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Intraspecies Variability of Cellular Fatty Acids among Soil and Intestinal Strains of *Desulfovibrio desulfuricans*

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A comparison of cellular fatty acid profiles of *Desulfovibrio desulfuricans* DSM 642 and 14 wild strains of this species, isolated from two completely different environments, soil and the human intestine, was carried out. All the *D. desulfuricans* strains grown on lactate and sulfate indicated the presence of considerable amounts of i-$C_{15:0}$, i-$C_{17:1}$ and $C_{16:0}$. Although differences in the quantities of individual fatty acids present in each strain were clear in the group of soil strains (similarity, 67.6%), in contrast to almost identical fatty acid patterns (similarity, near 100%) in the intestinal strains, the results were variable within the limits acceptable for species demonstration. The higher similarity of the fatty acid profiles of intestinal strains may be a result of the similarity of biocenoses in the human digestive tract. The coefficients of variability of i-$C_{17:1}$ and i-$C_{15:0}$ (the major branched-chain fatty acids), as well as clustering of the investigated strains compared with strains described in the literature after plotting percentages of i-$C_{17:1}$ fatty acid against i-$C_{15:0}$ fatty acid, confirmed a certain heterogeneity of cellular fatty acid profiles within the group of soil strains, in contrast to almost ideal homogeneity within the group of intestinal isolates. Intestinal strains contained a higher ratio of saturated to unsaturated fatty acids (2.2 ± 0.14) than did soil strains (1.6 ± 0.2; in one case, 2.7). We propose that intestinal *D. desulfovibrio* bacteria should be assumed to be a highly homogeneous group and should be represented by the strain *D. desulfuricans* subsp. *intestinus* in collections of microbial cultures.

Dissimilatory sulfate reduction occurs in sulfate-reducing bacteria (SRB) of the genera *Desulfovibrio*, *Desulfotomaculum*, *Desulfomonas*, *Desulfobacter*, *Desulfobulbus*, and some others (16, 25, 34). These bacteria can couple oxidative phosphorylation with reduction of sulfate to produce hydrogen sulfide. The growing interest in SRB arises from their role in the environment. The influence of SRB is both ecological and economic. SRB participate in the degradation of organic matter in the natural environment (19, 34) and play an important role in the removal of heavy metals, such as mercury, chromium, and copper, from various environments (11, 18). On the other hand, these bacteria are responsible for corrosion processes, especially in pumping equipment, storage tanks, and pipelines used in oil technologies (17, 25). Significant progress has been made in the understanding of their biology and of their role in the human intestine. The presence of SRB in human feces was demonstrated by Beerens and Romond (1) and by Moore et al. (23) in the 1970s, and more intensive investigations have been carried out since then (4, 9, 10, 15, 16). It was suggested by Gibson's group (10, 14–16) that SRB may play an important role in the development of ulcerative colitis.

The lipid membrane profiles of SRB populations grown in liquid Postgate medium (24) are widely used as a valuable property for the identification of different SRB (2, 7, 21, 28, 29, 31, 33). The published comparative studies of cellular fatty acid profiles of various wild strains and type strains of definite species, such as *Desulfovibrio desulfuricans*, do not give enough information about the degree of similarity or dissimilarity of strains belonging to this species because of the small number of strains tested (usually no more than four).

The aim of this work was to determine the similarity of the fatty acid composition of cells of 1 type strain and 14 wild strains of *D. desulfuricans*, isolated from two diametrically different environments: soil samples and human intestines. The bacteria were cultured and analyzed under identical conditions.

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MATERIALS AND METHODS

Bacterial strains, culture conditions, and harvest. Fifteen gram-negative *D. desulfuricans* strains were investigated. Eight wild strains (referred to as soil strains) were isolated from various excavations (strains DV-1/86, -3/86, -4/86, -5/86, and -7/86) and mud deposits (strains DV-2/86, -6/86, and -8/86) (6). Six wild strains (referred to as intestinal strains) were isolated from feces (strains DV-A/94, -B/94, -C/94, -H/94, and -I/94) or biopsy specimens (strain DV-I-1/94) obtained from patients with various gastrointestinal disorders (7, 13). The *D. desulfuricans* type strain Essex 6 (La 2226, ATCC 29577, NCIB 8307, or DSM 642) was obtained from the Swiss National Collection of Type Cultures, Lausanne, Switzerland.

