APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1992, p. 2053–2065
0099-2240/92/062053-13$02.00/0

Vol. 58, No. 6

Differentiation of Gram-Negative, Nonfermentative Bacteria Isolated from Biofilters on the Basis of Fatty Acid Composition, Quinone System, and Physiological Reaction Profiles

ANDRÉ LIPSKI,[1]* STEFAN KLATTE,[2] BERND BENDINGER,[1] AND KARLHEINZ ALTENDORF[1]

Abteilung Mikrobiologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Barbarastrasse 11, D-4500 Osnabrück,[1] and Deutsche Sammlung von Mikroorganismen GmbH, Mascheroder Weg 1b, D-3300 Braunschweig,[2] Germany

Received 20 November 1991/Accepted 16 March 1992

Gram-negative, nonfermentative bacteria isolated from biofilters for off-gas treatment of animal-rendering-plant emissions were differentiated by whole-cell fatty acid analysis, quinone analysis, and numerical taxonomy based on their physiological reaction profiles. The last system consisted of 60 physiological tests and was arranged as a microtest system on microtitration plates. Based on fatty acid analyses, 31 isolates were separated into six clusters and five single-member clusters. The isolates of two clusters were identified as *Alcaligenes faecalis* and *Pseudomonas diminuta*. The remaining nine clusters were characterized by their fatty acid profiles, quinone systems, and physiological reaction profiles. Clusters resulting from fatty acid analyses were compared with those resulting from physiological reaction profiles. Six clusters could be confirmed this way. The efficiency of the physiological test system was increased by the prearrangement of the isolates according to their quinone type.

Biofilters are used in increasing numbers for waste gas treatment because they are economical. In contrast to the extensive efforts to increase efficiency by improving the design or the packing material, few investigations are concerned with the naturally developing bacterial population responsible for degradation by a biofilter (1, 4). Although these bacteria were isolated by plate culture methods and therefore do not represent the complete bacterial population of biofilters, it is necessarily the first step to obtain bacteria for physiological studies. These studies can disclose the relation between the degradation capacities of biofilters and the bacteria responsible for the degradation process. Such studies are an important step toward the understanding of biofilters.

An important aspect of biofilters is the heterogeneous bacterial populations that naturally develop during exposure to waste gases. Effective differentiation criteria have to be found in order to detect newly appearing organisms, which are of great interest because of their presumable degradation properties. These criteria should characterize and, if possible, identify the strains in question. A reliable identification or a sufficiently precise characterization allows for comparison of isolates from various biofilters, from wastewater treatment plants, or from soils. Once a strain is identified, further investigations can take advantage of its known properties.

This study dealt with gram-negative, nonfermentative bacteria isolated from experimental biofilters used in odor abatement of animal-rendering-plant emissions. The proportion of these bacteria in the culturable population varied strongly depending on the waste gas composition (1). Isolates originating from different biofilters were pooled to establish methods useful for differentiation of a spectrum of gram-negative, nonfermentative bacteria from these sources. Until now, differentiation and identification of these bacteria were based on a limited number of physiological and morphological tests and allowed only a rough and sometimes uncertain classification (4). In this study, whole-cell fatty acid analyses and quinone analyses were applied to obtain more reliable information about the identity of the gram-negative, nonfermentative bacteria isolated from biofilters. These chemotaxonomic methods have already been used successfully for the characterization of *Thiobacillus* species (10), members of the family *Rhodospirillaceae* (11), *Moraxella* and related taxa (20), the *Flavobacterium-Cytophaga* complex (22), *Pseudomonas* species (23), and methanol-utilizing bacteria (25). Additionally, in this study, a physiological test system with microtitration plates was applied, and the results were compared with those obtained by the chemotaxonomic methods. As a result of that, it became clear that the efficiency of the physiological test system can be increased by prearranging the isolates based on chemotaxonomic markers.

MATERIALS AND METHODS

Chemicals. Chemicals were purchased from Merck (Darmstadt, Germany), Sigma (Deisenhofen, Germany), Riedel de Haën (Seelze, Germany), Difco (Detroit, Mich.), and GIBCO (Eggenstein, Germany).

Origin and cultivation of strains. The reference strains used were obtained from the Deutsche Sammlung von Mikroorganismen GmbH (DSM), Braunschweig, Germany; Institute for Fermentation, Osaka (IFO), Japan; and Laboratorium Microbiologie Rijksuniversiteit Gent (LMG), Ghent, Belgium. Strains were cultivated on antibiotic sulfonamide sensitivity test agar (ASS agar; no. 5392; Merck) at 30°C. Isolates were obtained from several experimental biofilters supplied with different kinds of off gases from an animal-rendering plant. The constituent classes of compounds in the rendering emissions were alkylsulfides, mainly dimethyldisulfide, furans, thiophenes and, in one kind of off gas, carbonyl compounds such as aldehydes and ketones as well as hydrogen sulfide. Additionally, ammonia and carbon dioxide occurred in high concentrations (1).

* Corresponding author.

Physiological tests. Sixty physiological tests were selected from a more extensive number of tests, from which those tests which gave negative results for all investigated strains and therefore supplied no information for differentiation were excluded. The tests were performed in microtitration plates (Greiner, Nürtingen, Germany). The vessels were filled with 50 μl of double-concentrated test medium. The following test media were used: for esculin hydrolysis, 5.0 g of peptone from meat (Merck) per liter, 3.0 g of meat extract (Merck) per liter, 1.0 g of esculin per liter, 0.5 g of ammonium ferric citrate per liter, pH adjusted to 7.0 with NaOH; for urease activity, 1.0 g of peptone from meat (Merck) per liter, 5.0 g of NaCl per liter, 2.0 g of KH_2PO_4 per liter, 0.08 g of bromthymol blue per liter, 1.0 g of glucose per liter, 20.0 g of urea per liter, pH adjusted to 6.5 with NaOH; for arginine dihydrolase, 2.0 g of peptone from casein (GIBCO) per liter, 1.0 g of yeast extract (GIBCO) per liter, 5.0 g of NaCl per liter, 0.3 g of $Na_2HPO_4 \cdot 7H_2O$ per liter, 0.08 g of bromthymol blue per liter, 0.5 g of glucose per liter, 10.0 g of L-arginine · HCl per liter, pH adjusted to 6.5 with HCl. Medium without arginine was used as a control. For acid production from carbohydrates, we used 0.2 g of peptone from casein (GIBCO) per liter, 0.1 g of yeast extract (GIBCO) per liter, 0.3 g of $Na_2HPO_4 \cdot 7H_2O$ per liter, 0.08 g of bromthymol blue per liter, and 5.0 g of NaCl per liter, and the pH was adjusted to 7.2 with HCl. The following carbohydrates were added to give a concentration of 15.0 g/liter: glucose, rhamnose, sucrose, xylose, fructose, lactose, and maltose. Medium without carbohydrates was used as a control. For citrate utilization with alkalinization, we used 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter, 1.0 g of $NH_4H_2PO_4$ per liter, 1.0 g of $K_2HPO_4 \cdot 3H_2O$ per liter, 2.0 g of Na citrate · $2H_2O$ per liter, 5.0 g of NaCl per liter, and 0.08 g of bromthymol blue per liter, and the pH was adjusted to 6.7 with NaOH. For malonate utilization with alkalinization, we used 1.0 g of yeast extract (GIBCO) per liter, 2.0 g of $(NH_4)_2SO_4$ per liter, 0.6 g of $K_2HPO_4 \cdot 3H_2O$ per liter, 0.4 g of KH_2PO_4 per liter, 2.0 g of NaCl per liter, 3.0 g of malonic acid per liter, 0.25 g of glucose per liter, and 0.08 g of bromthymol blue per liter, and the pH was adjusted to 6.7 with NaOH. For nitrate and nitrite reduction, we used 8.6 g of peptone from meat (Merck) per liter, 6.4 g of NaCl per liter, and 0.1 g of KNO_3 or KNO_2, respectively, per liter, and the pH was adjusted to 7.2 with NaOH. Medium without KNO_3 and KNO_2 was used as a control. Hydrolysis of chromogenic substrates was tested in phosphate buffer containing 3.5 g of $Na_2HPO_4 \cdot 7H_2O$ per liter, 1.5 g of KH_2PO_4 per liter, and 5.0 g of NaCl per liter; chromogenic substrates were added in final concentrations of 1 mM for the *para*-nitroanilide (pNA)-linked substrates and 2 mM for the *para*-nitrophenyl (pNP)-linked substrates; the pH was adjusted to 7.2. The chromogenic substrates used are listed in Table 5. The tests for carbon source utilization were performed in yeast nitrogen base (Difco) as the basal medium. Carbon sources were added to give a final concentration of 0.2%, and the pH was adjusted with NaOH or HCl to 7.0. The carbon sources used are listed in Table 5.

