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Polyphasic Taxonomic Study of the Emended Genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an Aerotolerant Bacterium Isolated from Veterinary Specimens

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The relationships of 77 aerotolerant *Arcobacter* strains that were originally identified as *Campylobacter cryaerophila* (now *Arcobacter cryaerophilus* [P. Vandamme, E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley, Int. J. Syst. Bacteriol. 41:88–103, 1991]) and 6 reference strains belonging to the taxa *Arcobacter nitrofigilis*, *Arcobacter cryaerophilus*, and "*Campylobacter butzleri*" were studied by using a polyphasic approach, in which we performed DNA-rRNA hybridizations, DNA-DNA hybridizations, a numerical analysis of whole-cell protein patterns after sodium dodecyl sulfate-polyacrylamide gel electrophoresis, an analysis of cellular fatty acid compositions, and a phenotypic analysis and determined DNA base ratios. Our results indicate that "*C. butzleri*" should be transferred to the genus *Arcobacter* as *Arcobacter butzleri* comb. nov., as was suggested by Kiehlbauch and coworkers (J. A. Kiehlbauch, D. J. Brenner, M. A. Nicholson, C. N. Baker, C. M. Patton, A. G. Steigerwalt, and I. K. Wachsmuth, J. Clin. Microbiol. 29:376–385, 1991). A rapid screening of all strains in which we used the sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique revealed five major groups, which were identified by using DNA-DNA hybridization data as *A. cryaerophilus* (two distinct electrophoretic subgroups), *A. butzleri*, *A. nitrofigilis*, and a new species, for which we propose the name *Arcobacter skirrowii*. The phylogenetic position within rRNA superfamily VI was established for each species. *A. butzleri* strains and strains belonging to one of the electrophoretic subgroups of *A. cryaerophilus* had similar fatty acid contents. An analysis of fatty acid compositions allowed clear-cut differentiation of all of the other groups. All of the species could be distinguished by using classical phenotypic tests, although erroneous identifications due to a shortage of clear-cut differentiating tests could occur.

In the late 1970s, Ellis and coworkers described spiral-shaped bacteria which were isolated from aborted bovine and porcine fetuses (5, 6). These strains were identified as campylobacters (23) and constituted two distinct biochemical groups; the so-called group 1 strains were identified as *Campylobacter fetus*, and the group 2 strains belonged to a previously undescribed *Campylobacter* taxon (22). Other investigators isolated bacteria that were similar to the group 2 strains from aborted porcine and bovine fetuses (10, 11), from the milk of cows with mastitis (17), and from a bovine preputial sheath washing (7). All of these organisms differed from the true campylobacters by their ability to grow in the presence of atmospheric oxygen after primary isolation in a microaerobic environment (22). Therefore, they were referred to as the aerotolerant campylobacters. The biochemical and physiological properties of 90 aerotolerant *Campylobacter* strains were examined by Neill et al. (21). Although these authors found a considerable degree of phenotypic heterogeneity, they proposed a single name, *Campylobacter cryaerophila*, for the organisms which were isolated from reproductive tracts and aborted fetuses of several species of farm animals, from animal feces, and from the milk of cows with mastitis (21). The pathogenicity of *C. cryaerophila* was unknown. A few years later, Lambert et al. (16) and Tee et al. (28) described the isolation of *C. cryaerophila*-like strains from human clinical sources. Preliminary DNA-DNA hybridization results of Vandamme et al. (32) showed that three atypical *C. cryaerophila* strains exhibited no significant DNA binding values with genuine *C. cryaerophila* strains. Kiehlbauch et al. (12) performed an extensive DNA-DNA hybridization study and phenotypic analysis of 78 aerotolerant *Campylobacter* strains and identified two DNA relatedness groups. Some of the strains which these authors studied belonged to *C. cryaerophila*, but most of their strains were members of a separate species that exhibited a level of DNA homology with *C. cryaerophila* of approximately 40%. The name "*Campylobacter butzleri*" was proposed by Kiehlbauch et al. (12) (as this name was not validly published, it is enclosed in quotation marks). Within the true species *C. cryaerophila*, two genotypic and phenotypic subgroups were identified (12). The phenotypic features which were used did not allow clear-cut differentiation between one of these subgroups (corresponding to DNA hybridization group 1B) and "*C. butzleri*" (12). The same authors showed that the three DNA homology groups ("*C. butzleri*," *C. cryaerophila* hybridization group 1A, and *C. cryaerophila* hybridization group 1B) could be differentiated by the presence of specific ribosomal DNA restriction fragments (13).

The phylogenetic relationships of the aerotolerant cam-

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pylobacters have been determined by using the DNA-rRNA hybridization technique (32) and comparative 16S rRNA sequencing (29). *C. cryaerophila* and *Campylobacter nitrofigilis* are members of a separate rRNA homology branch that deserves separate genus status (29, 32). The genus name *Arcobacter* has been proposed for this group, with *Arcobacter nitrofigilis* as the type species and *Arcobacter cryaerophilus* as an additional species. In an addendum in proof, Kiehlbauch et al. (12) concluded from their results that "*C. butzleri*" also belongs to the genus *Arcobacter* as it exhibits a level of DNA homology with *C. cryaerophila* (12) (now *A. cryaerophilus* [32]) of approximately 40%; the name of this organism should be *Arcobacter butzleri*. Below, we formally transfer "*C. butzleri*" to the genus *Arcobacter* as *A. butzleri* comb. nov. This name and the name *Arcobacter skirrowii*, which we propose for a new species of aerotolerant arcobacters, are used below.

In this study we used a polyphasic approach to determine the taxonomic structure and the phylogenetic relationships of 77 strains that were originally identified as *C. cryaerophila*.

MATERIALS AND METHODS

Isolation of *Arcobacter* strains. Of the 83 strains which we studied, 45 were our own isolates. *Arcobacter* strains are seldom isolated by using the routine culture methods used for campylobacters and *Campylobacter*-like bacteria. The first *Arcobacter* strains were isolated by Ellis et al. (5, 6), who used a semisolid *Leptospira* medium. A distinct growth zone below the surface was observed if arcobacters were present. Adding 100 μg of 5-fluorouracil per ml to the growth medium inhibited the contaminating flora but did not influence the growth of *Arcobacter* strains (6, 10). Neill et al. (24) described a two-stage isolation method. First, samples were inoculated into the semisolid medium described above. After growth was visible, a few drops from the growth zone below the surface was streaked onto blood agar base containing 125 μg of carbenicillin per ml. Other investigators have used direct filtration techniques (7).

In our study, strains LMG 9800 through LMG 9803 and LMG 11072 through LMG 11078 (all identified as *A. skirrowii* [see below]) were isolated from preputial fluids of bulls. The fluid was inoculated into a transport medium. The inoculated transport medium was taken to our laboratory and centrifuged. Then the supernatant was filtered through a membrane filter (pore size, 0.8 μm; Millex). The first drops were discarded, and then 5 or 6 drops were streaked onto the following media: (i) thioglycolate agar (catalog no. 0257-01-9; Difco Laboratories, Detroit, Mich.) supplemented with 10% sheep blood, 25 IU of bacitracin per ml, 5 IU of polymyxin B sulfate per ml, 0.005 mg of novobiocin per ml, and 0.05 mg of cycloheximide (Acti-Dione) per ml; (ii) the same medium without polymyxin B sulfate; (iii) tryptone soya agar (catalog no. CM131; Oxoid, Ltd., Basingstoke, United Kingdom) containing the *Brucella* selective supplement (catalog no. SR83; Oxoid); and (iv) MacConkey agar (catalog no. CM7; Oxoid). Positive cultures were obtained in the thioglycolate medium without polymyxin B sulfate. Two strains were also isolated from the same medium containing polymyxin B sulfate. No positive cultures were found on the other media. Grey, flat, watery colonies were visible after 3 to 4 days of incubation in a microaerobic atmosphere at 37°C.

Strains LMG 10209 through LMG 10244 were isolated mainly from the tissues of fetuses when we were searching for leptospires. The medium used was semisolid PLM-5 medium (catalog no. 0242-00; Armour Pharmaceutical Co., Kankakee, Ill.) supplemented with 200 μg of 5-fluorouracil per ml. The tubes were checked by using dark-field examination; when *Campylobacter*-like organisms were observed, subculturing was carried out on nutrient agar no. 2 (catalog no. CM67; Oxoid).