Cultures of isolated strains were enriched by the conventional batch method and were identified as *D. desulfuricans* by the number of criteria recommended in *Bergey's Manual of Systematic Bacteriology* (34) and by following directions given by other investigators (5, 25, 27, 33). Isolation and taxonomic determinations of the investigated strains have been described previously (6, 13). Generally, for isolation of *D. desulfuricans* in pure cultures, the fecal or biopsy samples were introduced into Postgate liquid medium B (25) containing lactate as the carbon source and sulfate as the terminal electron acceptor. After 3 weeks of incubation at 30°C under anaerobic conditions, the enriched slurries were used for preparation of the deep agar dilution series. The colonies that grew at greater depths were isolated and inoculated into liquid medium B. After blackening of the medium, the bacterial slurries were inoculated by the streak plate method on separate dishes containing Postgate solid medium B and incubated at 30°C. When single colonies were recognized by blackening, they were isolated, inoculated into liquid medium B, and incubated at 30°C until blackening of the medium. Then the cultures obtained were streaked on solid medium B in petri dishes by the reductive method. Incubation of agar plate cultures at the indicated temperature was performed in anaerostats saturated with a mixture of CO_2 and H_2 (5:95). After 3 to 5 days, single bacterial colonies causing blackening of the medium were separated and reinoculated onto solid medium. This procedure was repeated until homogeneous cultures were obtained. These cultures were maintained in a viable condition for several months and did not show any change in their characteristics. For long-term preservation, the culture specimens were lyophilized. Various culture, biochemical, and microscopic tests were performed to examine the taxonomic affiliations of the strains. All isolated intestinal *D. desulfuricans* strains were gram negative, mesophilic, motile rods, curved rods,

TABLE 1. Cellular fatty acid composition in soil and intestinal *D. desulfuricans* strains

Fatty acid	Cellular fatty acid composition (%) in[a]:														
	Soil strains								Intestinal strains						Type strain (DSM 642)
	DV-1/86	DV-2/86	DV-3/86	DV-4/86	DV-5/86	DV-6/86	DV-7/86	DV-8/86	DV-A/94	DV-B/94	DV-C/94	DV-H/94	DV-I/94	DV-I-1/94	
$C_{12:0}$	−	−	−	−	−	−	−	−	+	0.7	0.6	1.2	0.5	+	0.9
$C_{13:0}$	−	−	−	−	−	−	−	−	+	0.9	1.6	+	0.6	0.5	+
$C_{14:0}$	2.6	1.7	4.4	2.9	5.3	0.9	1.7	1.5	2.1	4.7	5.2	4.3	2.2	2.2	3.2
i-$C_{15:0}$	12.2	15.3	7.0	17.3	11.7	13.1	7.5	17.2	27.7	26.7	25.6	27.1	30.4	28.9	16.3
ai-$C_{15:0}$	7.0	6.4	12.6	10.2	9.7	7.4	7.6	9.7	5.6	5.0	4.0	6.9	9.1	5.1	2.3
$C_{15:0}$	2.8	2.7	+	0.6	3.3	0.7	2.7	0.6	0.6	1.4	1.9	1.7	0.9	0.7	1.6
$C_{16:1}$	9.7	10.1	4.1	10.9	8.5	9.1	5.3	7.3	5.6	5.7	3.6	3.6	5.1	4.3	13.8
i-$C_{16:0}$	1.6	1.5	1.3	2.7	1.2	2.7	2.2	2.6	0.5	0.4	0.5	+	0.4	0.4	0.7
$C_{16:0}$	14.2	14.6	26.6	14.1	14.5	12.9	13.2	12.5	22.4	18.7	18.3	19.1	20.0	21.7	19.7
i-$C_{17:1}$	23.8	24.9	17.2	19.6	29.4	24.0	20.9	21.9	26.1	23.1	24.1	22.8	23.8	26.3	20.9
i-$C_{17:0}$	7.5	5.3	5.2	6.6	4.9	8.9	9.7	7.9	7.6	5.9	8.8	7.6	5.2	7.3	7.9
ai-$C_{17:0}$	2.9	2.6	4.2	3.4	1.9	4.5	5.0	3.6	0.7	1.4	1.5	+	0.7	0.3	0.6
$C_{17:0}$	1.7	1.8	+	1.1	1.4	1.0	3.9	1.1	−	−	−	−	−	−	0.9
$C_{18:1}$	8.3	6.4	5.8	5.2	4.7	6.9	7.6	6.1	0.5	4.6	3.2	4.2	0.7	1.0	8.4
$C_{18:0}$	5.7	6.7	11.5	5.4	3.5	7.9	12.7	8.0	0.5	0.8	1.1	1.5	0.4	0.9	2.8