For the physiological tests, the reference strains and isolates from the biofilters were grown on ASS agar for 2 days at 30°C. Cell material was suspended in 0.9% NaCl to give an opacity equivalent to a McFarland standard of 0.5. A 50-μl sample of this cell suspension was added to each vessel in the microtitration plate. The media for urease and arginine dihydrolase activity and the corresponding control medium were overlaid with 3 drops of sterile paraffin. Microtitration plates were incubated for 3 days at 25°C. Two drops of nitrite reagent (dimethyl-α-naphthylamine, 0.6%; sulfanilic acid, 0.8%; acetic acid, 30%) (18) were added to the test media for nitrate reduction to nitrite and nitrite reduction and to the corresponding control medium. Results were read by a microtitration plate reader (Dynatech, Denkendorf, Germany). Tests for esculin hydrolysis, urease and arginine dihydrolase activity, acid production from carbohydrates, and citrate and malonate utilization with alkalinization were read at 630 nm. Tests for nitrite and nitrate reduction, hydrolysis of chromogenic substrates, and assimilation were read at 410 nm. For conversion into positive and negative results, threshold values were determined by measuring the extinction of all reference strains for each test. The threshold value was defined as that value with equal distances to the extinction of the closest positive and negative test result of the reference strains. Results were defined as positive if the differences between the extinction of the test medium and the control medium exceeded the threshold value. For the test of nitrite reduction, the result was defined as negative if the difference between the extinction of the test medium and the control medium exceeded the threshold value. The following threshold values were determined: for nitrate reduction to nitrite, 0.32; for nitrite reduction, 0.13; for urease activity, 0.17; for arginine dihydrolase activity, 0.10; and for acid production from carbohydrates, 0.14. For tests without corresponding control media, results were defined as positive if the extinction of the medium exceeded the threshold value. For esculin hydrolysis, the threshold value was set to 0.97; for citrate utilization with alkalinization, it was set to 0.35; and for malonate utilization with alkalinization, it was set to 0.29. Threshold values for the hydrolysis of chromogenic substrates and the assimilation tests were set to 0.30 and 0.07, respectively, according to the method of Kämpfer et al. (8). The transformation of the reaction profiles into similarity coefficients (Jaccard coefficient) and generation of dendrograms by using unweighted average linkage clustering were performed as described by Sneath and Sokal (24).

Quinone analysis. Cells were grown in shake cultures in 100 ml of brain heart infusion broth (Difco) at 30°C. Cultures were centrifuged, washed twice with deionized water, and lyophilized. Quinones were extracted and separated into menaquinones and ubiquinones by thin-layer chromatography with Silica Gel layer 60 F_{254} (Merck) (13). Further separation of the ubiquinones according to the different isoprenyl chain lengths was performed with HPTLC-RP 18 F_{254}s layer (Merck).

Fatty acid analysis. Cells were grown on Trypticase soy broth (Becton Dickinson, Heidelberg, Germany) with 1.5% agar for 3 days at 30°C. Saponification, methylation, extraction and identification of fatty acids, and the presentation of the results in the form of a dendrogram were performed as described by Kroppenstedt et al. (15).

RESULTS

Fatty acid analysis. Cluster analysis was applied to the fatty acid profiles of 31 gram-negative, nonfermentative rods isolated from the biofilters and 25 reference strains. The quantitative and qualitative differences in the fatty acid patterns were transformed into Euclidean distances and plotted as a dendrogram (Fig. 1). Three main groups, which could be distinguished by the following properties, were found. Group 1 included all strains containing mainly iso- and anteiso-fatty acids (Table 1), group 2 included all strains with a low content of octadecenoic acid (18:1-*cis* 11) and

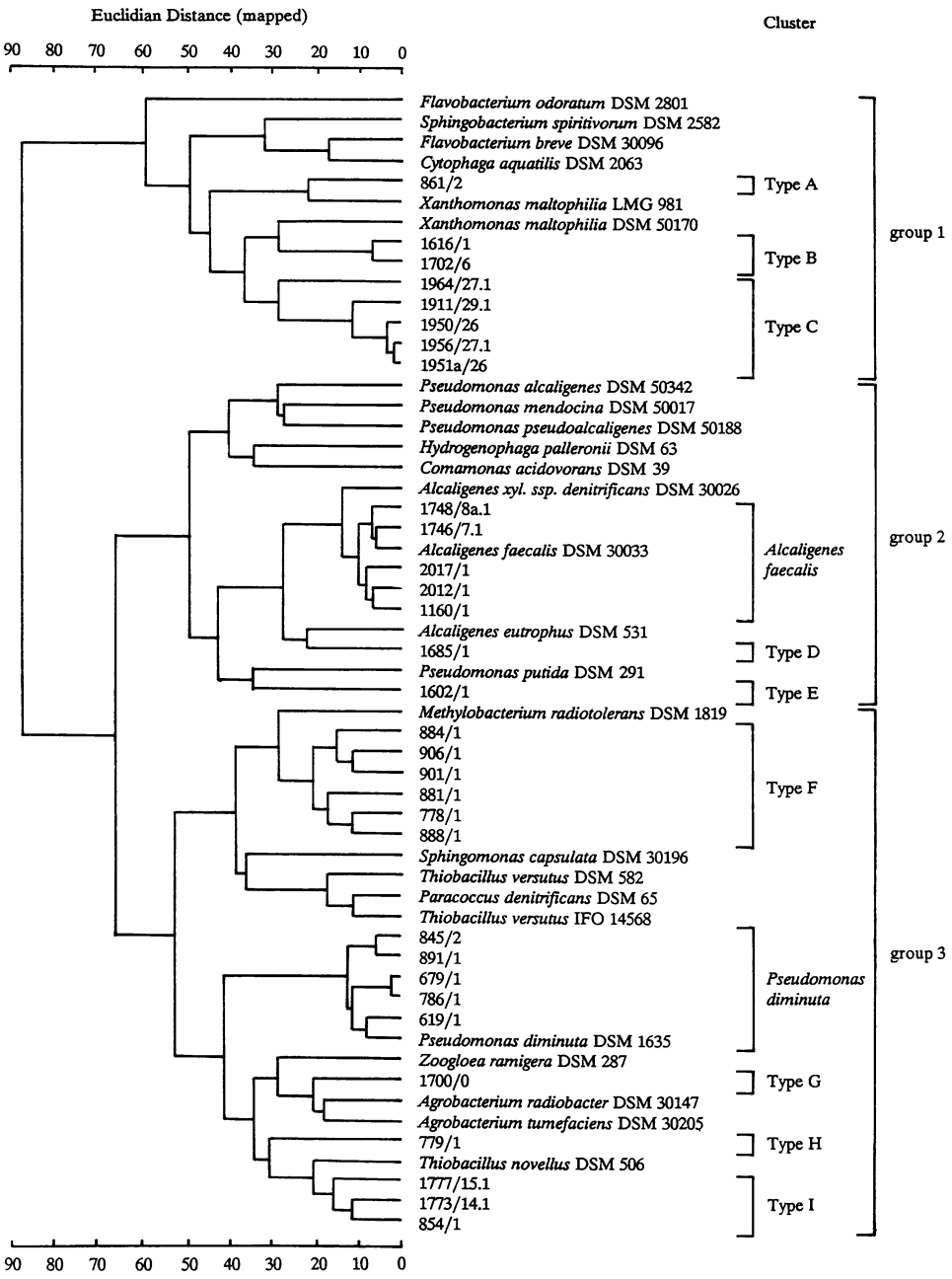

FIG. 1. Dendrogram generated from fatty acid composition.

methyleneoctadecanoic acid (19:0-cyclo 11-12) (Σ < 47%) (Table 2), and group 3 included all strains with a high content of the fatty acids 18:1-*cis* 11 and 19:0-cyclo 11-12 (Σ > 47%) (Table 3). Furthermore, the isolates could be divided into distinct clusters. All isolates which formed a cluster at a Euclidian distance smaller than 14 together with a reference strain were named after the species name of this reference strain. This applied to five isolates which were identified as *Alcaligenes faecalis* and five isolates which were identified as *Pseudomonas diminuta*. The remaining isolates showed a larger distance to the closest reference strain, and therefore no further assignment to any reference strain was made. These clusters were named type A-I.

