 1965	Ulva	1.707	48.5
11	T. D. Brock, Cape Reykjanes, Iceland, 1965	Laminaria	1.706	46.9

* Mean of two determinations.

preparations are shown in Table 1. The buoyant densities of the preparations were remarkably similar, and only strain Lm 11 showed a significant difference from the others, although even this strain varied only slightly from the mean. The mean GC content for the entire group was 48.6 $\pm$ 1.1 moles per cent, and if strain Lm 11 was excluded this value became 48.8 $\pm$ 0.7 moles per cent GC. It was thus concluded that the isolates form a quite homogeneous group in terms of DNA density. It seems likely that the species *L. mucor*, as defined morphologically, represents a homogeneous group of strains as defined physiologically and by DNA composition. This contrasts with the diversity of physiological and genetic types often found in other groups of morphologically similar bacteria, and probably reflects the fact that the cells and filaments of *L. mucor* are sufficiently large and distinctive so that the species can be readily recognized by simple microscopic examination.

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