[a] −, not detected; +, present at <0.1%.

and vibrios, indicating the presence of desulfoviridin. Cultures grew strongly in media containing lactate (with sulfate or nitrate), pyruvate (alone or with sulfate), or malate with sulfate. Weaker growth was observed in media containing choline (with and without sulfate) or malate without sulfate. All strains were resistant to hibitane at 20 mg/liter. The strong fermentative growth on pyruvate (without sulfate) and the ability to ferment choline, as well as the strong growth on malate with sulfate and the relatively high hibitane resistance, allowed us to classify our strains as *D. desulfuricans* and distinguish them from *D. vulgaris*. *D. vulgaris* does not grow on malate plus sulfate and is inhibited by about 10-fold-lower concentrations of hibitane than is *D. desulfuricans* (25).

Prior to fatty acid analysis, all strains were cultured in Postgate liquid medium B (25) containing lactate and sulfate. The medium was sterilized in an autoclave at 120°C for 20 min. The cultures were grown anaerobically in 2-liter vessels at 30°C. At the end of the intensive growth phase, the culture suspension was decanted from above the sediments, which contained insoluble components of culture medium and products of bacterial metabolism, and then the cells were harvested from the liquid medium by centrifugation at 14,000 × *g* for 30 min.

Preparation of fatty acid methyl esters. The bacterial pellets were suspended in 3 ml of Tris-HCl buffer (pH 7.5), and bacterial cells were separated by centrifugation at 34,000 × *g* for 20 min. The pellets were weighed and used as wet bacterial biomass.

Cellular lipids in the wet bacterial biomass were saponified in 1 ml of 6 N NaOH in 50% methanol under reflux for 30 min at 100°C. After cooling, 3 ml of 3 N HCl in 40% methanol and 1 ml of the BF_3-methanol reagent were added. The mixture was well shaken and heated at 80 to 85°C for 10 min under reflux. The fatty acid methyl esters were then extracted from the rapidly cooled mixture by washing the tube contents three times with 2 ml of petroleum ether for 10 min each. The combined ether layers contained the methyl esters of the total cellular fatty acids.

GC-MS identification. The methyl esters were identified by gas chromatography-mass spectrometry (GC-MS) with a Hewlett-Packard 5989A mass spectrometer (17a), a Hewlett-Packard 5890 series II gas chromatograph, and the Hewlett-Packard ChemStation program with an NIST/EPA/NIH 75K mass spectral database (35). The GC was performed with an HP-5MS fused-silica capillary column (30 m by 0.25 mm [inner diameter]; 0.25-μm film) containing 5% diphenyl and 95% dimethylpolysiloxane; the column temperature was 60°C for 2 min, increasing to 250°C at 15°C/min and holding for 15 min; the injector temperature was 220°C, and the helium flow rate was 2 ml/min. The MS conditions were as follows: electron energy, 70 eV; ion source temperature, 174°C, analyzer (quad) temperature, 100°C; GC/MS interface temperature, 250°C. A standard bacterial acid methyl esters CP mix was obtained from Matreya, Inc., Chalfont, Pa.

