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BASE COMPOSITION OF DEOXYRIBONUCLEIC ACID OF SULFATE-REDUCING BACTERIA DEDUCED FROM BUOYANT DENSITY MEASUREMENTS IN CESIUM CHLORIDE

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ABSTRACT

SAUNDERS, GRADY F. (University of Illinois, Urbana), L. LEON CAMPBELL, AND JOHN R. POSTGATE. Base composition of deoxyribonucleic acid of sulfate-reducing bacteria deduced from buoyant density measurements in cesium chloride. J. Bacteriol. **87**:1073-1078. 1964.—The base composition of the deoxyribonucleic acid (DNA) of sulfate-reducing bacteria was calculated from buoyant density measurements in CsCl. The sporulating sulfate-reducing bacteria fell into two groups: *Desulfovibrio orientis* with a DNA base composition of 42% guanine plus cytosine (G + C), and *Clostridium nigrificans* with a DNA base composition of 45% G + C. The mesophilic relative of *C. nigrificans* had a DNA base composition of 46% G + C. Thirty strains of nonsporulating sulfate-reducing bacteria called *D. desulfuricans* were studied. They fell into three groups as judged by DNA base composition: group I (11 strains), 60 to 62% G + C; group II (13 strains), 54 to 56% G + C; and group III (6 strains), 46 to 47% G + C. These data underline the need for a taxonomic revision of this group of microorganisms.

Schildkraut, Marmur, and Doty (1962) published base compositions derived from buoyant density measurements on deoxyribonucleic acid (DNA) from single representatives of four types of sulfate-reducing bacteria. Sigal et al. (1963) published analytical data on seven strains which, with the exception of *Desulfovibrio orientis*, were consistent with those of Schildkraut et al. (1962). The base composition of DNA promises to be a useful tool in elucidating the somewhat nebulous taxonomy of the sulfate-reducing bacteria, but more data are needed on the variation in base composition among strains of a putative species. As part of a wider taxonomic study of this group, the DNA base composition of a variety of strains was studied by the buoyant density procedure.

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MATERIALS AND METHODS

Organisms. Thirty strains of *D. desulfuricans* were studied. All were nonsporulating, mesophilic, anaerobic, sulfate-reducers; all contained a C_3-type cytochrome and desulfoviridin; and all were progressively motile, except for strains Holland D-6 and Teddington R which were nonmotile. The following 16 strains were of fresh-water origin: Hildenborough, from clay, Kent, England; Teddington M, from Thames River mud at Teddington, England; Marseille Gaz 54, from gas holder water, Marseille, France; Holland D-6, from ditch mud, Delft, Holland; Beckton, from sewage outfalls, Beckton, England; Furusaka, from a muck paddy field in Japan; Llanelly, from soil by a corroded gas main in Llanelly, Wales; Benghazi, from well water from Benghazi, Libya; Wandle, from Wandle river water near London, England; "cholinicus," from stagnant creek mud near Chapel Hill, N.C.; Teddington R, from Thames River water at Teddington, England; Essex 6, from a tar and sand mixture surrounding a gas main in clay in South Essex, England; Berre Eau and Berre Sol, from water and soil samples, respectively, from Etang de Berre, Marseille, France; Holland C-6, from canal mud, Delft, Holland; and Monticello 2, from duck-pond mud at Monticello, Ill. The following 14 strains were of salt-water origin: Walvis Bay, from Walvis Bay, S.W. Africa; Sylt 3, from marine mud, Hamburg, Germany; Norway 4, from Oslo Harbor water, Norway; El Agheila Z, from sulfur-lake mud near El Agheila, Cyrenaica, N. Africa; Canet 20, Canet 40, and Canet 41, from Etang de Canet, Perpignan, France; Venice 1, from the Grand Canal, Venice, Italy; Avonmouth, from an oil and water sample from an exploded petroleum tank, Avonmouth, England; British Guiana, from "sling mud" in

British Guiana; El Agheila C, from a mud sample from Ain-el-Braghi, Libya; California 43:11, from a mud core sample on Timbalier Island off the coast of Louisiana (this strain was isolated by workers at the Scripps Institution of Oceanography, La Jolla, Calif., and was designated California 43:11); California 43:63 from mud from Sorrento Slough, near Del Mar, Calif.; and Maizuru 1, from Maizuru Bay, Japan. A large spirilloid sulfate-reducer, strain Gigas, was also examined. This strain was isolated by J. LeGall from a water sample from the Etang de Berre, Marseille, France. It is a nonsporulating, mesophilic, anaerobic organism exhibiting progressive motility with lophotrichous flagella, and contains both a C_3-type cytochrome and desulfoviridin (LeGall, 1963).