Table 4 shows the diagnostic fatty acids of the clusters obtained with the biofilter isolates. The values of Table 4 give the arithmetic mean of the percent fatty acid composition of the members of the clusters. The *Alcaligenes faecalis* cluster was characterized by the main fatty acids hexadecanoic acid (16:0), methylenehexadecanoic acid (17:0-cyclo), and 3-hydroxytetradecanoic acid (14:0-3OH), which were present in amounts of 32, 32, and 11%, respectively. The *Pseudomonas diminuta* cluster contained the fatty acids 16:0 and 19:0-cyclo 11-12 in amounts of 34 and 32%, respectively, and the fatty acid 18:1-*cis* 11 in an amount of 21%. The type A, B, and C clusters contained mainly branched-chain fatty acids. The typical fatty acids of the type A cluster were

TABLE 1. Percent fatty acid composition of isolates and reference strains of group 1 with mainly iso- and anteiso-fatty acids

Cluster	Reference strain or isolate	Quinone type	%[a]																																
			10:0-iso	11:0-iso	11:0-anteiso	11:0-iso-3OH	12:0-iso-3OH	12:0-3OH	13:0-iso-3OH	14:0-iso	14:0	15:1-iso F[b]	15:1-iso G[b]	15:0-iso	15:0-anteiso	15:1-B[b]	15:0	15:0-iso-2OH	15:0-iso-3OH	16:0-iso	16:1 B[b]	16:1-*cis* 9	16:0	16:0-iso-3OH	16:0-3OH	17:1-iso F[b]	17:0-iso	17:0-anteiso	17:1 B[b]	17:1 C[b]	17:0-iso-3OH	18:1-*cis* 9	18:1-*cis* 11
	Flavobacterium ordoratum DSM 2801	MK												55	3				6			1			2	17	tr				11		
	Sphingobacterium spiritivorum DSM 2582	MK									tr	tr		33	tr	tr	tr	34	3				3		2	2				tr	14		
	Flavobacterium breve DSM 30096	MK									tr			24	tr	4	3		8	tr		16	8	tr		3	3	tr	tr	tr	15		
	Cytophaga aquatilis DSM 2063	MK									tr		3	32	tr	3	6		10			11	4	1	5	3	tr			1	11		
Type A	861/2	Q-8	1	4		1	4		1	23	2	2		16	4	tr	3			16	1	8	3			4	tr		2				
	Xanthomonas maltophilia LMG 981	Q-8	2	2	tr	2	3	tr	2	12	1	3		20	5		tr			21	tr	6	3			11	2		tr			tr	tr
	Xanthomonas maltophilia DSM 50170	Q-8		4		2	tr	3	5	tr	2	tr		43	9	tr	1			2	1	7	5			4	4	tr	tr			1	tr
Type B	1616/1	Q-8		6	1	8	tr	tr		tr	1	2		29	21		tr			2	tr	6	6			11	7	1	tr				
	1702/6	Q-8		6	1	8	tr	tr		tr	tr	1		22	23		2			3	1	7	8			11	4	2	tr				
Type C	1964/27.1	Q-8		9		8				tr	tr	3		48	2					3		tr	2		tr	17	6						
	1956/27.1	Q-8		8		7				tr	tr	2		49	2		tr			3		tr	2			18	7						
	1911/29.1	Q-8		10		6				tr	tr	1		38	2		tr			3		2	4		tr	23	9						
	1951a/26	Q-8		8		8				tr	tr	2		49	2		tr			3		tr	2		tr	17	6						
	1950/26	Q-8		6		7				tr	tr	3		49	2		tr			3		1	2			19	8						

[a] tr, less than 0.9%.
[b] F, G, B, C, location of the double bond unknown but different.

TABLE 2. Percent fatty acid composition of isolates and reference strains of group 2 with low levels of 18:1-*cis* 11 and 19:0-cyclo 11-12 (Σ < 47%)

Cluster	Reference strain or isolate	Quinone type	% [a]																			
			10:0	10:0-3OH	11:0	11:0-iso-3OH	12:0	12:0-2OH	12:0-3OH	14:0	14:0-2OH	14:0-3OH	15:1 B[b]	15:0	15:0-iso-2OH	16:1-*cis* 9	16:0	17:1 B[b]	17:0-cyclo	17:0	18:1-*cis* 11	18:0	19:0-cyclo 11-12
	Pseudomonas alcaligenes DSM 50342	Q-9	3		tr	tr	7			5			tr	2	4	21	15	1		tr	36	tr	
	Pseudomonas mendocina DSM 50017	Q-9	tr	5	tr		11	tr	6	tr			tr	2		16	17	1	4	1	33	tr	tr
	Pseudomonas pseudoalcaligenes DSM 50188	Q-9	tr		tr	tr	9	tr		tr			tr	2		15	16	2	6	2	43	tr	2
	Hydrogenophaga palleronii DSM 63	Q-8									tr				tr		36	30		9	tr	23	tr
	Comamonas acidovorans DSM 39	Q-8		3			2			1			4	9		25	30		14	3	8		
	Alcaligenes xylosoxydans subsp. *denitrificans* DSM 30026	Q-8					1	3	tr	5		8	tr	tr		10	34		27	tr	6	2	2
Alcaligenes faecalis	1748/8a.1	Q-8				tr	4	3	tr	2		11		tr		3	32		33	tr	4	1	3
	1746/7.1	Q-8					3	3	tr	2		11	tr	tr		5	30		35	tr	6	tr	2
	Alcaligenes faecalis DSM 30033	Q-8					3	2	tr	2		10	tr	tr		5	35		34	tr	4	tr	2
	2017/1	Q-8					3	2	tr	2		9		tr		9	34		30	tr	6	1	2
	2012/1	Q-8					3	3		1		11		tr		5	34		32	tr	7	2	2
	1160/1	Q-8					3	3		1		13		tr		7	28		29	tr	10	2	2
	Alcaligenes eutrophus DSM 531	Q-8					tr		tr	5	3	11	tr	tr		29	26	tr	12	tr	12	tr	tr
Type D	1685/1	Q-8					4		tr	4		9				17	34		23		6	tr	tr
	Pseudomonas putida DSM 291	Q-9	tr	4			4	7	5	tr				tr	6	10	29	tr	18	tr	14	tr	tr
Type E	1602/1	Q-9	tr	4		4	6	4	6	1						6	21		15		12	tr	19

[a] tr, less than 0.9%.
[b] B, location of the double bond unknown.

12-methyltridecanoic acid (iso-14:0) (23%) and 14-methylpentadecanoic acid (iso-16:0) (16%). The type B cluster was characterized by a high level of 12-methyltetradecanoic acid (anteiso-15:0) (22%). The main fatty acids of the type C cluster were 13-methyltetradecanoic acid (iso-15:0) (47%) and 15-methylhexadecenoic acid (iso-17:1) (19%). The type D isolate contained the fatty acids 16:0 (34%), 17:0-cyclo (23%), and hexadecenoic acid (16:1-*cis* 9) (17%). The type E isolate contained the fatty acids 16:0 (21%) 19:0-cyclo 11-12 (19%), 17:0-cyclo (15%), and 18:1-*cis* 11 (12%). The type F cluster contained the fatty acid 18:1-*cis* 11 (73%). A characteristic feature was the occurrence of nonadecanoic acid (19:0), which was detected only in members of this cluster. The amount of heptadecanoic acid (17:0) differed within this cluster. The amounts of 14% for two isolates and 6% for four isolates suggest a division into two subgroups. The type G cluster contained the predominating fatty acids 18:1-*cis* 11 (49%) and 19:0-cyclo 11-12 (16%). Moreover, 14:0-3OH, 3-hydroxyhexadecanoic acid (16:0-3OH), and 3-hydroxyoctadecanoic acid (18:0-3OH) were found. The type H isolate contained the fatty acids 18:1-*cis* 11 (54%), 16:0 (12%), 17:0 (11%), and the characteristic 3-hydroxy-9-methyldecanoic acid (iso-11:0-3OH) (3%). The type I cluster contained the fatty acids 18:1-*cis* 11 (58%) and 19:0-cyclo 11-12 (23%). For *Rhizobium leguminosarum* DSM 30132 and *Methylobacterium mesophilicum* DSM 1708, fatty acid composition could not be determined because they failed to grown on Trypticase soy broth agar.