Statistical evaluation of results. The results obtained for all *D. desulfuricans* strains investigated were used in numerical cluster analysis. Fatty acid profiles were computed to determine their similarity. A dendrogram based on the Pearson correlation coefficient as the distance metric was generated. Clustering of our *D. desulfuricans* strains was investigated after percentages of i-$C_{17:1}$ fatty acid had been plotted against those of i-$C_{15:0}$ fatty acid. Statistics were performed with the computer programme SYSTAT, version 5 (SAS Institute Inc., Evanston, Ill.).

RESULTS

To identify the fatty acid methyl esters obtained from the total cellular lipids of our *D. desulfuricans* strains, the results of the chromatographic analysis (by retention time) of these esters were compared with those obtained with a standard mixture containing known bacterial fatty acid methyl esters. Besides, the spectra obtained from GC-MS analysis indicated very high goodness of fit (higher than 90%) with those contained in the database program (35).

The cellular fatty acid composition of our *D. desulfuricans* strains is presented in Table 1. The fatty acid composition of wild strains (marked DV) was compared with that of the type strain DSM 642.

Data presented in Table 1 indicated the presence of straight-chain and branched-chain fatty acids, both saturated and unsaturated, in all investigated *D. desulfuricans* strains. Especially high concentrations of i-$C_{15:0}$, i-$C_{17:1}$, and $C_{16:0}$ were observed. Smaller amounts of ai-$C_{15:0}$, $C_{16:1}$, i-$C_{17:0}$, and $C_{18:1}$ were detected in all strains, and $C_{18:0}$ was present only in soil strains. Small amounts of $C_{14:0}$, $C_{15:0}$, i-$C_{16:0}$, and ai-$C_{17:0}$ were found in all strains, whereas $C_{17:0}$ was present only in the type strain and in soil strains. Strain DV-3/86 did not contain $C_{15:0}$. On the other hand, $C_{12:0}$ and $C_{13:0}$ were found only in the type strain, DSM 642, and in the intestinal strains. i-$C_{17:1}$ (biomarker for *D. desulfuricans*) was one of the major components in all the strains except DV-3/86, in which the highest concentration of $C_{16:0}$ was found. A considerable amount of $C_{16:0}$ was also detected in all the intestinal strains and in the type strain, DSM 642.

The results of similarity analysis of fatty acid composition revealed the presence of three distinct clusters among the *D. desulfuricans* strains (Fig. 1). Cellular fatty acid profiles were approximately identical (98.2 to 99.8% similarity for all intestinal strains (group 1). A 92.4% similarity was evaluated in group 2, containing soil strains DV-1/86, DV-2/86, DV-4/86, DV-5/86, DV-6/86, and DV-8/86. The members of this group were 87% similar to type soil strain DSM 642. All intestinal strains and soil strains mentioned above are similar at the 85.5% level. A pair of strains, DV-3/86 and DV-7/86 (group 3),