Two strains of *D. orientis* were examined; Singapore 1 was isolated from a soil sample near a rising main at Sungei Whampa, Rangoon Road, Singapore, and Singapore 2 was isolated from soil from Ulu Pandan, Singapore. These strains are sporulating, mesophilic, anaerobic, sulfate-reducing vibrios; neither contains a C_3-type cytochrome nor desulfoviridin; both are multiflagellate and exhibit nonprogressive motility (Adams and Postgate, 1959).

Two unclassified sporulating sulfate-reducers isolated from the heated rumen fluid of a sheep, strain Coleman 42 and strain DL, were examined. These strains are mesophilic, anaerobic, sulfate-reducing rods closely related to *Clostridium nigrificans;* they do not contain a C_3-type cytochrome or desulfoviridin; they exhibit nonprogressive motility with peritrichous flagella (Postgate and Campbell, 1963).

Two strains of *C. nigrificans* were studied; Teddington Garden strain was isolated from garden soil at Teddington, England; NCA strain was isolated from spoiled canned food, Washington, D.C. These strains are thermophilic, anaerobic, sulfate-reducing rods; they do not contain a C_3-type cytochrome or desulfoviridin; they exhibit nonprogressive motility with peritrichous flagellation (Postgate, 1956; Campbell, Frank, and Hall, 1957).

The following strains called *D. desulfuricans* were obtained from G. H. Booth, National Chemical Laboratory, Teddington, England: Beckton, Canet 20, Berre Sol, Avonmouth, and Benghazi; strain "cholinicus" was obtained from H. Hayward and T. C. Stadtman, National Heart Institute, Bethesda, Md.; strain Furusaka was obtained from C. Furusaka, Tohoku University, Sendai, Japan; strain Maizuru I was obtained from H. Kadota, Kyoto University, Japan; strain Monticello 2 was isolated locally (Postgate, 1963). Strain Holland C-6 of *D. desulfuricans* and the NCA strain of *C. nigrificans* were obtained from the American Type Culture Collection, Washington, D.C. The strains of the mesophilic relative of *C. nigrificans*, Coleman 42 and DL, were obtained from G. S. Coleman, ARC Institute for Animal Physiology, Babraham, Cambridge, England. All other strains were obtained through the courtesy of J. M. Shewan from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland. All cultures were checked for purity by the agar shake-tube method described by Postgate (1953).

Media. Stock cultures were maintained in Baars' (1930) medium supplemented with yeast extract (0.1%, w/v) and, for strains of marine origin, with NaCl (2.5%, w/v).

The sporulating organisms were grown in a modification of the medium C of Butlin, Adams, and Thomas (1949) which was described by Baker, Papiska, and Campbell (1962); for the thermophilic strains, sodium pyruvate (0.6%, w/v) replaced sodium lactate as the carbon source. The nonsporulating strains were grown either in modified medium C or in medium N. Medium N was developed in a study of the growth yields of these organisms (Campbell and Postgate, *unpublished data*) and contained: $MgCl_2 \cdot 6H_2O$, 0.06 g; sodium citrate $\cdot 2H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.06 g; KH_2PO_4, 0.5 g; NaCl, 1.0 g; $(NH_4)_2SO_4$, 7.0 g; yeast extract (Difco), 1.0 g; sodium lactate, 6.0 g; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.01 g; dissolved in the order listed per liter of distilled water. The pH was adjusted to 7.5 prior to sterilization by autoclaving. Filter-sterilized (Millipore) Na_2S (1 mM) was added to poise the E_h, except in the cases of the Gigas strain, for which sodium ascorbate (0.5 mM) was used, and of *D. orientis*, for which sodium thioglycolate (1 mM) was used. For strains of marine origin, all media contained 2.5% (w/v) NaCl except where mentioned.

Cultivation. Mesophiles were grown at 30 C and thermophiles at 55 C. Anaerobiosis was obtained with pyrogallol plugs made alkaline with a solution containing K_2CO_3 (15%, w/v) and NaOH (10%, w/v). After incubation for 24 to 36 hr,

populations equivalent to 10 to 50 mg (dry weight) of organisms were harvested by centrifugation and stored as a frozen pellet until needed.