Bacterial strains and growth conditions. All of the strains which we used are listed in Table 1. We received the type strain of *A. butzleri* (strain LMG 10828) and the reference strain of *A. cryaerophilus* hybridization group 1B (strain LMG 10829) from J. Kiehlbauch, Centers for Disease Control, Atlanta, Ga. Two reference strains of *A. nitrofigilis* (strains LMG 7604^T [T = type strain] and LMG 7547) and the type strain of *A. cryaerophilus* (strain LMG 7536) were sent by E. Falsen from the Culture Collection of the University of Göteborg, Göteborg, Sweden. A second subculture of the type strain of *A. cryaerophila* (strain LMG 9904) was sent by S. D. Neill from the Veterinary Research Laboratories, Belfast, Northern Ireland.

Most aerotolerant strains (except the strains that were identified as *A. butzleri*) grew weakly on the common blood agar bases, including 5% (vol/vol) horse blood in Mueller-Hinton agar (catalog no. CM337; Oxoid), nutrient agar no. 2 (catalog no. CM67; Oxoid), Columbia agar (catalog no. CM331; Oxoid), and brain heart infusion agar (catalog no. CM375; Oxoid). Growth was not markedly enhanced on chocolate agar plates. We obtained good growth on a medium that contained (per liter) 10 g of Special Peptone (catalog no. L72; Oxoid), 5 g of Lab Lemco powder (catalog no. L29; Oxoid), 5 g of yeast extract (catalog no. L21; Oxoid), 5 g of sodium chloride (catalog no. 6404; Merck, Darmstadt, Germany), 2 g of sodium succinate hexahydrate (catalog no. RPL 1785; RPL, Leuven, Belgium), 2 g of sodium L-glutamate monohydrate (catalog no. 6445; Merck), 1 g of magnesium chloride hexahydrate (catalog no. 5833; Merck), and 16 g of agar no. 3 (catalog no. L13; Oxoid); the final pH was 7.0. We refer to this growth medium below as the standard medium. This standard medium was supplemented with 5% (vol/vol) horse blood or with 0.4% (wt/vol) bacteriological charcoal (catalog no. L9; Oxoid), and the supplemented media supported similar growth. If the media were stored for more than 1 week, the charcoal-based medium gave better growth results than the blood agar. *A. nitrofigilis* strains were cultured on nutrient agar no. 2 (catalog no. CM67; Oxoid) supplemented with 1% sodium chloride (catalog no. 6404; Merck).

All of the strains were incubated at 36 to 37°C in a microaerobic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 unless indicated otherwise. Including 7.5% hydrogen in the microaerobic atmosphere (containing approximately 5% O_2, 3.5% CO_2, 7.5% H_2, and 84% N_2) enhanced the growth of only a few strains.

Bacteriological purity was checked by plating and examining living and Gram-stained cells. For mass cultures, cells were grown on approximately 20 petri dishes (diameter, 9 cm).

PAGE of whole-cell proteins. The cell yields of three or four petri dishes were required to obtain the amount of wet cell weight needed for polyacrylamide gel electrophoresis (PAGE) (approximately 70 mg), and we used Mueller-Hinton agar (catalog no. CM337; Oxoid) supplemented with 5% horse blood as the growth medium. A data base for the identification of campylobacters and related organisms (such as *Arcobacter* and *Helicobacter* strains) by means of a numerical analysis of whole-cell protein profiles has been accumulated, and the same growth medium and incubation

TABLE 1. Strains used

Strain[a]	Other designation(s)[a]	Depositor[a,b]	Source
A. skirrowii LMG 6621^T	Skirrow 449/80^T, CCUG 10374^T	CCUG	Feces, lamb with diarrhea
A. skirrowii LMG 8538	Skirrow 1018/79, CCUG 10375	CCUG	Bovine
A. skirrowii LMG 9800	Hommez 9(1837.10)		Preputial fluid, bull (Belgium)
A. skirrowii LMG 9801	Hommez 12(1837.8)		Preputial fluid, bull (Belgium)
A. skirrowii LMG 9802	Hommez 16(m)		Preputial fluid, bull (Belgium)
A. skirrowii LMG 9803	Hommez 15(i)		Preputial fluid, bull (Belgium)
A. skirrowii LMG 9911	Neill 02777, S/314/T	Neill	Thoracal fluid, aborted porcine fetus (Ireland)
A. skirrowii LMG 9912	Neill 02745, A169/P	Neill	Placenta, aborted bovine fetus (Ireland)
A. skirrowii LMG 10234	Higgins 88-2104		Lung and liver, aborted porcine fetus (Canada)
A. skirrowii LMG 10236	Higgins 88-2363		Lung and liver, aborted bovine fetus (Canada)
A. skirrowii LMG 10238	Higgins 88-2649		Feces, 2.5-mo-old calf with hemorrhagic colitis (Canada)
A. skirrowii LMG 10239	Higgins 88-3208		Feces, 1-yr-old cow with diarrhea (Canada)
A. skirrowii LMG 11072	Hommez BN2015m		Preputial fluid, bull (Belgium)
A. skirrowii LMG 11073	Hommez BN2077f		Preputial fluid, bull (Belgium)
A. skirrowii LMG 11074	Hommez BN2165a		Preputial fluid, bull (Belgium)
A. skirrowii LMG 11075	Hommez BN2198a		Preputial fluid, bull (Belgium)
A. skirrowii LMG 11076	Hommez BN2198b		Preputial fluid, bull (Belgium)
A. skirrowii LMG 11078	Hommez BN2198d		Preputial fluid, bull (Belgium)
A. butzleri LMG 6620	CCUG 10373, Skirrow 996/79	CCUG	Blood, 65-yr-old woman
A. butzleri LMG 9910	Neill 02834, LC/78/35/3, D2892	Neill	Deadborn piglet (United Kingdom)
A. butzleri LMG 9869	Neill 02832, LC7835/1, CCUG 17812	CCUG	Deadborn piglet (United Kingdom)
A. butzleri LMG 9906	Neill 04955, S/568/E, D2885	Neill	Eye, aborted porcine fetus (Ireland)
A. butzleri LMG 9939	ADRI 1009	Garcia	Aborted fetus (Canada)
A. butzleri LMG 10220	Higgins 87-41-2063		Feces, pig with hemorrhagic diarrhea (Canada)
A. butzleri LMG 10223	Higgins 87-3295		Bovine liver (Canada)
A. butzleri LMG 10240	Higgins 88-3250		Diarrheic equine feces (Canada)
A. butzleri LMG 10243	Higgins 88-3830		Feces, 2-yr-old cow with diarrhea (Canada)
A. butzleri LMG 10828^T	D2686^T, ATCC 49616^T	CDC	Feces, human with diarrhea (United States)
A. butzleri LMG 10902	SL3900	Lauwers	Spoiled meat (Belgium)
A. butzleri LMG 11118	SL4091	Lauwers	Human feces (Italy)
A. cryaerophilus subgroup 1 strains			
A. cryaerophilus LMG 6622	Neill 02774, B1904/K, CCUG 12018, PC367, D2081	CCUG	Kidney, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 7536^T	Neill 02766^T, A169/B^T, CCUG 17801^T, D2079^T	CCUG	Brain, aborted bovine fetus (Ireland)
A. cryaerophilus LMG 9065	Neill 02797, CCUG 12019, B750/P, D2077	CCUG	Placenta, aborted ovine fetus (Ireland)
A. cryaerophilus LMG 9066	Neill 02772, CCUG 12020, S613/E(b), D2078	CCUG	Eye, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 9862	Neill 02748, N1033/P, CCUG 17803	CCUG	Bovine placenta (Ireland)
A. cryaerophilus LMG 9863	Neill 02799, B938, CCUG 17806	CCUG	Placenta, aborted ovine fetus (Ireland)
A. cryaerophilus LMG 9865	Neill 02771, S613(a)E, CCUG 17808	CCUG	Eye, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 9871	Neill 02732, A175/K, CCUG 17814, LMG 9905	CCUG	Kidney, aborted bovine fetus (Ireland)
A. cryaerophilus LMG 9904^T	Neill 02766^T, A169/B^T, LMG 7536^T, CCUG 17801^T	Neill	Brain, aborted bovine fetus (Ireland)
A. cryaerophilus LMG 9905	Neill 02732, A175/K, LMG 9871	Neill	Kidney, aborted bovine fetus (Ireland)
A. cryaerophilus LMG 10210	Higgins F148-2		Aborted bovine fetus (Canada)
A. cryaerophilus subgroup 2 strains			
A. cryaerophilus LMG 7537	Neill 02828, B1056/P	CCUG	Aborted ovine fetus (Ireland)
A. cryaerophilus LMG 9861	Neill 02824, A102/Pt, CCUG 17802	CCUG	Peritoneal fluid, aborted bovine fetus (Ireland)
A. cryaerophilus LMG 9864	Neill 02790, S568/E, CCUG 17807	CCUG	Eye, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 9866	Neill 02792, B3286/F, CCUG 17809, LMG 9908	CCUG	Porcine feces (Ireland)
A. cryaerophilus LMG 9867	Neill 02830, B837/F, CCUG 17810	CCUG	Spleen, aborted equine fetus (Ireland)