Quinone analysis. The results of the quinone analyses are shown in Tables 1 to 3. Sixteen isolates contained ubiquinone 10 (Q-10), 1 isolate contained ubiquinone 9 (Q-9), and 14 isolates contained ubiquinone 8 (Q-8). Menaquinones (MK) were never found in the isolated strains. The occurrence of Q-10 was strictly associated with the presence of high levels of 18:1-*cis* 11 and 19:0-cyclo 11-12 (Σ > 47%). Quinone types of *Xanthomonas maltophilia* DSM 50170, *Xanthomonas maltophilia* LMG 981, and *Methylobacterium mesophilicum* DSM 1708 were obtained from published data (26, 29).

Physiological tests. Table 5 shows the reaction profiles of the reference strains and the biofilter isolates obtained with the physiological microtest system. The dendrogram which was generated from these data is shown in Fig. 2. In accordance with the cluster arrangement based on the fatty acid composition, the *Pseudomonas diminuta* cluster, the type A cluster, the type B cluster, the type F cluster, the type G cluster, and the type I cluster could be confirmed by using only physiological data. However, the *Alcaligenes faecalis* cluster contained the reference strains *Alcaligenes xylosoxydans* subsp. *denitrificans* DSM 30026 and *Pseudomonas mendocina* DSM 50017. The type C cluster also contained the isolate of the type D cluster, the isolate of the type H cluster, and the reference strain *Flavobacterium odoratum* DSM 2801. The reaction profile of the type E isolate is similar to that of *Pseudomonas pseudoalcaligenes* DSM 50188, which is in contrast to the results of the fatty acid analysis. For the dendrograms shown in Fig. 3, the same physiological data were used, but the reaction profiles were prearranged by the quinone type of the different strains. This led to a better reproduction of the clusters

TABLE 3. Percent fatty acid composition of isolates and reference strains of group 3 with high levels of 18:1-*cis* 11 and 19:0-cyclo 11-12 (Σ > 47%)

Cluster	Reference strain or isolate	Quinone type	% [a]																	
			10:0-3OH	11:0-iso-3OH	12:0-3OH	14:0	14:0-2OH	14:0-3OH	15:0	16:1-*cis* 9	16:0	16:0-3OH	17:1 B [b]	17:0-cyclo	17:0	18:1-*cis* 11	18:0	18:0-3OH	19:0-cyclo 11-12	19:0	ECL [c] 19.368	ECL 19.828
	Methylobacterium radiotolerans DSM 1819	Q-10						tr		1	4				1	88	6					
Type F	884/1	Q-10	4	3				4	tr		tr				5	76	5		2		1
	906/1	Q-10	3	tr				3					tr		8	73	4		5		2
	901/1	Q-10	4					3			tr				4	80	4		2		3
	881/1	Q-10	4	tr				3		tr					6	77	4		2		1
	778/1	Q-10	4	tr				3			tr		tr		13	61	5		5		1
	888/1	Q-10	3	1				3			tr		tr		14	64	5		5		1
	Sphingomonas capsulata DSM 30196	Q-10				tr	15		tr	6	9					62						
	Thiobacillus versutus DSM 582	Q-10	5					3		tr	13				tr	61	2		11			
	Paracoccus denitrificans DSM 65	Q-10	5					4		tr	10		tr		tr	70	2		3			
	Thiobacillus versutus IFO 14568	Q-10	3					3		tr	9				tr	79	1		tr			
Pseudomonas diminuta	845/2	Q-10			3	1			1	4	34		tr		1	15	1		38			
	891/1	Q-10			3	1			1	3	34		1		2	14	1		40			
	679/1	Q-10			3	tr			1	5	32		tr		1	29	tr		27			
	786/1	Q-10			3	tr			1	5	34		tr		1	27	tr		29			
	619/1	Q-10			3	1			1	2	34		1		3	24	1		28			
	Pseudomonas diminuta DSM 1635	Q-10			3	2			1	1	39		tr		2	18	1		31			
	Zoogloea ramigera DSM 287	Q-10						1	tr	3	15	8	tr	tr	1	52	2	1	11		4	
Type G	1700/0	Q-10				tr		9	1	4	8	2	2	2	2	49	tr	1	16			
	Agrobacterium radiobacter DSM 30147	Q-10				1		10	tr	4	12	6		6		43			18			
	Agrobacterium tumefaciens DSM 30205	Q-10						6		2	8	4		2		62			14			
Type H	779/1	Q-10		3							12		2		11	54	7		9			
	Thiobacillus novellus DSM 506	Q-10									9				2	69	2		18			
Type I	1777/15.1	Q-10			1						10				1	65	3		16		2	
	1773/14.1	Q-10			tr						10		tr	1	1	53	4		25		3	
	854/1	Q-10			tr						7			1	tr	56	4		27		3	

[a] tr, less than 0.9%.
[b] B, location of the double bond unknown.
[c] ECL, for unknown fatty acids, the respective ECL is given.

which were defined by fatty acid analysis (Fig. 3). Improvements were obtained for the type H cluster, which was separated from the type C and type D clusters. *Pseudomonas mendocina* DSM 50017 was separated from the *Alcaligenes faecalis* cluster. However, the reference strain *Alcaligenes xylosoxydans* subsp. *denitrificans* DSM 30026 could not be separated from the *Alcaligenes faecalis* cluster. The physiological reaction profile of *Thiobacillus novellus* DSM 506 could not be determined because the strain failed to grow on ASS agar.

DISCUSSION

Based on the fatty acid analyses, the investigated biofilter isolates were differentiated into six clusters and five single-member clusters (Fig. 1). Compared with these results, the physiological test system could reproduce four clusters and two single-member clusters, and when it was combined with quinone analysis, a third single-member cluster (type H) could be reproduced (Fig. 3). This shows that fatty acid analysis is a more efficient method for differentiation than the analysis of physiological reaction profiles alone. The clusters were characterized by their diagnostic fatty acids.

Identification was successful for 10 isolates, which were distributed over two clusters. These clusters contained reference strains which allowed their identification down to species level as *Pseudomonas diminuta* and *Alcaligenes faecalis* (Fig. 1). These results were confirmed by the physiological reaction profiles for the *Pseudomonas diminuta* cluster and, with some reservation, for the *Alcaligenes faecalis* cluster, which could not be separated from *Alcaligenes xylosoxydans* subsp. *denitrificans* DSM 30026 by numerical analysis of the physiological data. However, the failure of growth on adipate allowed an assignment of the isolates of the *Alcaligenes faecalis* cluster to *Alcaligenes faecalis* DSM 30033 (12) (Table 5). The fatty acid profiles of

TABLE 4. Diagnostic fatty acids of the clusters of biofilter isolates (arithmetic average values in percentages)

Group	Cluster	No. of strains	Quinone type	10:0-3OH	11:0-iso	11:0-anteiso	11:0-iso-3OH	12:0	12:0-2OH	12:0-3OH	14:0-iso	14:0-3OH	15:0-iso	15:0-anteiso	16:0-iso	16:0	16:1-*cis* 9	16:0-3OH	17:1-iso F[b]	17:0-iso	17:0-anteiso	17:0-cyclo	17:0	18:1-*cis* 11	18:0	18:0-3OH	19:0-cyclo 11-12	19:0	ECL[c] 19.828
				%[a]																									
1	Type A	1	Q-8		4		1				23		16	4	16	3	8		4	tr									
	Type B	2	Q-8		6	1	8				tr		25	22	3	7	6		11	5	1								
	Type C	5	Q-8		8		7				tr		47	2	3	2	1		19	7									
2	*Alcaligenes faecalis*	6	Q-8					3	3			11				32	6					32	tr	6	1		2		
	Type D	1	Q-8					4		tr		9				34	17					23		6	tr		tr		
	Type E	1	Q-9	4			4	6	4	6						21	6					15		12	tr		19		
3	Type F	6	Q-10	4			1					3				tr							6/14	73	5			3	2
	Pseudomonas diminuta	5	Q-10							3						34	3						2	21	1		32		
	Type G	1	Q-10									9				8	4	2				2		49	1	1	16		
	Type H	1	Q-10				3									12							11	54	7		9		
	Type I	3	Q-10							tr						8	1						tr	58	4		23		2