DNA isolation. DNA was isolated by an abbreviated and scaled-down version of the method of Marmur (1961). The frozen pellets were resuspended in 2.5 ml of saline-EDTA (0.15 M NaCl-0.1 M ethylenediaminetetraacetate; pH 8). The cells were lysed, as evidenced by an increase in viscosity, by the addition of sodium lauryl sulfate (0.2 ml of a 25% solution); following lysis, 0.6 ml of 5 M $NaClO_4$ and 3.3 ml of 4% (v/v) iso-amyl alcohol in chloroform were added to the tubes. The emulsion was shaken for 30 min, centrifuged, and the supernatant layer precipitated with 2 volumes of 95% ethanol. The fibrous precipitate was drained and redissolved in 0.5 to 2.5 ml of SSC buffer (0.15 M NaCl-0.015 M trisodium citrate; pH 7). The product was a crude DNA concentrate suitable for CsCl density gradient centrifugation. For determining the thermal denaturation temperature (Tm) of the DNA, purified DNA was prepared by the more rigorous procedure of Marmur (1961), and the final product was dialyzed overnight at 5 C against SSC.

Buoyant density (ρ) measurements. Portions of the crude DNA concentrates were centrifuged to equilibrium in a Spinco model E analytical ultracentrifuge at 44,770 rev/min at 25 C for 18 to 24 hr in approximately 5.7 M CsCl-0.02 M tris(hydroxymethyl)aminomethane (tris) buffer (pH 8.5), with N^{15}-labeled *Pseudomonas aeruginosa* DNA (ρ, 1.742 g/cc) as a reference standard. The banded DNA was photographed with the use of ultraviolet absorption optics. Photographs were traced with a Joyce-Lobel double-beam recording microdensitometer. Buoyant densities and base compositions were calculated according

TABLE 1. *Deoxyribonucleic acid base composition of sporulating strains of sulfate-reducing bacteria deduced from buoyant density determinations*

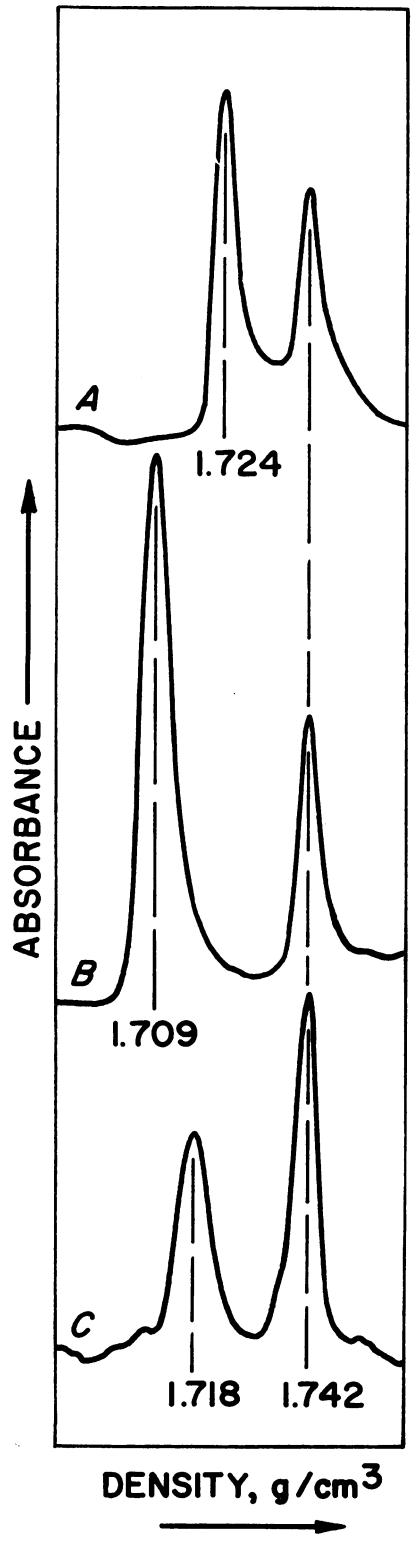
Strain	Buoyant density	Guanine + cytosine
	g/cc	%
Clostridium nigrificans		
Teddington Garden (8351)	1.708	44.7
NCA (7946)*	1.708	44.7
Unclassified strains		
Coleman 42 (8452)	1.709	45.6
DL	1.709	45.6
Desulfovibrio orientis		
Singapore 1 (8382)	1.705	41.7
Singapore 2 (8445)	1.705	41.7

* Refers to the American Type Culture Collection. All other strain numbers refer to the National Collection of Industrial Bacteria.