A. cryaerophilus LMG 9870	Neill 02776, S1010/E, CCUG 17813, LMG 9907	CCUG	Eye, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 9907	Neill 02776, S/1010/E, LMG 9870	Neill	Eye, aborted porcine fetus (Ireland)
A. cryaerophilus LMG 9908	Neill 02792, LC/78/35/4, LMG 9866	Neill	Milk, cow with mastitis (United Kingdom)
A. cryaerophilus LMG 9909	Neill 04957, LC/78/35/1, D2891	Neill	Deadborn piglet (United Kingdom)
A. cryaerophilus LMG 9937	ADRI 1007	Garcia	Aborted fetus (Canada)
A. cryaerophilus LMG 9944	ADRI 1014	Garcia	Aborted fetus (Canada)
A. cryaerophilus LMG 9947	ADRI 1018	Garcia	Aborted fetus (Canada)
A. cryaerophilus LMG 9948	ADRI 1019	Garcia	Aborted fetus (Canada)
A. cryaerophilus LMG 10209	Higgins F103		Aborted bovine fetus (Canada)
A. cryaerophilus LMG 10211	Higgins F174		Aborted bovine fetus (Canada)
A. cryaerophilus LMG 10212	Higgins F197		Aborted bovine fetus (Canada)
A. cryaerophilus LMG 10213	Higgins 82-233		Aborted porcine fetus (Canada)
A. cryaerophilus LMG 10215	Higgins 82-783		Aborted porcine fetus (Canada)
A. cryaerophilus LMG 10216	Higgins 87-1801		Placenta, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10217	Higgins 87-1870A		Placenta, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10218	Higgins 87-1870B		Placenta, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10219	Higgins 87-1928		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10221	Higgins 87-2168		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10222	Higgins 87-2477		Placenta, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10224	Higgins 87-3445		Deadborn piglet (Canada)
A. cryaerophilus LMG 10225	Higgins 87-3499		Porcine colon (Canada)
A. cryaerophilus LMG 10226	Higgins 87-3993		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10227	Higgins 87-4647		Aborted porcine fetus (Canada)
A. cryaerophilus LMG 10228	Higgins 87-4984		Liver, lung, and spleen, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10229	Higgins 87-5154		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10230	Higgins 88-56-025		Feces, 15-week-old piglet with grey diarrhea (Canada)
A. cryaerophilus LMG 10231	Higgins 88-643		Lung and liver, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10232	Higgins 88-1219		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10233	Higgins 88-2073		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10235	Higgins 88-2139		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10237	Higgins 88-2612		Lung and liver, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10241	Higgins 88-3421R		Kidney, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10242	Higgins 88-3778		Lung, liver, and spleen, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10244	Higgins 88-4073		Lung and liver, aborted porcine fetus (Canada)
A. cryaerophilus LMG 10829	D2610, ATCC 49615	CDC	Human blood (United States)
A. nitrofigilis LMG 7547	CCUG 12022, PC371	CCUG	Roots or root-associated sediments from *Spartina alterniflora* (United States)
A. nitrofigilis LMG 7604^T	CCUG 15893^T, CCUG 15892^T, CI^T	CCUG	Roots from *Spartina alterniflora* (Canada)

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control, Atlanta, Ga.; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; Garcia, M. Garcia, Animal Diseases Research Institute (ADRI), Nepean, Ontario, Canada; Lauwers, S. Lauwers, Department of Microbiology, University Hospital, Free University of Brussels, Brussels, Belgium; Neill, S. D. Neill, Veterinary Research Laboratories, Stormont, Belfast, Northern Ireland.

[b] Organisms were our own isolates unless specified otherwise.

conditions were used for nearly all of the strains (35). For this reason, all of the strains were incubated in a microaerobic atmosphere containing approximately 5% O_2, 3.5% CO_2, 7.5% H_2, and 84% N_2. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate (SDS)-PAGE was performed by using the procedure of Laemmli (15), modified slightly as described previously (31).

Numerical analysis of protein gel electropherograms. The densitometric analysis, normalization, and interpolation of the protein profiles were performed as described by Pot et al. (25). A numerical analysis was performed as described by Vauterin et al. (36) on points 10 to 120 and points 150 to 310 of the 400 points of each interpolated trace. The profiles were recorded and stored on a PC-AT computer. The levels of similarity between all pairs of traces were expressed by using the Pearson product moment correlation coefficient (r), and clustering was performed by using the unweighed pair group method using average linkage (26).

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared from 1.5 to 3 g (wet weight) of cells. Bacterial cells were harvested and washed in saline-EDTA buffer (0.15 M NaCl plus 0.1 M EDTA, pH 8). A 1-g portion of cells was suspended in approximately 30 ml of buffer. Proteinase K (catalog no. 24568; Merck) (0.625 mg/g [wet weight] of cells) and 1.5 ml of 25% SDS were added. The resulting solution was incubated for 15 to 30 min at 37°C. The total NaCl concentration was adjusted to 1 M, and the DNA solution was vigorously shaken for a few seconds. The DNA emulsion was then gently shaken for 30 min at room temperature and centrifuged, and the supernatant was removed. The DNA was precipitated by adding 2 volumes of ethanol, spooled on a glass rod, and dissolved at 45°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7). Then 1.25 ml of a 0.2% solution of RNase (catalog no. 34390; Serva Feinbiochemica GMBH & Co., Heidelberg, Germany) per g (wet weight) was added, and the mixture was incubated for 30 min at 45°C. The deproteinization step described above was repeated, and the DNA was finally suspended in 1 to 3 ml of 0.1× SSC and stored at −80°C.

DNA-DNA hybridization experiments. The degree of DNA-DNA binding, expressed as a percentage, was determined spectrophotometrically by using the initial renaturation rate method of De Ley et al. (3). Each value given below is the average of values from at least two hybridization experiments. DNA binding values of 30% and less do not indicate significant DNA homology. The total DNA concentration was about 39 μg/ml, and the optimal renaturation temperature in 2× SSC was 61.6°C.

DNA base compositions. All of the guanine-plus-cytosine (G+C) values were determined by the thermal denaturation method and were calculated by using the equation of Marmur and Doty (18), as modified by De Ley (2).

DNA-rRNA hybridization experiments. In vivo radioactively labeled rRNAs from *A. nitrofigilis* LMG 7547 and *A. butzleri* LMG 6620 were prepared as described previously (32). Fixation of single-stranded DNA on membrane filters, chemical determinations of the amounts of DNAs on the filters, saturation hybridization, RNase treatment, and thermostability measurements of hybrids were performed as described by De Ley and De Smedt (4). Each DNA-rRNA hybrid was characterized by determining the $T_{m(e)}$ value (the melting temperature of elution), the temperature at which 50% of the DNA-rRNA hybrid was denatured. A homologous duplex was formed between DNA and rRNA from the same strain; a heterologous hybrid was formed between DNA and rRNA from different strains. The higher the $T_{m(e)}$ of a heterologous hybrid, the more closely the two strains were related. The $T_{m(e)}$ values obtained from reciprocal hybridizations in which we used all of the strains of each rRNA branch were used to calculate the average linkage levels between pairs of rRNA branches.