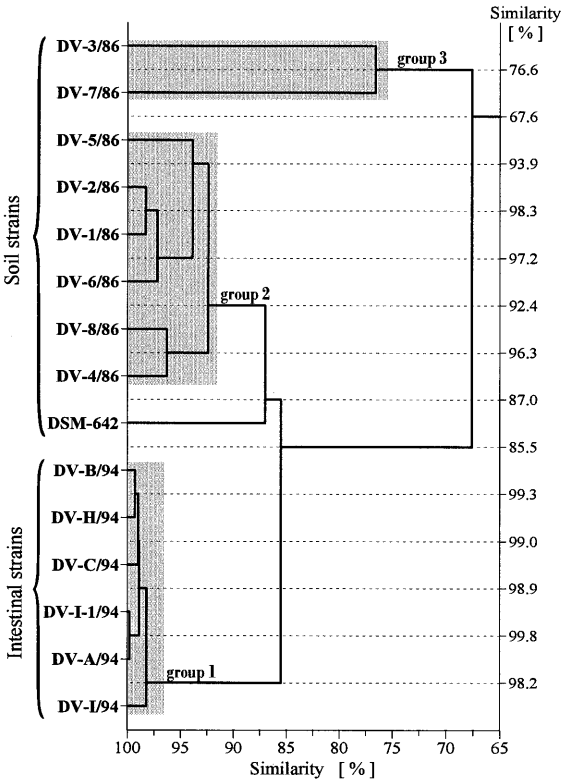

FIG. 1. Dendrogram generated by numerical cluster analysis of cellular fatty acid profiles of *D. desulfuricans* strains.

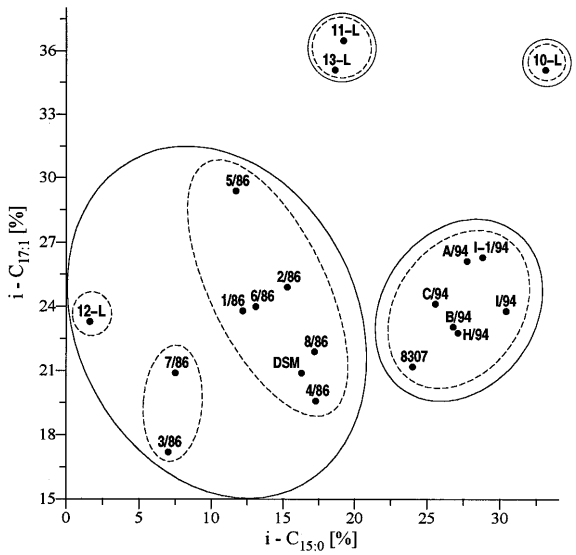

FIG. 2. Clustering of *D. desulfuricans* (DV) strains after plotting percentages of i-$C_{17:1}$ against those of i-$C_{15:0}$. The DSM strain and strains marked 1/86 through 8/86 are soil strains (DV) investigated in this study. Strains marked A/94 through I/94 are intestinal strains (DV) investigated in this study. 8307 is the NCIB 8307 type strain described by Ueki and Suto (31). Strains marked 10L through 13L are strains described by Vainshtein et al. (33) (see the text). Note that DSM = DSM 642 = 8307 = NCIB 8307 = 10L. Symbols: – – – –, six-cluster analysis; ———, four-cluster analysis.

had 76.6% similarity to each other and were similar to all other investigated strains at the 67.6% level.

Taking into account the concentrations of i-$C_{17:1}$ and i-$C_{15:0}$, the two main branched-chain fatty acids detected in investigated bacterial cultures, statistical analysis of the variability in the concentration of these acids among soil and intestinal strains of *D. desulfuricans* was carried out. The results are presented in Table 2. The i-$C_{17:1}$ and i-$C_{15:0}$ concentrations were very similar in the group of intestinal strains, with a standard deviation of ±1.5 to 1.7% and a coefficient of variation of about 6%. In the group of soil strains, a standard deviation of ±3.7 to ±3.9% was calculated and the coefficient of variation was 16.3 and 30.7% for i-$C_{17:1}$ and i-$C_{15:0}$, respectively.

The results of numeric group analysis of the concentrations of these two fatty acids in the *D. desulfuricans* strains, presented as a cluster picture obtained after plotting the percentage of i-$C_{17:1}$ against that of i-$C_{15:0}$, are shown in Fig. 2. Data taken from literature are also used in this figure for comparison with our results. All wild intestinal strains were again clustered in one group, which include also strain NCIB 8307, described by Ueki and Suto (31). The second group contained six wild soil *D. desulfuricans* strains (DV-1/86, -2/86, -4/86, -5/86, -6/86, and -8/86) and the soil type strain, DSM 642. Two wild soil strains (DV-3/86 and DV-7/86) were clustered in the third group. Strains 11-L (DSM 1924) and 13-L (DSM 4369), described by Vainshtein et al. (33), were clustered in the fourth group. These strains were isolated, respectively, from creek mud in the United States and from termite gut in Africa. Strains 10-L (DSM 642, isolated from soil in England) and 12-L (DSM 1926, isolated from sulfurous lake mud in Libya), also described by Vainshtein et al. (33), each formed a single cluster. When four-cluster analysis was used, all the wild soil strains were grouped with the type strain, DSM 642, and with strain 12-L (DSM 1926) described in the literature (33). The remaining strains formed the same clustering patterns as in six-cluster analysis.