[a] tr, less than 0.9%.
[b] F, location of the double bond unknown.
[c] ECL, for the unknown fatty acid, the ECL is given.

the *Pseudomonas diminuta* and the *Alcaligenes faecalis* clusters are in accord with published data. For *Alcaligenes faecalis*, Dees and Moss (3) found the fatty acids 16:0, 17:0, and 14:0-3OH in amounts comparable to those found in this study. However, the levels of the fatty acids 16:1 and 18:1 were reported to be about threefold higher. This may be due to the shorter growth period of the cells used by Dees and Moss (3). The decrease in the amount of monounsaturated fatty acids with an increase in the incubation time was already described by Law et al. (17). For *Pseudomonas diminuta*, the characteristic fatty acid profile, which corresponds well to our results, has been described before (6, 19, 23). These positive correlations confirmed the validity of the identification methods used. They also showed that a comparison between the unidentified clusters and the increasing number of published bacterial fatty acid profiles is feasible, disclosing possible taxonomic relations between the unidentified clusters and already established taxa. In addition, the physiological reaction profiles and the quinone types provided valuable clues as to the confirmation or rejection of an assumed taxonomic relation. With this approach, the resemblance of the type A, B, and C isolates to the genus *Xanthomonas* and the resemblance of the type G isolate to the genus *Agrobacterium* has been disclosed.

The isolates of the type A, B, and C clusters possessed branched-chain fatty acids. Usually, this is a typical feature for gram-positive bacteria; only a few gram-negative bacteria such as *Spirochaeta*, *Xanthomonas*, *Legionella*, *Thermus*, *Flavobacterium*, *Cytophaga*, *Sphingobacterium*, *Bacteroides*, and *Desulfovibrio* species contain branched-chain fatty acids (9). The fatty acid composition of the type A, B, and C clusters differed from that of the *Flavobacterium-Sphingobacterium-Cytophaga* complex in the occurrence of 9-methyldecanoic acid (iso-11:0) and iso-11:0-3OH and the lack of 3-hydroxy-13-methyltetradecanoic acid (iso-15:0-3OH) as well as 3-hydroxy-15-methylhexadecanoic acid (iso-17:0-3OH). Moreover, for the type A, B, and C clusters, the Q-8 quinone type was found. This is in contrast to the *Flavobacterium-Sphingobacterium-Cytophaga* complex, which contained the MK type (Table 1). The fatty acid patterns of the type A, B, and C clusters showed similarities to those of the reference strains of the species *Xanthomonas maltophilia*. The type A isolate was more related to *Xanthomonas maltophilia* LMG 981 (subj. syn.: *Pseudomonas pictorum* Gray and Thornton 1928 [16]) according to the higher levels of iso-14:0 and iso-16:0, whereas the type B cluster was more related to *Xanthomonas maltophilia* DSM 50170, as indicated by the lower levels of these fatty acids. Ikemoto et al. (7) used this feature for a subdivision of the species *Xanthomonas maltophilia* (formerly *Pseudomonas maltophilia*) into two biovars. Biovar I, including the methionine-requiring strains, is further characterized by growth on sucrose but not on DL-β-hydroxybutyrate, whereas biovar II, including the methionine-independent strains, is characterized by growth on DL-β-hydroxybutyrate but not on sucrose. According to these characteristics, *Xanthomonas maltophilia* DSM 50170 and the type B cluster could be designated biovar I strains and *Xanthomonas maltophilia* LMG 981 and the type A isolate could be designated biovar II strains (Table 5). However, numerical analysis of the physiological reaction profiles of the type A, B, and C clusters showed equal similarities of the type B cluster to both reference strains of *Xanthomonas maltophilia* and low similarities of the type A and C clusters to these reference strains (Fig. 3).

The type D isolate showed a fatty acid composition similar to that of *Alcaligenes eutrophus* DSM 531 (Table 2) and had

TABLE 5. Physiological reaction profiles of reference strains and isolates

Reference strain or cluster	% Positive															Hydrolysis of:									
	Esculin	Urease	Arginine dihydrolase	Citrate, alkalinization	Malonate, alkalinization	Acid from glucose	Acid from rhamnose	Acid from sucrose	Acid from xylose	Acid from fructose	Acid from lactose	Acid from maltose	Nitrate to nitrite	Nitrite reduction	pNP-*N*-acetyl-β-D-glucosaminide	pNP-β-D-galactopyranoside	pNP-β-D-glucopyranoside	pNP-β-D-glucuronide	pNP-α-D-maltoside	pNP-β-D-xyloside	Bis-pNP-phosphate	pNP-phosphocholine	Thymidine-5′-monophosphate-pNP-ester	L-Alanine-pNA
Alcaligenes faecalis cluster[a]	0	0	0	100	100	0	0	0	0	0	0	0	0	100	20	0	20	0	0	0	0	0	0	100
Alcaligenes faecalis DSM 30033	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Alcaligenes xylosoxydans subsp. *denitrificans* DSM 30026	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Pseudomonas diminuta cluster[a]	0	0	0	0	80	0	0	0	0	0	0	0	40	20	0	0	0	0	0	0	100	0	80	100
Pseudomonas diminuta DSM 1635	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
Type A cluster	0	0	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	100
Type B cluster	50	0	0	0	0	100	0	0	100	100	100	100	0	100	100	100	100	50	100	100	0	0	0	100
Type C cluster	20	0	0	20	0	0	0	0	0	0	0	0	0	100	0	0	20	0	20	0	0	0	20	0
Type D cluster	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100
Type E cluster	0	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	100
Type F cluster	0	0	0	0	33	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100
Type G cluster	0	0	0	0	0	0	100	0	100	0	100	100	0	100	100	0	100	0	0	0	0	0	0	100
Type H cluster	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100
Type I cluster	0	0	0	0	33	33	0	0	67	100	0	0	0	67	0	0	0	0	0	0	0	0	0	100
Agrobacterium radiobacter DSM 30147	+	+	−	−	−	−	+	+	+	+	−	+	−	+	+	−	+	−	+	+	+	−	−	+
Agrobacterium tumefaciens DSM 30205	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	−	−	−	+
Alcaligenes eutrophus DSM 531	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Comamonas acidovorans DSM 39	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+
Cytophaga aquatilis DSM 2063	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+
Flavobacterium breve DSM 30096	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+
Flavobacterium odoratum DSM 2801	−	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	−
Hydrogenophaga palleronii DSM 63	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Methylobacterium mesophilicum DSM 1703	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Methylobacterium radiotolerans DSM 1819	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Paracoccus denitrificans DSM 65	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Pseudomonas alcaligenes DSM 50342	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+
Pseudomonas mendocina DSM 50017	−	−	+	+	+	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Pseudomonas pseudoalcaligenes DSM 50188	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+
Pseudomonas putida DSM 291	−	−	+	+	+	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Rhizobium leguminosarum DSM 30132	−	+	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+
Sphingobacterium spiritivorum DSM 2582	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	+
Sphingomonas capsulata DSM 30196	+	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	+	−	+	−	+	−	+
Thiobacillus versutus DSM 582	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Thiobacillus versutus IFO 14568	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Xanthomonas maltophilia LMG 981	+	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	+	−	+	−	−	−	+	−
Xanthomonas maltophilia DSM 50170	+	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	+	−	+	−	−	−	+	+
Zoogloea ramigera DSM 287	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	+	−	+	−	−	−	−	+

[a] Cluster without reference strain.