Gas chromatographic analysis of FAMEs. To prepare fatty acid methyl esters (FAMEs), one or two petri dishes (diameter, 9 cm) containing the standard medium supplemented with 5% horse blood were inoculated for each strain. The cultures were incubated for 72 h at 37°C in a microaerobic atmosphere containing approximately 5% O_2, 3.5% CO_2, 7.5% H_2, and 84% N_2. A loopful of well-grown cells of each strain was harvested with a sterile plastic loop (diameter, 4 mm) and transferred to a test tube capped with a Teflon-lined screw cap. To prepare FAMEs, the procedure described by Stead (27) was used. FAMEs were separated by gas-liquid chromatography by using a model 5890A instrument (Hewlett-Packard Co., Avondale, Pa.) and fused silica capillary column (25 m by 0.2 mm) that was coated with methyl phenyl silicone (Hewlett-Packard). The computer-controlled parameters for the instrument were the same as those described by Korn-Wendisch et al. (14). FAME fingerprints were identified by using the Microbial Identification System software package (MIS version no. 3.2) obtained from Microbial ID, Inc., Newark, Del., and a calibration mixture of known standards (Hewlett-Packard). FAME profiles were compared and grouped by using principal-component analysis. Within each individual FAME group, the fatty acid mean percentages and standard deviations were calculated.

Phenotypic tests. Phenotypic tests were performed on all *Arcobacter* strains. The morphology of cells was evaluated by Gram staining. Motility was observed in young cultures by examining wet mounts in broth by phase-contrast microscopy. The following tests were performed as described previously (9): temperature tolerance; growth under aerobic and anaerobic conditions; presence of oxidase and catalase; reduction of nitrate and nitrite; hydrogen sulfide production in triple sugar iron agar and as determined with the rapid H_2S test; oxidation and fermentation of carbohydrates; hydrolysis of hippurate, DNA, urea, and indoxyl acetate; susceptibility to nalidixic acid and cephalothin (30-μg discs); and growth in the presence of 1% glycine, 1% oxgall, 1.5 and 3.5% NaCl, and 0.04% 2,3,5-triphenyltetrazolium chloride (TTC). Tests for growth in the presence of 8% glucose, growth in MacConkey agar, and hydrogen sulfide production from cysteine were performed as described by Barrett et al. (1). The test for growth on VB medium consisted of growing the organisms on the previously described selective medium at 37°C in a microaerobic atmosphere (8).

RESULTS

PAGE of whole-cell proteins. We prepared duplicate protein extracts of several strains to check the reproducibility of the growth conditions and the preparation of the extracts. The level of correlation (r) between duplicate protein patterns was ≥0.95.

For four strains we received two subcultures that originated from different depositors (Table 1). The two subcultures of each pair (except the subcultures of the type strain of *A. cryaerophilus*) produced identical protein profiles; these subcultures grouped in the numerical analysis at r levels of ≥0.95 (Fig. 1). Minor differences between the protein patterns of the two subcultures of the type strain of

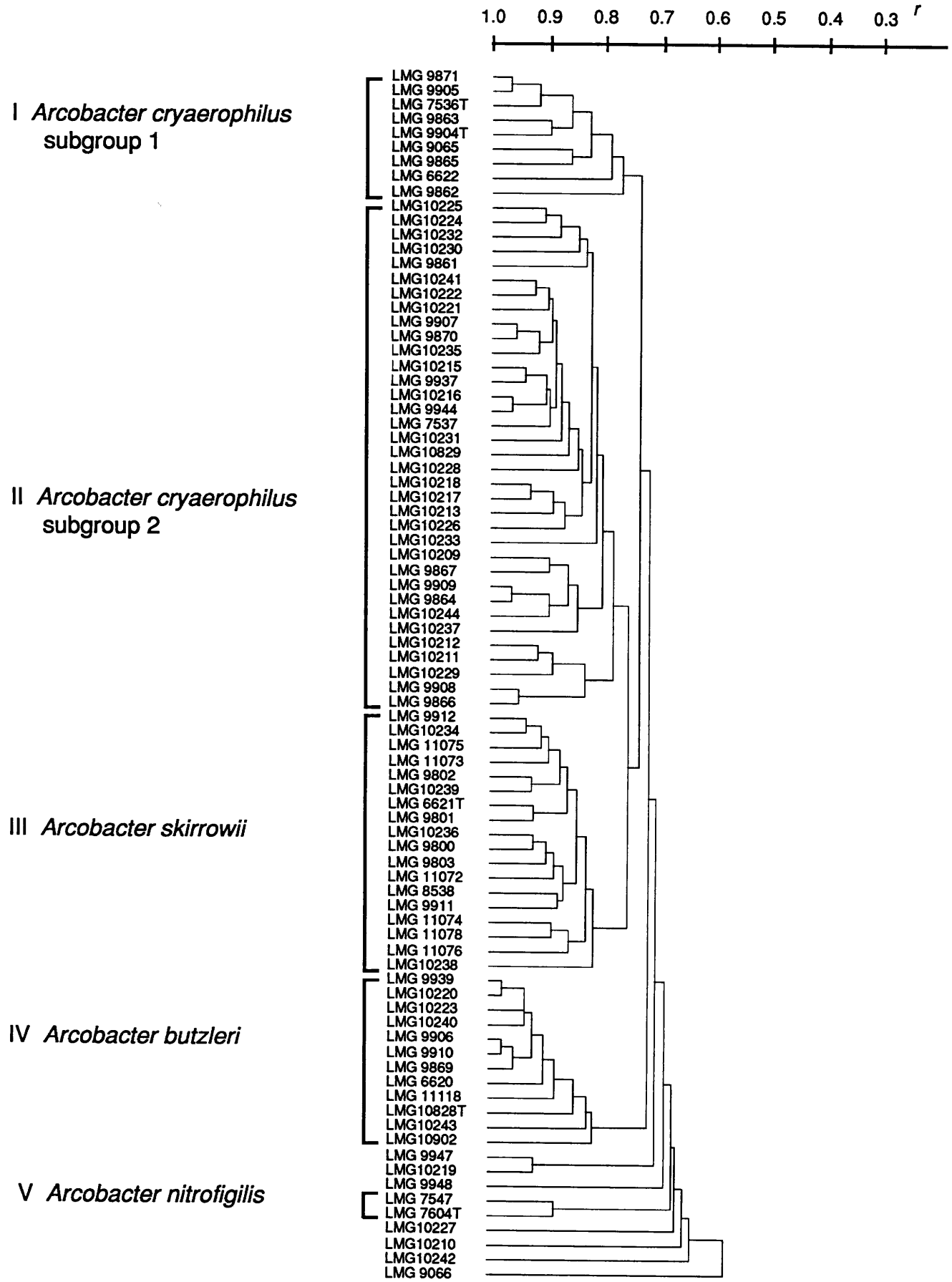

FIG. 1. Dendrogram derived from unweighed pair group average linkage of *r* values for the reduced protein patterns (points 10 through 120 and 150 through 310) of all of the strains studied. The roman numerals are cluster numbers.