TABLE 2. Variability in the concentrations of main branched-chain fatty acids in soil and intestinal strains of *D. desulfuricans*[a]

Source of *D. desulfuricans* strain (no. of isolates)	Fatty acid	$\bar{X}_n$ (%)	s (%)	k (%)
Soil (n = 8)	i-$C_{17:1}$	22.7	3.7	16.3
	i-$C_{15:0}$	12.7	3.9	30.7
Intestines (n = 6)	i-$C_{17:1}$	24.4	1.5	6.2
	i-$C_{15:0}$	27.7	1.7	6.1

[a] $\bar{X}_n$, arithmetic mean; s, standard deviation; k, coefficient of variation (k = 100s/$\bar{X}$).

The percentages of total saturated fatty acids (SFA) and total unsaturated fatty acids (UFA), as well as the SFA/UFA ratios in our *D. desulfuricans* strains, are presented in Table 3. The data show that the concentrations of SFA in all strains were higher than the concentrations of UFA. The SFA/UFA ratios calculated for the soil strains were within the range of 1.3 to 1.9 (except for strain DV-3/86, for which the SFA/UFA ratio was 2.7). SFA/UFA ratios of 2.0 to 2.4 were determined for intestinal strains.

DISCUSSION

It is generally accepted that characteristic patterns of cellular fatty acid profiles in various bacterial strains, as well as their high reproducibility and relative stability during the growth cycle, enable taxonomists to use fatty acids as chemotaxonomic

TABLE 3. SFA/UFA ratios for *D. desulfuricans* strains

Strain designation	% of SFA	% of UFA	SFA/UFA ratio
Type strain			
DSM 642	56.9	43.1	1.3
Soil strains			
DV-1/86	58.2	41.8	1.4
DV-2/86	58.6	41.4	1.4
DV-3/86	72.9	27.1	2.7
DV-4/86	64.3	35.7	1.8
DV-5/86	57.4	42.6	1.3
DV-6/86	60.0	40.0	1.5
DV-7/86	66.2	33.8	1.9
DV-8/86	64.7	35.3	1.8
Intestinal strains			
DV-A/94	67.8	32.2	2.1
DV-B/94	66.6	33.4	2.0
DV-C/94	69.1	30.9	2.2
DV-H/94	69.4	30.6	2.3
DV-I/94	70.4	29.6	2.4
DV-I-1/94	68.0	31.6	2.2

markers (22, 26, 28, 30–33). On the other hand, it is known that changes of cultivation and analytical conditions are reflected in the quantitative distribution of the fatty acids in the bacterial strains (28, 33). Vainshtein et al. (33) reported that repeated analyses of the same bacterial cell biomass were characterized by almost identical fatty acid profiles. The standard deviation calculated for the main fatty acids ranged up to ±12%. Similar calculations including results obtained from the long-term batch cultures (18 months) indicated an increase in the standard deviation up to ±17%. There is still only scarce information, however, about the intraspecies variability of fatty acid composition, especially for strains obtained from highly different environments.

In this study, we analyzed the cellular fatty acid composition of 15 strains of *D. desulfuricans*. The wild strains were isolated from two very different types of environments, i.e., soil and the human intestine. Although the cellular fatty acid compositions of bacterial strains belonging to various species have already been presented in numerous studies, there is a lack of information concerning *D. desulfuricans* strains isolated from the human digestive tract and their comparison with isolates obtained from other environments. Such information may be very important for the interpretation of fatty acid profiles of intestinal microbial communities. We assumed that a higher level of similarity of fatty acid profiles would be obtained in the group of intestinal strains than among the soil strains, because of the similarity of biocenoses in the human digestive tract (8). On the other hand, the greater difference in the fatty acids in strains that grow under the highly variable soil conditions could be diminished by use of identical growth medium and laboratory conditions during cultivation of the strains. Thus, it was difficult or even impossible to predict any differences or similarities in fatty acid profiles of the investigated strains.