TABLE 5—*Continued*

% Positive																												
Hydrolysis of:							Growth with:																					
L-Arginine–pNA	γ-L-Glutamate–pNA	Glycine-pNA	L-Leucine–pNA	L-Lysine–pNA	L-Proline–pNA	L-Valine–pNA	*N*-Acetylglucosamine	L-Arabinose	D-Cellobiose	D-Fructose	D-Galactose	Gluconate	D-Glucose	D-Maltose	D-Mannose	D-Ribose	D-Sucrose	D-Trehalose	I-Inosite	D-Mannite	Sorbite	Acetate	Propionate	T-aconitate	Adipate	Citrate	DL-3-Hydroxybutyrate
0	0	0	40	0	0	0	0	0	0	0	0	20	20	0	0	20	0	0	0	0	0	40	80	0	0	100	100
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+
100	100	0	100	100	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
0	100	0	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
100	100	0	100	100	0	0	100	0	100	0	100	0	50	100	100	0	100	100	0	0	0	50	0	0	0	50	0
0	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	100	0	67	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	17	0	0	0	100
0	0	100	100	0	0	0	100	0	100	100	0	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	0
0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	33	100	0	0	0	100	0	0	100	0	0	100	0	100	0	0	0	100	0	0	33	100	0	33	0	100
−	−	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−
−	−	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
−	+	−	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+
−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	+
+	+	−	+	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+
−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	+	−	−	−	+	−	−	+	−	+	+	+	−	−	+	+	−	−	+	−	+	−	−	−	+
−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	+	−	+	+
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	+	+
−	−	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
+	−	−	+	+	−	+	+	−	+	+	−	−	+	+	+	−	+	+	−	+	−	−	−	−	−	−	−
+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	+	+	−	+	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	−	−	−	−	+
−	−	−	−	−	−	−	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−	+
−	+	−	+	−	−	−	+	−	+	+	−	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+
−	+	−	−	−	−	−	+	−	+	+	−	−	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−
−	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	−	−	−	−

Continued on following page

TABLE 5—*Continued*

Reference strain or cluster	% Positive: Growth with: DL-Lactate	L-Malate	Pyruvate	L-Histidine	L-Hydroxyproline	L-Ornithine	L-Proline	Putrescine
Alcaligenes faecalis cluster[a]	100	80	60	40	0	20	100	0
Alcaligenes faecalis DSM 30033	+	−	+	−	−	−	+	−
Alcaligenes xylosoxydans subsp. *denitrificans* DSM 30026	+	−	+	−	−	−	+	−
Pseudomonas diminuta cluster[a]	0	0	0	100	0	0	20	0
Pseudomonas diminuta DSM 1635	−	−	−	−	−	−	−	−
Type A cluster	100	0	0	0	100	0	100	0
Type B cluster	0	0	0	0	0	0	0	0
Type C cluster	0	0	0	0	0	0	0	0
Type D cluster	0	0	0	0	0	0	0	0
Type E cluster	100	0	0	0	0	0	0	0
Type F cluster	100	17	50	67	67	0	67	0
Type G cluster	100	0	100	100	100	100	100	0
Type H cluster	0	0	0	100	0	0	0	0
Type I cluster	100	0	0	100	67	100	100	0
Agrobacterium radiobacter DSM 30147	+	−	−	+	+	+	+	−
Agrobacterium tumefaciens DSM 30205	+	−	+	+	+	+	+	−
Alcaligenes eutrophus DSM 531	+	−	+	+	−	−	−	−
Comamonas acidovorans DSM 39	+	+	+	+	−	−	+	−
Cytophaga aquatilis DSM 2063	−	−	−	−	−	−	−	−
Flavobacterium breve DSM 30096	−	−	−	−	−	−	−	−
Flavobacterium odoratum DSM 2801	−	−	−	−	−	−	−	−
Hydrogenophaga palleronii DSM 63	+	−	−	−	−	−	+	−
Methylobacterium mesophilicum DSM 1703	−	−	+	−	−	−	−	−
Methylobacterium radiotolerans DSM 1819	−	+	+	−	−	−	−	−
Paracoccus denitrificans DSM 65	+	+	−	+	+	−	+	−
Pseudomonas alcaligenes DSM 50342	−	−	−	−	−	−	−	−
Pseudomonas mendocina DSM 50017	+	+	+	+	−	−	+	+
Pseudomonas pseudoalcaligenes DSM 50188	+	−	−	−	−	−	−	+
Pseudomonas putida DSM 291	+	−	−	+	+	+	+	+
Rhizobium leguminosarum DSM 30132	−	−	−	−	−	−	−	−
Sphingobacterium spiritivorum DSM 2582	−	−	−	−	−	−	−	−
Sphingomonas capsulata DSM 30196	−	−	−	+	−	−	−	−
Thiobacillus versutus DSM 582	+	−	+	+	−	−	−	−
Thiobacillus versutus IFO 14568	+	−	+	+	−	−	+	−
Xanthomonas maltophilia LMG 981	−	−	−	−	−	−	−	−
Xanthomonas maltophilia DSM 50170	+	+	−	−	−	−	−	−
Zoogloea ramigera DSM 287	+	−	−	+	+	+	+	−

the same quinone type. However, the physiological reaction profile showed that no relationship to this reference strain exists. Therefore, the assignment of this isolate to *Alcaligenes eutrophus* DSM 531 or to the other reference strains of this genus could not be confirmed.

The type E isolate was the only one containing Q-9, and it was therefore chemotaxonomically far separated from the other isolates, which contained Q-8 or Q-10. The fatty acid profile of this isolate differed from that of the other Q-9-containing reference strains of the genus *Pseudomonas* in a high content of 19:0-cyclo 11-12 (19%) and the occurrence of the fatty acid iso-11:0-3OH (4%) (Table 2). The physiological reaction profile of this isolate formed a cluster with the profiles of *Pseudomonas alcaligenes* DSM 50342 and *Pseudomonas pseudoalcaligenes* DSM 50188. However, since the Jaccard similarity coefficient was used, this similarity was based only on the positive test results of the hydrolysis of L-alanine–pNA and nitrate reduction to nitrite (Table 5). A physiological marker of the type E isolate was urease activity, which could not be shown for any other isolate (Table 5). With respect to the different fatty acid composition, the type E isolate could not be assigned to *Pseudomonas pseudoalcaligenes* DSM 50188 and *Pseudomonas alcaligenes* DSM 50342.

The type F cluster was characterized by its content of the fatty acid 19:0 of about 3%, which could not be detected in any other isolate or reference strain (Table 3). The presence of 3-hydroxydecanoic acid (10:0-3OH), 19:0, and an unknown fatty acid with an equivalent chain length (ECL) (14) of 19.828 distinguished this cluster from *Methylobacterium radiotolerans* DSM 1819. Although Urakami and Komagata (26) reported the occurrence of 19:0 in some isolates which they placed into the genus *Protomonas* (which was later reclassified as *Methylobacterium* [2]), there is no report of the occurrence of 10:0-3OH within this genus (5, 25–27). This leads to a clear distinction from the genus *Methylobacterium* which was even more pronounced when using the physiological reaction profiles (Fig. 2).

Based on the fatty acid profile, the type G isolate showed similarity to the reference strains *Agrobacterium radiobacter* DSM 30147 and *Agrobacterium tumefaciens* DSM 30205. Especially, the presence of the fatty acids 14:0-3OH and 16:0-3OH was described by Veys et al. (28) as a characteristic criterion for *Agrobacterium radiobacter* within the group of clinical relevant bacteria. Yokota (30) described the hydroxy fatty acids as useful indicators for the differentiation of members of the family *Rhizobiaceae* and reported that 14:0-3OH and 16:0-3OH are typical predominating hydroxy fatty acids for the genus *Agrobacterium*. The combination of these two fatty acids was found in the reference strains of the genus *Agrobacterium*, the type G isolate, and also in the reference strain *Zoogloea ramigera* DSM 287 (Table 3). The last strain was similar in fatty acid composition and physiological reaction profile to the reference strains of the genus *Agrobacterium*. Based on the fatty acid composition, *Zoogloea ramigera* DSM 287 could be separated from the *Agrobacterium* cluster by the lower content of 14:0-3OH (1%) and an unknown fatty acid with an ECL of 19.368 (Table 3). The physiological reaction profiles reproduced the cluster arrangement of these four strains and confirmed, therefore, the relationship of the type G isolate to the genus *Agrobacterium*.

The type H isolate could not be assigned to any reference strain by means of the fatty acid composition and the physiological reaction profile (Fig. 1 and 2).

