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Deoxyribonucleic Acid Base Composition of Isolates of the Genus *Chlorobium*

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Until the description of the monotypic genus *Chloropseudomonas* (Shaposhnikov, Kondratieva, and Fedorov, Nature **187**:167, 1960), the genus *Chlorobium* Nadson represented the only green sulfur bacterium which had been studied in pure culture. The first successful axenic cultivation of *C. limicola* was achieved by Van Niel (Arch. Mikrobiol. **3**:1, 1931) and of *C. thiosulfatophilum* by Larsen (J. Bacteriol. **64**:187, 1952). The two species have been the only representatives of the genus and have been distinguished solely by the ability of the latter to utilize thiosulfate as an electron donor. The question of a third type, "*thiophilum*," was disposed of by Larsen (Kgl. Norske Videnskab. Selskabs Skrifter **1**:1953). A large number of pure cultures of green sulfur bacteria have been isolated recently from North American and European environments. The method of cultivation was described by Pfennig [Zentr. Bakteriol. Parasitenk. Abt. I (Suppl.): 179, 1964]. These isolates have been characterized as *C. thiosulfatophilum* or *C. limicola* on the basis of the ability to utilize thiosulfate. The deoxyribonucleic acid (DNA) of each isolate was examined for base composition to determine whether the species separation based on this single criterion could be further supported (Marmur, Falkow, and Mandel, Ann. Rev. Microbiol. **17**: 329, 1963). The chemically determined DNA base composition of one representative of *C. thiosulfatophilum* has been reported as 57.8%

TABLE 1. *Characteristics of Chlorobium isolates and their isolated deoxyribonucleic acid*

Strain	Thiosulfate utilization	T_m	GC*	DNA buoyant density†	GC‡
		C	%	*g/cc*	%
Gilroy Hot Spring	−	91.3	50	1.710	51
Zeulenrode	−			1.7105	51.5
Klein-Kalden	−	92.1	51	1.7105	51.5
Reiershausen	−			1.711	52
Lüneberg	+			1.7115	52.5
Golf Pond	+			1.7115	52.5
Moss Landing	−	92.2	51	1.7125	53.5
Sehestedt	+			1.7125	53.5
Ps	−	92.3	52	1.714	55
Berkeley	+	94.1	56	1.7155	56.5
Carmel R. 17	−			1.716	57
Carmel R. 24	+			1.7165	57.5
Rothamstedt	+			1.7165	57.5
Federsee	−	94.2	56	1.717	58
Bennhausen	−			1.717	58
Carmel R. 3	+			1.717	58
Tassajara	+			1.717	58

* Calculated from T_m.
† Average of duplicate determinations.
‡ Calculated from buoyant density.

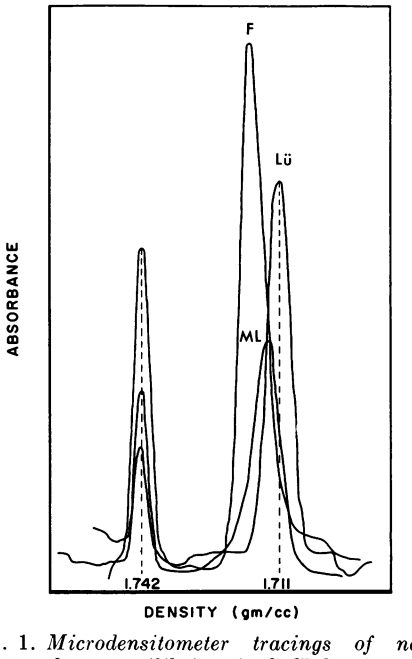

FIG. 1. *Microdensitometer tracings of native DNA samples at equilibrium in CsCl density gradient. The tracings are superimposed so that the centers of the bands of the reference DNA (from bacteriophage SP8) coincide. Sample F from strain Federsee, ML from Moss Landing, Lü from strain Lüneberg.*

guanine plus cytosine (GC) (Vanyushin and Belozersky, Dokl. Acad. Nauk SSSR **135**:197, 1960).

DNA was isolated from cells harvested from 200 to 400 ml of growth medium by a modification of the method of Marmur (J. Mol. Biol. **3**:208, 1961). The modification involved immediate phenol extraction of the detergent-lysed cells and elimination of the isopropanol precipitation when low yields were apparent. The base composition of the DNA was estimated from the buoyant density in CsCl in analytical ultracentrifugation (Schildkraut, Marmur, and Doty, J. Mol. Biol. **3**:595, 1961) with DNA from bacteriophage SP8 as a reference standard. Melting curves of representative samples were performed in the standard saline citrate buffer as described by Marmur and Doty (J. Mol. Biol. **5**:109, 1962) for more highly purified samples to determine the regularity of structure of the DNA samples and to confirm the observed differences in GC content inferred from the density measurements. Good agreement between the two physicochemical parameters of base composition was realized (Table 1).

The microdensitometer tracings of the DNA bands of three of the cultures are shown in Fig. 1; in all cases, similar unimodal bands of native molecules have been observed in the CsCl density gradient, and heat-denatured samples likewise demonstrate a unimodal band of appropriately increased density.

The data in Table 1 demonstrate the existence of organisms having different DNA base compositions, with at least three types being discernible: a group of organisms with 52% GC, another with 57% GC, and a smaller group with 53 to 55% GC. It is also obvious that these groupings do not correlate with the *limicola-thiosulfatophilum* division. This suggests that there are at least three species, perhaps more, of green sulfur bacteria, and that the distinctions based on ability to utilize thiosulfate or polythionates may be relatively trivial insofar as speciation of this group is concerned. But much more information on the biochemical, physiological, and morphological properties of the different strains is needed before further conclusions can be formed on the taxonomy of *Chlorobium*.

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