In our study, the profiles of cellular fatty acid obtained for all investigated strains (1 type strain and 14 wild strains) of *D. desulfuricans* were sufficiently similar to confirm the identification of these strains as members of *D. desulfuricans*. Although the quantities of individual fatty acids present in the strains were variable within certain limits, the standard deviations calculated for the key fatty acids (i-$C_{17:1}$ and i-$C_{15:0}$) were no higher than ±4 and ±2%, respectively, for soil and intestinal strains (Table 2). Our data (Table 1) indicating that i-$C_{17:1}$, i-$C_{15:0}$, and $C_{16:0}$ are the main fatty acids in *D. desulfuricans* are in good agreement with those obtained by Ueki and Suto (31) for the type strain NCIB 8307 (DSM 642) and also described by Ueki et al. (30) for two wild strains isolated from drains. Cellular fatty acid profiles presented here and in the papers mentioned above are different from those obtained by Taylor and Parkes (28) for a *D. desulfuricans* strain isolated from the anaerobic sediments of Loch Eil, Scotland, and cultivated on lactate or under an atmosphere of H_2-CO_2 (80:20). This strain had a higher concentration of i-$C_{17:1}$ (about 40%) and considerably lower concentration of $C_{16:0}$. Also, Vainshtein et al. (33) found higher (above 35%) concentrations of i-$C_{17:1}$ in various *D. desulfuricans* strains, including the type strain DSM 642. We assumed that the observed discrepancy may be due to differences in the composition of culture media used in the different investigations and/or to differences in procedures of cellular fatty acid methyl ester preparation and analytical conditions used.

All the intestinal strains examined in our experiments had almost identical fatty acid patterns (similarity, near 100%). Surprisingly, all the soil strains maintained their individual fatty acid profiles (Fig. 1) regardless of cultivation under identical laboratory conditions, in contrast to the findings of Petersen and Klug (24) in a study of the phospholipid fatty acid profiles of soil microbial communities. Thus, the differences found among soil isolates may reflect the genetic makeup of the strains. In our study, the type strain DSM 642, isolated from soil (25), showed high similarity (above 90%) to the group of six wild soil strains (DV-1/86, -2/86, -4/86, -5/86, -6/86, and -8/86). The remaining two wild soil strains (DV-3/86 and DV-7/86) had about 25% lower similarity to all other tested strains. Although there were clear differences in the fatty acid patterns in the group of soil strains and the group of intestinal ones, it must be emphasized that all our results are variable within the limits acceptable for species demonstration.

Interesting information was obtained from the plot of the concentration of i-$C_{17:1}$ against that of i-$C_{15:0}$ (Fig. 2). The *D. desulfuricans* strains were grouped similarly to the groups in Fig. 1, in which all the detected fatty acids are included. The type strain designated NCIB 8307, investigated by Ueki and Suto (31), has been included in the cluster containing all wild intestinal strains. The same type strain deposited in the German collection and designated DSM 642 was described by Vainshtein et al. (33). This strain, labeled 10-L in Fig. 2, is separated from the other strains, even from the two strains 11-L and 13-L described in the literature (33), which are considered to be correctly classified as *D. desulfuricans* species. In the opinion of Vainshtein et al. (33), *D. desulfuricans* DSM 1926 (El Agheila Z), labeled 12-L in Fig. 2, may be misclassified and may not belong to *D. desulfuricans*. The distances between the separate clusters in Fig. 2 as well as the values of coefficients of variation for i-$C_{17:1}$ and i-$C_{15:0}$ concentrations (Table 2) may confirm that there are certain differences among soil strains of *D. desulfuricans* but high similarity for intestinal strains belonging to this species.