The isolates of the type I cluster had a fatty acid profile

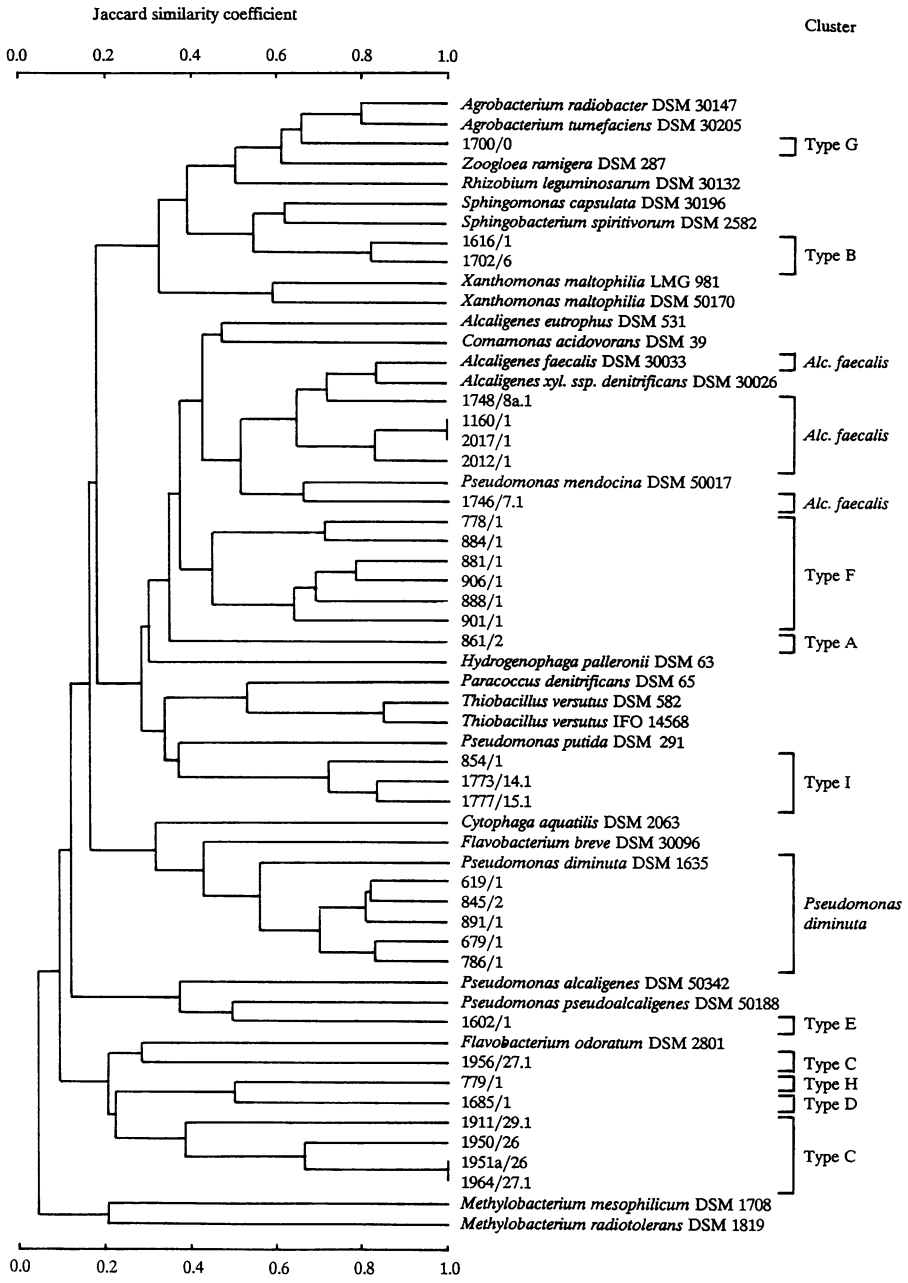

FIG. 2. Dendrogram generated from physiological reaction profiles.

which was similar to that of the reference strain *Thiobacillus novellus* DSM 506, except that trace amounts of the fatty acid 12:0-3OH and an unknown fatty acid with an ECL of 19.828 were detected in the type I cluster (Table 3). *Thiobacillus novellus* DSM 506 contained no hydroxy fatty acids, which was in accordance with the results of Katayama-Fujimura et al. (10). They found *Thiobacillus novellus* to be the only species of the genus *Thiobacillus* lacking hydroxy fatty acids. The physiological properties of the type I isolate differed clearly from those of *Thiobacillus novellus* DSM 506 since this reference strain failed to grown on ASS agar.

Fatty acid analysis proved to be a useful tool for the differentiation and characterization of gram-negative, nonfermentative bacteria isolated from biofilters. The physiological test system reproduced most of the clusters, which could be regarded as confirmation of these clusters. This applied to isolates showing a reaction profile with at least six positive test results, equivalent to 10% of the reactions tested. However, for the isolates with a smaller number of positive test results (type C, D, E, and H isolates), major differences occurred in comparison with the fatty acid analyses. This indicates that the physiological test systems are of limited usefulness when applied as a single method for the differentiation of isolates from biofilters and possibly from other environments as well, such as wastewater treatment plants or soils. Actually, many bacteria can be isolated from these sources, which exhibit low reactivity in the usually applied physiological identification systems. The prearrangement of

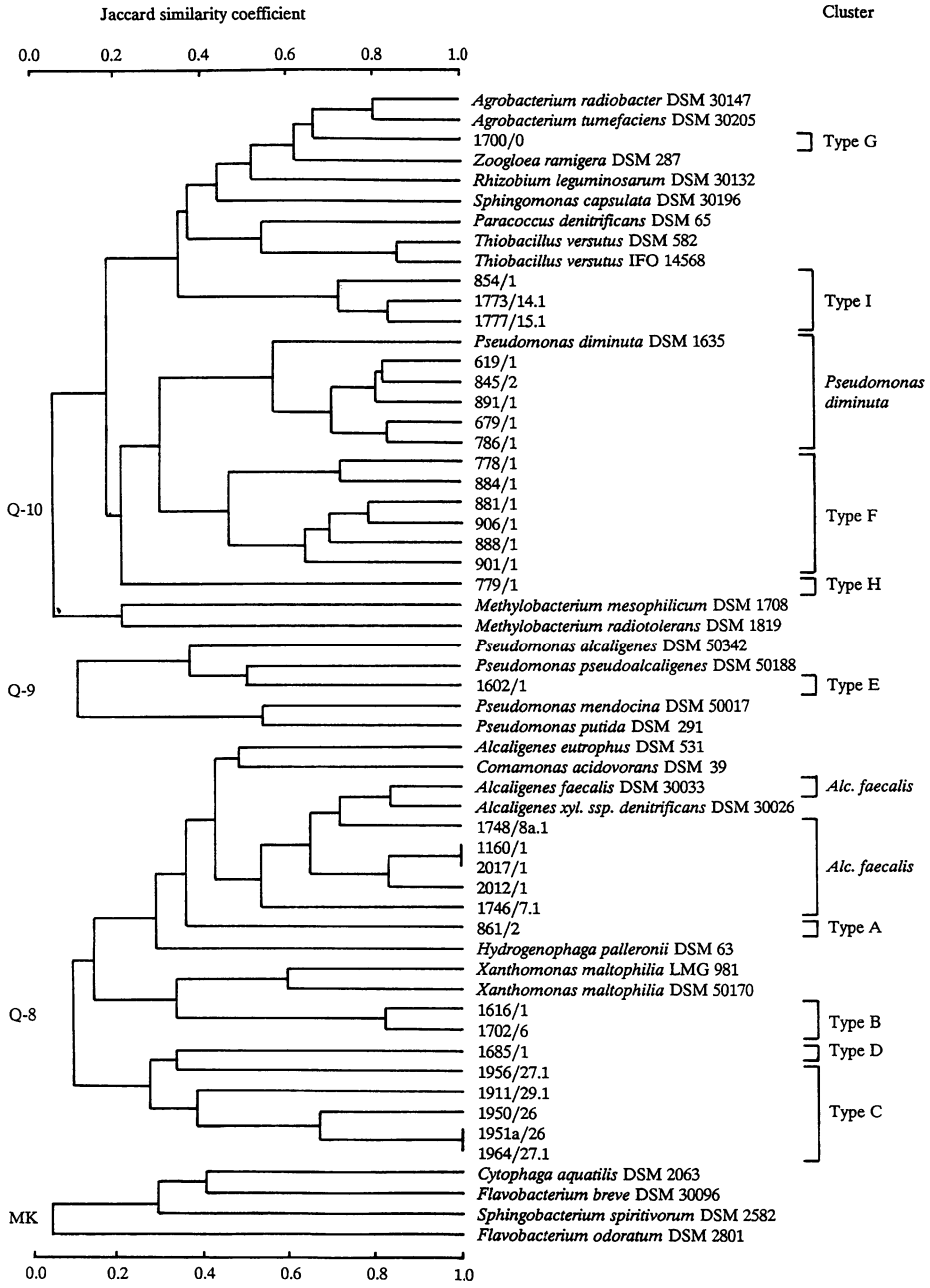

FIG. 3. Dendrogram generated from physiological reaction profiles with prearrangement according to the quinone type of the strains.

the biofilter isolates into distinct groups according to their quinone type improved the coincidence with the clusters obtained by fatty acid analysis. This suggests that the application of physiological test systems should be restricted to those groups of organisms which are prearranged, preferably by a chemotaxonomic marker such as the quinone type.