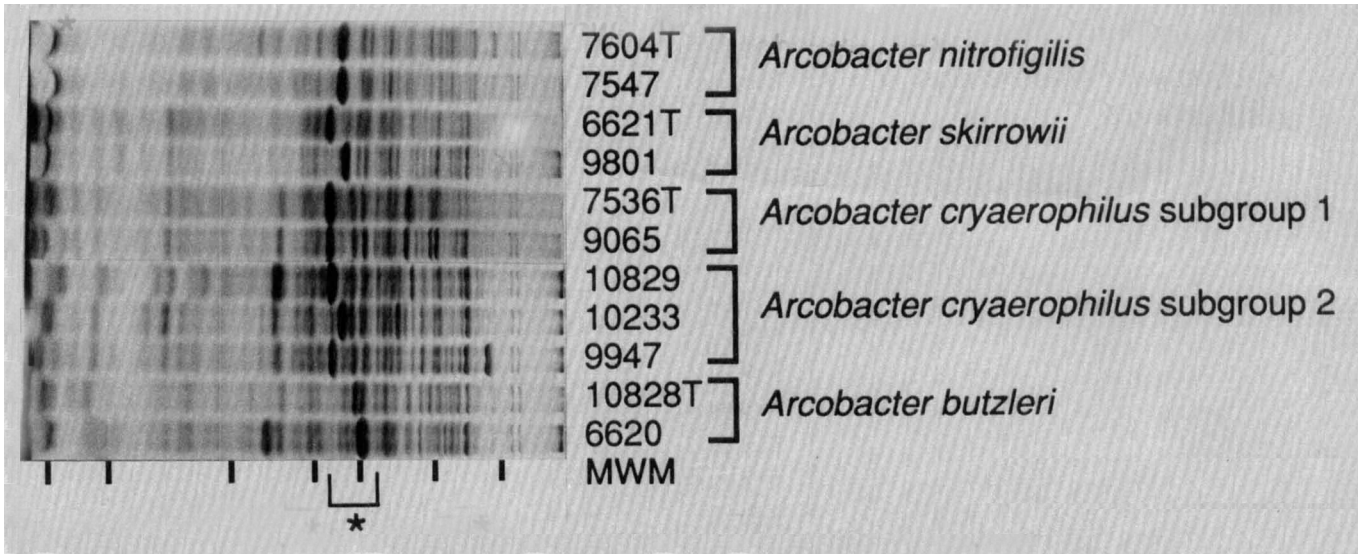

FIG. 2. Electrophoretic protein profiles of representative strains belonging to each electrophoretic cluster and a strain that occupied a separate position on the dendrogram (strain LMG 9947). All strain designations are LMG numbers (see Table 1, footnote *a*). The positions of the following molecular weight markers (track MWM) are indicated (from left to right): lysozyme (molecular weight, 14,500), trypsin inhibitor (20,100), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), bovine albumin (66,000), and β-galactosidase (116,000). The bracket marked with an asterisk indicates a variable dense-band region that was omitted from the numerical analysis.

A. cryaerophilus (strains LMG 7536 and LMG 9904) were found; the *r* level for these two profiles was 0.87 (Fig. 1).

Our numerical analysis of the complete protein patterns (points 10 to 310) was distorted by the presence of a variable dense-band region similar to the principal dense-band region previously described for *Campylobacter* strains (35). This region was situated in the 40,000- to 58,000-molecular-weight range (points 121 to 149 of the profile) (Fig. 2). Therefore, these points were omitted from the numerical analysis, which resulted in clear-cut grouping of nearly all of the strains studied. It has been shown previously that the clusters obtained in such a restricted analysis are also genotypically homogeneous (34, 35).

Above an *r* level of 0.77, five major clusters and seven strains having separate positions were delineated (Fig. 1). Cluster I contained the two subcultures of the type strain of *A. cryaerophilus* and seven aerotolerant strains; these organisms grouped at an *r* level of more than 0.78. We refer to these strains below as the *A. cryaerophilus* subgroup 1 strains. Cluster II consisted of 35 aerotolerant *Arcobacter* strains, including the reference strain of *A. cryaerophilus* hybridization group 1B (strain LMG 10829) (12), which grouped at an *r* level of more than 0.78; below, we refer to these strains as the *A. cryaerophilus* subgroup 2 strains. Cluster III contained 18 aerotolerant isolates that grouped at an *r* level of more than 0.83; below, we show that these strains are members of a new *Arcobacter* species, for which we propose the name *Arcobacter skirrowii*. Cluster IV ($r >$ 0.84) contained *A. butzleri* LMG 10828^T (T = type strain) and 11 aerotolerant isolates which were identified as *A. butzleri* strains. The two *A. nitrofigilis* strains constituted cluster V at an *r* level of 0.88. Strains LMG 9066, LMG 9947, LMG 9948, LMG 10210, LMG 10219, LMG 10227, and LMG 10242 had separate positions at an *r* level of 0.77. Strains

Protein electrophoretic cluster no.	Taxon	LMG no	%(G+C)	%DNA binding
Cluster I	*Arcobacter cryaerophilus* subgroup 1	7536T	28	100
		6622	28	57
		9065	28	67
		9862		56
Cluster II	*Arcobacter cryaerophilus* subgroup 2	10829	27	55
		7537	29	
		9909	30	66
		10209	29	
		10212		
		10215	29	
		10218		51
		10229		69
		10231	29	
		10235		
		10241		48
		9066	28	
		10210	28	55

100																				
57	100																			
67		100																		
56	60		100																	
55		66		100																
		46		76	100															
66				68		100														
				71			100													
								100												
							79			100										
51				80	78		98			100										
69								56			100									
						67			69			100								
						75			70			71	100							
48	63				81					81	67	78		100						
							86								100					
55										58				61	72	100				

FIG. 3. DNA-DNA hybridization results and DNA base compositions of *A. cryaerophilus* strains. Each DNA-DNA hybridization value is the average degree of binding from at least two hybridization experiments. LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium.

Protein electrophoretic cluster no.	Taxon	LMG no.	%(G+C)	%DNA binding													
Cluster V	*Arcobacter nitrofigilis*	7604T	29	100													
		7547	29	98	100												
Cluster IV	*Arcobacter butzleri*	10828T		19		100											
		6620	28			79	100										
		9906	28			79		100									
		9939	28				81	76	100								
		10243	29	9		64	82	83	78	100							
		11118	28			70	80			76	100						
Cluster III	*Arcobacter skirrowii*	6621T	29	8			19					100					
		8538	29									96	100				
		9800	29			19						79		100			
		9911	29								29	92		84	100		
		10236	30									80		89	80	100	
		10238		6			21							76	88	85	100

FIG. 4. DNA-DNA hybridization results and DNA base compositions of *A. nitrofigilis*, *A. butzleri* and *A. skirrowii* strains. Each DNA-DNA hybridization value is the average degree of binding from at least two hybridization experiments. LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium.

LMG 9947 and LMG 10219 grouped at an *r* level of 0.94 (Fig. 1). The overall protein profiles of strains LMG 9066 and LMG 10210 were very similar to the profiles of the cluster I strains, while the protein profiles of strains LMG 9947, LMG 9948, LMG 10219, LMG 10227, and LMG 10242 corresponded to the profiles of the cluster II strains (this is illustrated for strain LMG 9947 in Fig. 2). These strains had an atypical dense protein band in the high-molecular-weight region (molecular weight, approximately 100,000) which disturbed the result of the numerical analysis. Nevertheless, these strains were closely related to the strains in their respective clusters (34). Similar protein bands have been described previously for *A. cryaerophilus*, *Campylobacter sputorum* and *Campylobacter fetus* (34, 35), and *Campylobacter concisus* strains (33).

The protein patterns of representative strains belonging to clusters I through V are shown in Fig. 2.

DNA-DNA hybridization results. We chose a number of representative strains from each electrophoretic cluster to perform DNA-DNA hybridizations; we used 4 strains from cluster I (*A. cryaerophilus* subgroup 1), 11 strains from cluster II (*A. cryaerophilus* subgroup 2), 6 strains from cluster III (*A. skirrowii*) and cluster IV (*A. butzleri*), and two strains from cluster V (*A. nitrofigilis*). The type strain of each taxon was included. We also prepared DNAs from two strains that had separate positions on the dendrogram (strains LMG 9066 and LMG 10210) and could be allocated visually to *A. cryaerophilus* subgroup 1 (see above) (Fig. 2).

The hybridization results revealed that there were four DNA homology groups (Fig. 3 and 4). The *A. cryaerophilus* subgroup 1 and 2 strains (clusters I and II, respectively) were related at the species level (the DNA binding values ranged from 46 to 69%) (Fig. 3); within these groups, DNA binding values of more than 56% were found (Fig. 3). Strains of *A. cryaerophilus* exhibited no significant DNA binding with strains of the other *Arcobacter* species (data not shown). The DNA binding values versus *A. skirrowii*, *A. butzleri*, and *A. nitrofigilis* strains (clusters III, IV, and V, respectively) were 32 ± 10, 21 ± 6, and 16 ± 1% (means ± standard deviations of 12, 6, and 2 binding values, respectively). Within *A. skirrowii*, *A. butzleri*, and *A. nitrofigilis*, DNA binding values greater than 76, 64, and 98%, respectively, were found (Fig. 4). Strains of these *Arcobacter* species exhibited no significant DNA binding values (<30%) with strains belonging to other *Arcobacter* species (Fig. 4).