The composition of fatty acids present in phospholipids plays an important role in the cell membranes of microorganisms, because a change in the SFA/UFA ratio is one of several factors regulating the fluidity of the membrane (3). This fluidity is necessary for the function of membrane-related metabolic processes. Membranes composed of only saturated fatty acids are too rigid to play a proper role in vital cells at moderate temperatures (20). Some bacteria are able to change the portion of UFAs resulting after the growth temperature is decreased (12). This effect can be achieved by enhanced de-

saturase activity or by an increase in the amount of anteiso fatty acids (2).

Data presented in Table 3 show that all soil *D. desulfuricans* strains investigated in our experiments (excluding DV-3/86) had SFA/UFA ratios similar to those calculated for strains described in the literature. The wild strains DV-1/86, DV-2/86, DV-5/86, and DV-6/86 and the type strain, DSM 642, are characterized by an SFA/UFA ratio of 1.3 to 1.5, which is almost identical to those calculated by us from data obtained by Ueki et al. (30) and by Taylor and Parkes (28) for wild *D. desulfuricans* strains. Wild strains DV-4/86, DV-7/86, and DV-8/86 had SFA/UFA ratios (1.8 to 1.9) identical to that calculated by us for type strain NCIB 8307 (DSM 642), described by Ueki and Suto (31). Type strain DSM 642 (NCIB 8307) used in our experiments has been characterized by an SFA/UFA ratio about 28% lower than that calculated for the above-mentioned NCIB 8307 strain. The SFA/UFA ratios calculated for our intestinal strains were about 16 to 30% higher than the ratios obtained for soil isolates. Taking into account all calculated values of the SFA/UFA ratio (Table 3), an average value of 1.55 ± 0.24 (excluding data obtained for DV-3/86) could be assumed as being characteristic of soil *D. desulfuricans* strains whereas a value of 2.20 ± 0.14 was characteristic of intestinal strains belonging to the same species.

We supposed that the higher SFA/UFA ratios for intestinal strains may be caused by a higher temperature in the human intestine, from which these bacteria were isolated. The higher temperature of the environment in which the bacteria existed before their isolation and cultivation under laboratory conditions may also be the reason for the higher SFA/UFA ratio (2.7) indicated for the DV-3/86 soil strain, isolated from soil obtained during excavation under a central heating system. On the other hand, the temperature stability in the human body may explain the much smaller deviations from mean values in intestinal strains than in soil ones. However, this hypothesis requires further investigations to elucidate the flexibility with which the organisms can react temperature changes.

Although we found that the soil and intestinal *D. desulfuricans* strains could be grouped and distinguished by the similarity of cellular fatty acid profiles and by the relative amounts of i-$C_{17:1}$ and i-$C_{15:0}$ as key branched-chain fatty acids, we cannot tell at present which other significant differences exist between the strains. Their morphological characteristics, positive tests for desulfoviridin, nutritional properties examined by the methods of Postgate (25) and Widdel and Pfenning (34), ability to use nitrate as a respiratory substrate, and cellular fatty acid profiles enable us to assign all the strains to *D. desulfuricans* species. On the other hand, we indicated a certain heterogeneity of cellular fatty acid profiles within the group of soil strains, in contrast to almost ideal homogeneity for this characteristic within the group of intestinal isolates. We propose that intestinal *D. desulfovibrio* bacteria should be assumed to be a highly homogeneous group that should be represented by *D. desulfuricans* subsp. *intestinus* in collections of microbial cultures. It seems very probable that other bacterial species existing in human intestines will form very homogeneous groups because of the remarkable similarity of colonic biocenoses of individual persons. Another investigation is now being carried out to determine the degree of phenotypic and genotypic diversity within various bacterial strains belonging to the same species, taking into account the influence of many environmental circumstances on the expression of genes in bacterial cells. Such complex investigations could probably help to improve the characterization of microbial communities occupying various defined habitats, such as the human digestive tract.

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