TABLE 2. *Deoxyribonucleic acid base composition of nonsporulating strains of sulfate-reducing bacteria deduced from buoyant density determinations*

Group	Strain and number	Buoyant density	Guanine + cytosine
		g/cc	%
I	Beckton (8319), Monticello 2 (9442), Furusaka	1.726	62.1
	Holland D-6 (8311), Gaz 54 (8386), Wandle (8305)	1.725	61.2
	Hildenborough (8303), Teddington M (8302), Llanelly (8446), Gigas (9332), Walvis Bay (8397), Benghazi (8401)	1.724	60.2
II	Sylt 3 (9335), "cholinicus" (13541)*	1.720	56.3
	Norway 4 (8310), Teddington R (8312), Holland C-6 (7757)*, Canet 20 (8391), Canet 40 (8363), Canet 41 (8393), Berre Sol (8388)	1.719	55.3
	Berre Eau (8387), Essex 6 (8307), El Agheila Z (8380), Venice I (8322)	1.718	54.4
III	Avonmouth (8398)	1.710	46.6
	British Guiana (8403), California 43:11 (8365), California 43:63 (8364), El Agheila C (8308), Maizuru I	1.709	45.6

* Refers to the American Type Culture Collection. All other strain numbers refer to the National Collection of Industrial Bacteria.



to the equations of Sueoka (1961); DNA from strain El Agheila Z was run five times, and gave a mean value of 54.4% guanine + cytosine (G + C) and a standard deviation of ± 0.3%.

Determination of the Tm. Thermal denaturation of highly purified DNA was followed in an Optica spectrophotometer equipped with a heated cuvette holder and temperature-control unit. Base compositions were calculated according to the relationship of McDonald et al. [1963; per cent of G + C = (Tm-69) 2.439]. The values obtained agree well with those obtained from a plot of Tm vs. per cent G + C made with the data of Marmur and Doty (1962).

RESULTS AND DISCUSSION

The buoyant density and per cent G + C values for the DNA of the sporulating sulfate-reducing bacteria are presented in Table 1. The values of 42% G + C obtained for the two strains of *D. orientis* differ from that for Singapore 1 (45%) quoted by Schildkraut et al. (1962), but are self-consistent and agree with the analytical value of 42% G + C quoted by Sigal et al. (1963). They also differ from the values (45 and 46% G + C) obtained for the DNA of *C. nigrificans* and its mesophilic relatives, Coleman 42 and DL (Postgate and Campbell, 1963). The value for *C. nigrificans* differs from that quoted by Schildkraut et al. (1961) for strain Delft 74 T (48% G + C), perhaps because a different method was used for calculating the per cent G + C.

Table 2 presents the buoyant density and per cent G + C values for the DNA of the non-sporulating sulfate-reducing bacteria. They fall into three groups: group I, 60 to 62% G + C; group II, 54 to 56% G + C; and group III, 46 to 47% G + C. Microdensitometer tracings of the banded DNA of a representative strain of each group are shown in Fig. 1. The Tm values and the per cent G + C of the DNA of these strains are presented in Table 3. The per cent G + C

FIG. 1. *Microdensitometer tracings of deoxyribonucleic acid (DNA) samples from representative strains of nonsporulating sulfate-reducing bacteria equilibrated in a CsCl density-gradient formed by centrifugation at 44,770 rev/min at 25 C. A, Hildenborough (8303); B, British Guiana (8403); C, El Agheila Z (8380). The band at the right in each case is N^{15}-labeled Pseudomonas aeruginosa DNA to provide a density reference.*

TABLE 3. *Deoxyribonucleic acid base composition of representative strains of nonsporulating sulfate-reducing bacteria calculated from thermal denaturation (Tm) measurements*

Strain*	Tm	Guanine + cytosine†
	C	%
Hildenborough (8303)...	93.6 ± 0.2	60.0
El Agheila Z (8380).....	90.5 ± 0.2	52.4
British Guiana (8403)...	87.1 ± 0.3	44.1

* Strain numbers refer to the National Collection of Industrial Bacteria.

† Base compositions were calculated by the equation of McDonald et al. (1963): % G + C = (Tm-69) 2.439.

calculated from the Tm agrees with the values obtained by the buoyant density procedure. The DNA base compositions of the nonsporulating sulfate-reducing bacteria obtained by the buoyant density procedure also agree essentially with the per cent G + C values quoted from chemical determinations by Sigal et al. (1963) for the strains examined in common. Sigal et al. (1963) divided these bacteria into two groups, corresponding to our groups I and II; however, they did not examine any of the strains which are in our group III.