DNA base compositions. The DNA base ratios which we determined are shown in Fig. 3 and 4. All of the *Arcobacter* strains had G+C contents between 27 and 30 mol% (Fig. 3 and 4).

DNA-rRNA hybridizations. The DNA-rRNA hybridization results are shown in Table 2 and are represented as a dendrogram based on the $T_{m(e)}$ values of the hybrids in Fig. 5. Some of these results have been published previously (32). As shown previously, *Arcobacter* strains belong to rRNA superfamily VI, a separate eubacterial lineage within the class *Proteobacteria* (30). *A. nitrofigilis* and *A. butzleri* strains constitute separate rRNA branches that are linked at a $T_{m(e)}$ of 72.4 ± 1.3°C. *A. cryaerophilus* and *A. skirrowii* strains had similar $T_{m(e)}$ values versus both labeled rRNAs (averages ± standard deviations, 71.6 ± 1.0 and 71.5 ± 0.9°C, respectively).

FAME composition. The average FAME composition of the strains of each *Arcobacter* taxon is shown in Table 3. Values for those fatty acids for which the average amount was less than 1% for all strains of the taxon (18:0 and unidentified fatty acids with equivalent chain lengths of

TABLE 2. $T_{m(e)}$ values of DNA-rRNA hybrids

Organism used for DNA isolation	$T_{m(e)}$ (°C) when hybridized with rRNA from:	
	A. nitrofigilis LMG 7547	*A. butzleri* LMG 6620
A. nitrofigilis LMG 7604^T	76.3	72.7
A. nitrofigilis LMG 7547	76.7	73.3
A. butzleri LMG 6620	73.8	76.8
A. butzleri LMG 10828^T	70.1	75.6
A. butzleri LMG 11118	72.6	75.7
A. skirrowii LMG 6621^T	70.1	71.9
A. skirrowii LMG 8538	72.4	71.4
A. cryaerophilus subgroup 1 strains		
A. cryaerophilus LMG 7536^T	71.8	72.6
A. cryaerophilus LMG 6622	72.3	71.8
A. cryaerophilus LMG 9065	71.9	70.6
A. cryaerophilus subgroup 2 strains		
A. cryaerophilus LMG 7537	72.5	72.4
A. cryaerophilus LMG 10241	70.0	70.0

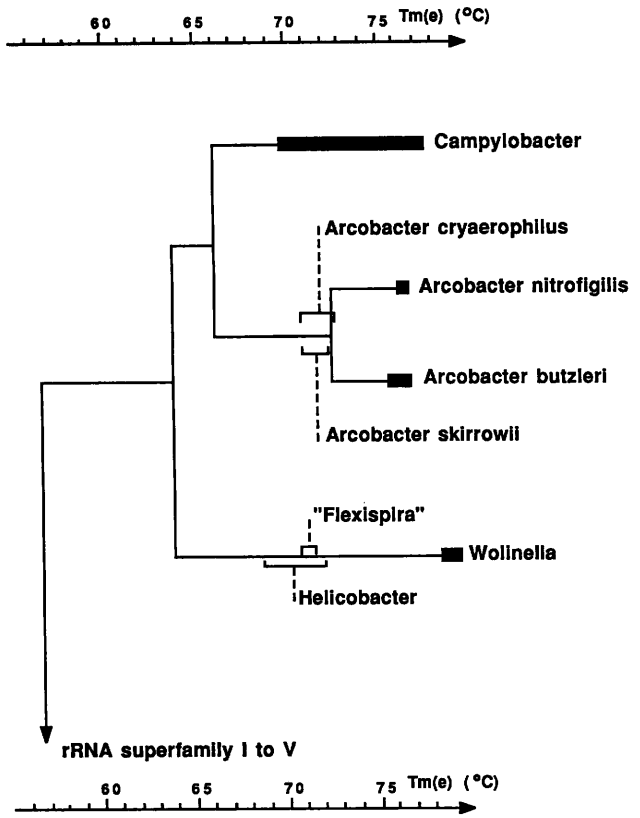

FIG. 5. Simplified rRNA cistron similarity dendrogram of rRNA superfamily VI. The solid bars indicate the $T_{m(e)}$ ranges observed within species or small groups. The dashed lines indicate rRNA branches for which no labeled rRNA is available yet.

11.798, 12.483, 13.951, and 14.503) are not given; therefore, the percentages for individual groups do not always total 100%. The fatty acids with equivalent chain lengths of 13.815, 15.485, 15.853, and 17.822 were identified as 14:1 *cis* 7, 14:0 3OH, 16:1 *trans* 9, and 18:1 *cis* 11, respectively, by using previously published data of Moss and Lambert-Fair (20). All of the arcobacters had 12:0, 14:0 3OH, 16:1 *cis* 9, 16:0, and 18:1 *cis* 11 as major components.

Phenotypic analysis. All of the strains which we studied were gram-negative, motile, curved rods. Alpha-hemolysis was common in *A. skirrowii* strains. With the exception of a few *A. butzleri* strains that were also alpha-hemolytic, all of the other strains were nonhemolytic. The colony color could be estimated best on the charcoal-based medium. The color of *A. cryaerophilus* colonies varied from beige to yellow. *A. nitrofigilis* colonies were white, *A. butzleri* colonies were whitish to beige, and *A. skirrowii* colonies were greyish. The results of the phenotypic tests are summarized in Table 4.

DISCUSSION

Differentiation between *Arcobacter* species. In this study, the taxonomic relationships of 83 *Arcobacter* strains were determined by using a polyphasic approach that included DNA-rRNA hybridization experiments, DNA-DNA hybridization experiments, determinations of DNA base compositions, SDS-PAGE of whole-cell proteins, an analysis of fatty acid compositions, and a phenotypic analysis consisting of approximately 30 classical phenotypic tests.

TABLE 3. Average fatty acid compositions of *Arcobacter* strains grouped according to electrophoretic cluster

Taxon	Electrophoretic cluster	% of:									
		12:0	14:1 *cis* 7	12:0 3OH	14:0	ECL 15.276[a]	14:0 3OH	16:1 *cis* 9	16:1 *trans* 9	16:0	18:1 *cis* 11
A. cryaerophilus subgroup 1	I	3.7 ± 2.8	9.2 ± 4.2	—[b]	1.8 ± 0.8	—	9.4 ± 1.5	45.2 ± 5.0	—	18.7 ± 2.5	10.6 ± 3.5
A. cryaerophilus subgroup 2	II	7.7 ± 2.6	9.2 ± 4.3	—	1.7 ± 0.8	—	14.3 ± 5.4	19.0 ± 5.2	18.8 ± 4.8	17.8 ± 5.2	10.8 ± 3.0
A. skirrowii	III	9.1 ± 2.1	1.4 ± 1.6	—	2.4 ± 1.3	2.5 ± 0.6	9.2 ± 1.3	22.8 ± 3.3	7.8 ± 3.6	22.2 ± 3.0	19.7 ± 4.1
A. butzleri	IV	6.8 ± 1.9	4.9 ± 2.2	—	3.2 ± 0.4	—	17.4 ± 4.8	19.2 ± 4.6	13.8 ± 6.9	19.6 ± 3.6	11.6 ± 2.8
A. nitrofigilis	V	7.2 ± 1.2	—	5.5 ± 0.6	4.8 ± 0.8	1.1 ± 0.1	5.3 ± 1.0	30.9 ± 1.3	—	32.0 ± 0.1	12.8 ± 1.4

[a] ECL, equivalent chain length.
[b] —, Absent or present in trace amounts (less than 1%).