An important result of the identification procedure was the reliable proof of *Alcaligenes faecalis* and *Pseudomonas diminuta* among the isolates from biofilters for off-gas treatment of animal-rendering-plant emissions. The involvement of these species in the biofilter population and possibly in the degradation process of the specific waste gas compounds could supplement the known properties of these species by interesting aspects. On the other hand, the identification of the biofilter isolates was successful only for 10 of 31 strains. This indicates that physiological data bases, which usually do not consider the species present in a biofilter population, should be used with great caution for identification procedures.

Information about the whole biofilter population has to remain fragmentary when using the methods applied in this study. Investigations of plate counts of bacteria from organic soil showed that about 1% of the total counts could be recovered by culture techniques (21). Additional techniques such as direct extraction of DNA, RNA, or chemotaxonomic markers can give valuable contributions for future ap-

proaches of population analyses. Nevertheless, in view of the low costs, the quick and simple method, and the large extent of automation of fatty acid analysis, efforts should be made to optimize the culturing methods for the bacteria established in biofilters. Not only would this make the greatest possible number of bacteria available for this advantageous differentiation method, but also it is necessary for further physiological studies which are important steps toward the understanding and future optimizing of the degradation capacities of biofilters.

ACKNOWLEDGMENT

We thank A. Ahrens, Universität Osnabrück, for providing a number of isolates from experimental biofilters.

REFERENCES

1. **Bendinger, B., R. M. Kroppenstedt, H. Rijnaarts, H. R. Van Langenhove, R. C. Oberthür, and K. Altendorf.** 1990. Studies on the microbiology and the degradation capacities of a biofilter, p. 529–533. *In* D. Behrend and P. Krämer (ed.), DECHEMA Biotechnology Conferences, vol. 4. VCH Verlagsgesellschaft, Weinheim, Germany.
2. **Bousfield, I. J., and P. N. Green.** 1985. Reclassification of bacteria of the genus *Protomonas* Urakami and Komagata 1984 in the genus *Methylobacterium* (Patt, Cole, and Hanson) emend. Green and Bousfield 1983. Int. J. Syst. Bacteriol. **35:**209.
3. **Dees, S. B., and C. W. Moss.** 1975. Cellular fatty acids of *Alcaligenes* and *Pseudomonas* species isolated from clinical specimens. J. Clin. Microbiol. **1:**414–419.
4. **Hippchen, B.** 1985. Stuttgarter Berichte zur Siedlungswasserwirtschaft, vol. 94. Mikrobiologische Untersuchungen zur Eliminierung organischer Lösungsmittel im Biofilter. Kommissionsverlag R. Oldenbourg, Munich.
5. **Ikemoto, S., K. Katoh, and K. Komagata.** 1978. Cellular fatty acid composition in methanol-utilizing bacteria. J. Gen. Appl. Microbiol. **24:**41–49.
6. **Ikemoto, S., H. Kuraishi, K. Komagata, R. Azuma, T. Suto, and H. Murooka.** 1978. Cellular fatty acid composition in *Pseudomonas* species. J. Gen. Appl. Microbiol. **24:**199–213.
7. **Ikemoto, S., K. Suzuki, T. Kaneko, and K. Komagata.** 1980. Characterization of strains of *Pseudomonas maltophilia* which do not require methionine. Int. J. Syst. Bacteriol. **30:**437–447.
8. **Kämpfer, P., W. Bette, and W. Dott.** 1987. Phenotypic differentiation of members of the family *Vibrionaceae* using miniaturized biochemical tests. Zentralbl. Bakteriol. Mikrobiol. Hyg. **266:**425–437.
9. **Kaneda, T.** 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev. **55:**288–302.
10. **Katayama-Fujimura, Y., N. Tsuzaki, and H. Kuraishi.** 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. J. Gen. Microbiol. **128:**1599–1611.
11. **Kato, S., T. Urakami, and K. Komagata.** 1985. Quinone systems and cellular fatty acid composition in species of *Rhodospirillaceae* genera. J. Gen. Appl. Microbiol. **31:**381–398.
12. **Kersters, K., and J. De Ley.** 1984. Genus *Alcaligenes* Castellani and Chalmers 1919, p. 361–373. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
13. **Kroppenstedt, R. M.** 1982. Anwendung chromatographischer HP-Verfahren (HPTLC und HPLC) in der Bakterien-Taxonomie. Lab. Med. **5:**266–275.
14. **Kroppenstedt, R. M.** 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms, p. 173–199. *In* M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. SAB technical series no. 20. Academic Press, Inc. (London), Ltd., London.
15. **Kroppenstedt, R. M., E. Stackebrandt, and M. Goodfellow.** 1990. Taxonomic revision of the actinomycete genera *Actinomadura* and *Microtetraspora*. Syst. Appl. Microbiol. **13:**148–160.
16. **Laboratorium Microbiologie Rijksuniversiteit Gent.** List of cultures 1989 bacteria. Laboratorium Microbiologie Rijksuniversiteit Gent, Ghent, Belgium.
17. **Law, J. H., H. Zalkin, and T. Kaneshiro.** 1963. Transmethylation reactions in bacterial lipids. Biochim. Biophys. Acta **70:** 143–151.
18. **MacFaddin, J.** 1980. Biochemical tests for identification of medical bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.
19. **Moss, C. W., and S. B. Dees.** 1976. Cellular fatty acids and metabolic products of *Pseudomonas* species obtained from clinical specimens. J. Clin. Microbiol. **4:**492–502.
20. **Moss, C. W., P. L. Wallace, D. G. Hollis, and R. E. Weaver.** 1988. Cultural and chemical characterization of CDC groups EO-2, M-5, and M-6, *Moraxella* (*Moraxella*) species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. J. Clin. Microbiol. **26:**484–492.
21. **Olsen, R. A., and L. R. Bakken.** 1987. Viability of soil bacteria: optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb. Ecol. **13:**59–74.
22. **Oyaizu, H., and K. Komagata.** 1981. Chemotaxonomic and phenotypic characterization of the strains of species in the *Flavobacterium-Cytophaga* complex. J. Gen. Appl. Microbiol. **27:**57–107.
23. **Oyaizu, H., and K. Komagata.** 1983. Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. J. Gen. Appl. Microbiol. **29:**17–40.
24. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman & Company, San Francisco.
25. **Urakami, T., and K. Komagata.** 1979. Cellular fatty acid composition and coenzyme Q system in Gram-negative methanol-utilizing bacteria. J. Gen. Appl. Microbiol. **25:**343–360.
26. **Urakami, T., and K. Komagata.** 1984. *Protomonas*, a new genus of facultatively methylotrophic bacteria. Int. J. Syst. Bacteriol. **34:**188–201.
27. **Urakami, T., and K. Komagata.** 1987. Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids in Gram-negative methanol-, methane-, and methylamine-utilizing bacteria. J. Gen. Appl. Microbiol. **33:**135–165.
28. **Veys, A., W. Callewaert, E. Waelkens, and K. van den Abbeele.** 1989. Application of gas-liquid chromatography to the routine identification of nonfermenting gram-negative bacteria in clinical specimens. J. Clin. Microbiol. **27:**1538–1542.
29. **Yamada, Y., H. Takinami-Nakamura, Y. Tahara, H. Oyaizu, and K. Komagata.** 1982. The ubiquinone systems in the strains of *Pseudomonas* species. J. Gen. Appl. Microbiol. **28:**7–12.
30. **Yokota, A.** 1989. Taxonomic significance of cellular fatty acid composition in *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* species. Inst. Ferment. Osaka Res. Commun. **14:**25–39.