The value of 54% G + C obtained for El Agheila Z DNA, a marine strain, was also obtained for the DNA of a substrain adapted to fresh water. A mixture of the DNA from the parent and adapted strain gave a single band with a buoyant density of 1.718 g/cc and 54% G + C. Similarly, in an experiment kindly performed by J. Marmur, the DNA base composition of a substrain of Hildenborough adapted to grow in a medium containing 2.5% NaCl did not differ from that of the parent strain. Hence, the characters acquired or lost by these bacteria by training to and from a marine environment (Ochynski and Postgate, 1963) are not reflected in their DNA base compositions.

Both marine and fresh-water strains appear in groups I and II, but so far all those in group III are obligate marine strains (Campbell and Vermeulen, *unpublished data*). However, this character does not separate an *aestuarii* and a *desulfuricans* type, because Sylt 3, which is in group II, is also an obligate marine strain (Stüven, 1960). The only nonmotile strains tested, Holland D-6 and Teddington R, are in groups I and II, respectively. With the exception of Monticello 2, the organisms so far examined in group I are incapable of sulfate-free growth in pyruvate media (Postgate, 1952; 1963). A firm correlation between specific fermentative properties and DNA base compositions may be possible when more data on the substrate specificities of these bacteria are available. Meanwhile, the present data show that the nonsporulating sulfate-reducing bacteria fall into well-defined groups, as judged by their DNA base composition, and underline the need for a taxonomic revision of this group of microorganisms.

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LITERATURE CITED

ADAMS, M. E., AND J. R. POSTGATE. 1959. A new sulphate-reducing vibrio. J. Gen. Microbiol. **20**:252–257.

BAARS, J. K. 1930. Over sulfaatreduktie door bacterien. Ph.D. Thesis. Technical University, Delft, The Netherlands.

BAKER, F. D., H. R. PAPISKA, AND L. L. CAMPBELL. 1962. Choline fermentation by *Desulfovibrio desulfuricans*. J. Bacteriol. **84**:973–978.

BUTLIN, K. R., M. E. ADAMS, AND M. THOMAS. 1949. The isolation and cultivation of sulphate-reducing bacteria. J. Gen. Microbiol. **3**:46–59.

CAMPBELL, L. L., JR., H. A. FRANK, AND E. R. HALL. 1957. Studies on thermophilic sulfate reducing bacteria. I. Identification of *Sporovibrio desulfuricans* as *Clostridium nigrificans*. J. Bacteriol. **73**:516–521.

LE GALL, J. 1963. A new species of *Desulfovibrio*. J. Bacteriol. **86**:1120.

MARMUR, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.

MARMUR, J., AND P. DOTY. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.

MCDONALD, W. C., I. C. FELKNER, A. TURETSKY,

AND T. S. MATNEY. 1963. Similarity in base compositions of deoxyribonucleates from several strains of *Bacillus cereus* and *Bacillus anthracis*. J. Bacteriol. **85**:1071–1073.

OCHYNSKI, F. W., AND J. R. POSTGATE. 1963. Some biochemical differences between fresh and salt water strains of sulphate-reducing bacteria, p. 426–441. *In* C. H. Oppenheimer [ed.], Symposium on marine microbiology. Charles C Thomas, Publisher, Springfield, Ill.

POSTGATE, J. R. 1952. Growth of sulphate-reducing bacteria in sulphate free media. Research (London) **5**:189.

POSTGATE, J. R. 1953. On the nutrition of *Desulphovibrio desulphuricans:* a correction. J. Gen. Microbiol. **9**:440–444.

POSTGATE, J. R. 1956. Sulphate-reducing bacteria which are deficient in cytochrome. J. Gen. Microbiol. **15**:viii.

POSTGATE, J. R. 1963. A strain of *Desulfovibrio* able to use oxamate. Arch. Mikrobiol. **46**: 287–295.

POSTGATE, J. R., AND L. L. CAMPBELL. 1963. Identification of Coleman's sulfate-reducing bacterium as a mesophilic relative of *Clostridium nigrificans*. J. Bacteriol. **86**:274–279.

SCHILDKRAUT, C. L., J. MARMUR, AND P. DOTY. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**:430–443.

SIGAL, N., J. C. SENEZ, J. LE GALL, AND M. SEBALD. 1963. Base composition of the deoxyribonucleic acid of sulfate-reducing bacteria. J. Bacteriol. **85**:1315–1318.

STÜVEN, K. 1960. Beiträge zur Physiologie und Systematik sulfatreduzierender Bakterien. Arch. Mikrobiol. **35**:152–180.

SUEOKA, N. 1961. Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of old and new data. J. Mol. Biol. **3**:31–40.