TABLE 4. Physiological, biochemical, and nutritional characteristics of *Arcobacter* species

Characteristic[a]	*A. cryaerophilus* subgroup 1 (11 strains)		*A. cryaerophilus* subgroup 2 (40 strains)		*A. butzleri* (12 strains)		*A. skirrowii* (18 strains)		*A. nitrofigilis* (2 strains)	
	% of strains positive	Reaction of strain LMG 7536^T	% of strains positive	Reaction of strain LMG 10829	% of strains positive	Reaction of strain LMG 10828^T	% of strains positive	Reaction of strain LMG 6621^T	% of strains positive	Reaction of strain LMG 7604^T
Catalase activity	100	+	100	+	100 (W)[b]	W	100	+	100	+
Nitrate reduction	36	−	30	−	100	+	100	+	100	+
DNase activity	64	+	72	+	92	+	100	+	100	+
Growth on VB medium (37°C)	55	+	93	+	100	+	100	+	50	−
Growth on MacConkey agar	27	+	43	−	100	+	0	−	50	−
Growth at 37°C	55	+	95	+	100	+	100	+	50	−
Growth at 42°C	18	−	0	−	67	+	33	−	0	−
Production of H_2S from cysteine	0	−	0	−	25	−	0	−	50	−
Growth in the presence of:										
1% Glycine	9	−	23	−	58	+	78	−	0	−
1% Oxgall	27	−	35	−	42	+	0	−	0	−
1.5% NaCl	9	−	0	−	83	+	6	−	100	+
3.5% NaCl	0	−	5	−	42	+	61	−	50	+
8% Glucose	45	−	48	−	100	+	50	−	100	+
0.04% TTC	18	−	20	+	67	+	6	−	0	−
Susceptibility to:										
Nalidixic acid	100	+	97	+	92	+	100	+	100	+
Cephalothin	27	−	28	−	17	−	78	+	50	+

[a] All strains were positive for the following characteristics: gram-negative curved rods; actively motile; microaerophilic growth (with and without hydrogen) at 25 and 30°C; aerobic growth at 30°C; anaerobic growth at 35 to 37°C; hydrolysis of indoxyl acetate; and oxidase activity. All strains were negative for the following characteristics: hydrolysis of hippurate; urease activity; hydrogen sulfide production in the rapid test or in triple sugar iron agar; reduction of nitrite; and fermentation or oxidation of D-glucose.

[b] W, weak reaction (oxygen was produced more than 10 s after H_2O_2 was added).

All of the strains were first grouped by using SDS-PAGE of whole-cell proteins. We identified four major electrophoretic clusters, which grouped with the reference strains of *A. nitrofigilis* (cluster V), *A. cryaerophilus* hybridization group 1A (cluster I) (12), *A. cryaerophilus* hybridization group 1B (cluster II) (12), and *A. butzleri* (cluster IV) (Fig. 1). A fifth cluster (cluster III) contained no reference strains. The results of DNA-DNA hybridization experiments showed that (i) despite their rather different protein profiles, all cluster I and II strains belong to *A. cryaerophilus*, (ii) all cluster IV strains belong to *A. butzleri*, and (iii) all cluster III strains belong to a new species, for which the name *Arcobacter skirrowii* is proposed below. Representative strains of all species were selected to determine their DNA base compositions. We did not observe significant differences between the *Arcobacter* species as all strains had G+C contents between 27 and 30 mol%.

The phylogenetic affiliations of representative strains of all five electrophoretic clusters were determined by using DNA-rRNA hybridization experiments. From the results of previous studies it was clear that the genus *Arcobacter* is closely related to the genus *Campylobacter* (32), and therefore, both genera were placed in the family *Campylobacteraceae* (30). *A. nitrofigilis* was shown to constitute a separate rRNA branch within the *Arcobacter* rRNA homology group (Fig. 5) (32). In a previous study, we included three atypical *A. cryaerophilus* strains (32). Strain LMG 6620 (= CCUG 10373) occupied a separate position on the $T_{m(e)}$ dendrogram; in this study, this strain was identified as an *A. butzleri* strain (Fig. 1, 2, and 4). Other representatives of this species, including the type strain, had similar $T_{m(e)}$ values versus radioactively labeled *Arcobacter* rRNAs (Table 2) and thus occupied similar positions on the $T_{m(e)}$ dendrogram (Fig. 5). These DNA-rRNA hybridization results support and extend the DNA-DNA hybridization results of Kiehlbauch et al. (12). They indicate that the organism that was formerly called "*C. butzleri*" is indeed a true member of the genus *Arcobacter*; it is transferred to this genus, renamed *A. butzleri* comb. nov., and described below.

The two remaining *A. cryaerophilus*-like strains that were included in our previous study (strains LMG 6621^T [= CCUG 10374^T] and LMG 8538 [= CCUG 10375]) (32) were identified in this study as *A. skirrowii* strains. The *A. skirrowii* strains had similar $T_{m(e)}$ values versus both *Arcobacter* rRNAs (Table 2 and Fig. 5). *A. cryaerophilus* subgroup 1 and 2 strains (electrophoretic clusters I and II, respectively) had similar $T_{m(e)}$ values versus rRNAs from *A. nitrofigilis* and *A. butzleri* (Table 2 and Fig. 5).

The fatty acid compositions of all *Arcobacter* strains were determined to evaluate the usefulness of this characteristic for differentiation and identification of *Arcobacter* taxa. The two electrophoretic subgroups of *A. cryaerophilus* could be differentiated easily from each other by the amounts of the two isomers of 16:1 (Table 3). *A. cryaerophilus* subgroup 1 strains (electrophoretic cluster I) had no 16:1 *trans* 9 and a very high percentage of 16:1 *cis* 9 (±45%), which allowed clear-cut differentiation from all other arcobacters. *A. cryaerophilus* subgroup 2 strains (electrophoretic cluster II) had equal amounts of both 16:1 isomers (±19%). The overall fatty acid compositions of the *A. cryaerophilus* subgroup 2 strains were very similar to those of the *A. butzleri* strains (Table 3). The *A. skirrowii* strains could be differentiated easily from most other taxa by the presence of an unknown fatty acid with an equivalent chain length of 15.276 and by a high percentage of 18:1 *cis* 11. Additional differentiating fatty acids are shown in Table 3. The *A. nitrofigilis* strains had an overall fatty acid profile that was very different from the profiles of the other arcobacters. Unexpectedly, one *A. skirrowii* strain (strain LMG 11073) had a fatty acid composition which corresponded to the composition of the *A. butzleri* and *A. cryaerophilus* subgroup 2 strains.

When the classical phenotypic tests were used, *A. nitrofigilis* could be differentiated from the other arcobacters by its nitrogenase activity (19) and by its typical colony morphology. *A. nitrofigilis* colonies are whitish and round, while the colonies of other arcobacters are beige to yellow and have a more irregular watery morphology. Furthermore, *A. nitrofigilis* could also be differentiated from *A. cryaerophilus* and *A. skirrowii* by its ability to grow in the presence of 1.5% NaCl and from *A. butzleri* by its strong catalase reaction. *A. skirrowii* colonies are greyish and alpha-hemolytic, a characteristic that occurs in only a few *A. butzleri* strains and is absent in all other arcobacters. *A. skirrowii* could be differentiated from *A. cryaerophilus* by its ability to grow in the presence of 1% glycine (14 of 18 strains versus 10 of 51 strains) and its susceptibility to cephalothin (30-μg discs) (14 of 18 strains versus 14 of 51 strains). *A. skirrowii* differs from *A. butzleri* by its inability to grow on MacConkey agar and by the fact that most *A. skirrowii* strains do not grow in the presence of 1.5% NaCl (1 of 18 strains versus 10 of 12 strains) and are susceptible to cephalothin (30-μg discs) (14 of 18 strains versus 2 of 12 strains). *A. butzleri* differs from *A. cryaerophilus* by its ability to grow in the presence of 1.5% NaCl (10 of 12 strains versus 1 of 51 strains) and from all other arcobacters by its weak catalase activity. A total of 68% of all *A. cryaerophilus* strains do not reduce nitrate, in contrast with all other arcobacters. Additional characteristics that are helpful for differentiating *Arcobacter* species by means of classical phenotypic tests are shown in Table 4. A number of discrepancies between our results and those of Kiehlbauch et al. (12) were found. In general, we found higher percentages of growth for *A. cryaerophilus* strains than Kiehlbauch et al. found (e.g., growth in the presence of 1% glycine, growth in the presence of 0.04% TTC, growth at 42°C). This might be explained by the higher number of strains investigated in our study (51 *A. cryaerophilus* strains versus 14 strains). Surprisingly, only 16 of 51 *A. cryaerophilus* strains reduced nitrate to nitrite.

We found that differentiating among *Arcobacter* species by using classical phenotypic tests is rather difficult. Using phenotypic criteria might give erroneous results because of a shortage of clear-cut differentiating tests, a phenomenon which has also been observed in the closely related genus *Campylobacter* (30).

Descriptions of new taxa. Within *A. cryaerophilus*, we identified two subgroups of strains. We believe that our subgroups correspond to the two subgroups of Kiehlbauch et al. (12). Indeed, six *A. cryaerophilus* strains were included in both studies; four of our subgroup 1 strains (strains LMG 6622, LMG 7536^T, LMG 9065, and LMG 9066) belonged to the first genotypic and phenotypic subgroup of Kiehlbauch et al. (12), and two of our subgroup 2 strains (strains LMG 9909, and LMG 10829) belonged to the second genotypic and phenotypic subgroup of these authors. Strain LMG 10829 is the reference strain of Kiehlbauch et al. (12). The two subgroups could not be differentiated in the DNA-DNA and DNA-rRNA hybridization studies. Subgroup 1 strains (electrophoretic cluster I) and subgroup 2 strains (electrophoretic cluster II) have similar DNA base compositions, occupy similar positions on the $T_{m(e)}$ dendrogram, and exhibit 46 to 69% DNA binding, while within the subgroups DNA binding values of 56 to 100% were found. The two subgroups differ in

their protein profiles (Fig. 1 and 2), their fatty acids compositions (Table 3), and their DNA restriction fragment length patterns (13). Therefore, although these two subgroups may deserve separate taxonomic status, we do not propose subspecies or infrasubspecific names because so far there are no descriptive phenotypic features to differentiate the two subgroups. The reference strain of Kiehlbauch et al. (12), strain LMG 10829 (= CDC D2610 = ATCC 49615), was also a typical representative of its group in the SDS-PAGE study and in the fatty acid analysis. The description of *A. cryaerophilus* is the description of *C. cryaerophila* given by Kiehlbauch et al. (12), with the alterations described below. All strains produce catalase. Growth occurs at 35 to 37°C under anaerobic conditions. Variable growth occurs at 37 and 42°C under microaerobic conditions. Variable growth occurs on MacConkey agar and on VB medium. Poor growth occurs in the presence of 1% glycine. Reduction of nitrates is variable. Growth in the presence of 1% oxgall is variable. Susceptibility to cephalothin (30-μg discs) is variable.

Emended description of the genus *Arcobacter*. *Arcobacter* (Ar'co.bac.ter.L.n.*arcus*, bow; Gr.n.*bacter*, rod; M. L. masc. n. *Arcobacter*, bow-shaped rod) cells are gram-negative nonsporeforming rods (0.2 to 0.9 μm wide and 1 to 3 μm long) that are usually curved, S-shaped, or helical. Motile with a darting, corkscrewlike movement by means of a single polar, unsheathed flagellum. Growth occurs at 15, 25, and 30°C; variable growth occurs at 37 and 42°C. Optimal growth occurs under microaerobic conditions (3 to 10% O_2). There is no hydrogen requirement for microaerobic growth. Aerobic growth occurs at 30°C. Anaerobic growth occurs at 35 to 37°C. Carbohydrates are neither fermented nor oxidized. Organic acids and amino acids are utilized as carbon sources. Growth occurs in the presence of 1% (wt/vol) pteridine vibriostatic compound 0/129. Growth is inhibited in the presence of 0.1% TTC. A TTC concentration of 0.04% is inhibitory to some strains. Reduction of nitrate, hydrolysis of DNA, and growth in the presence of 1% glycine, 1% oxgall, 1.5 and 3.5% NaCl, and 8% glucose are variable. The strains have oxidase and catalase activities. Negative reactions in methyl red and Voges-Proskauer tests, no reduction of nitrite, no urease activity, no hydrolysis of hippurate, and no production of indole. No production of hydrogen sulfide from triple sugar iron agar or in the rapid H_2S test; variable production of hydrogen sulfide from cysteine. Esculin and starch are not hydrolyzed, and gelatin is not liquified. Most strains are nonhemolytic; alpha-hemolysis is often observed with *A. skirrowii* strains. Nearly all strains are susceptible to nalidixic acid; susceptibility to cephalothin (30-μg discs) is variable. For all species examined so far, menaquinone 6 and a second atypical menaquinone 6, the identity of which remains to be established, are the major respiratory quinones. All strains have 12:0, 14:0 3OH, 16:1 *cis* 9, 16:0, and 18:1 *cis* 11 as major fatty acids.

Strains have been isolated from root-associated sediments and roots of salt marsh plants, from aborted fetuses of several species of farm animals, and from various other animal and human sources. The pathogenicity of these organisms is unknown.

The type species is *Arcobacter nitrofigilis*. The DNA base composition ranges from 27 to 30 mol%.

Description of *Arcobacter skirrowii* sp. nov. *Arcobacter skirrowii* (ski.ro'wi.i.) N. L. gen. n. *skirrowii*, of Skirrow, in honor of M. B. Skirrow, a British microbiologist who was the first to describe a simple isolation technique for *Campylobacter jejuni* obtained from stool specimens, which enabled most routine laboratories to culture the organisms. Cells are 1 to 3 μm long and 0.2 to 0.4 μm wide. After 3 days of incubation on blood agar, colonies are 2 to 3 mm in diameter and greyish and have a flat irregular shape on moist media. Most characteristics are as given above for the genus. Additional characteristics are given below. Most strains produce alpha-hemolysis on blood agar. Growth occurs on VB medium; no growth occurs on MacConkey agar. No growth occurs in the presence of 1% oxgall. No production of hydrogen sulfide from cysteine. Most strains (14 of 18 strains tested) grow in the presence of 1% glycine. Most strains (17 of 18 strains tested) do not grow in the presence of 0.04% TTC and 1.5% NaCl. All strains are susceptible to nalidixic acid (30-μg discs), and most strains (14 of 18 strains tested) are susceptible to cephalothin (30-μg discs).

The DNA base composition of *A. skirrowii* is 29 to 30 mol%. The major fatty acids are 12:0, 14:0 3OH, 16:1 *cis* 9, 16:1 *trans* 9, 16:0, and 18:1 *cis* 11. Additional fatty acids that are present in smaller quantities are 14:1 *cis* 7, 14:0, and an unidentified fatty acid with an equivalent chain length of 15.276.

So far, *A. skirrowii* strains have been isolated mainly from preputial fluids of bulls; the other strains are bovine, porcine, and ovine isolates obtained from aborted fetuses and diarrheic feces. The clinical significance of this new species remains to be established.

The type strain is strain LMG 6621 (= Skirrow 449/80 = CCUG 10374), which was isolated from the feces of a lamb with diarrhea. Characteristics of the type strain are summarized in Table 4. Its G+C content is 29 mol%. All of our *A. skirrowii* strains have been deposited in the Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium.

Description of *Arcobacter butzleri* comb. nov. *Arcobacter butzleri* cells are 0.2 to 0.4 μm wide and 1 to 3 μm long. After 3 days of incubation on blood agar plates, colonies are 2 to 4 mm in diameter and predominantly round, and most are whitish. Most characteristics are as given above for the genus. Additional characteristics are described below. Weak catalase activity. All strains grow on VB medium and MacConkey agar and reduce nitrate. Growth occurs in the presence of 8% glucose. Most strains (11 of 12 strains tested) produce DNase, and most strains (10 of 12 strains tested) grow in the presence of 1.5% NaCl. Growth at 42°C is variable (8 of the 12 strains tested grow), and hydrogen sulfide production from cysteine is variable (3 of the 12 strains tested produce H_2S). The following characteristics are variable: growth in the presence of 1% oxgall (5 of the 12 strains tested are positive), growth in the presence of 3.5% NaCl (5 of the 12 strains tested are positive), growth in the presence of 1% glycine (7 of the 12 strains tested are positive), and growth in the presence of 0.04% TTC (8 of the 12 strains tested are positive). Most strains (11 of 12 strains tested) are susceptible to nalidixic acid, and most strains (10 of 12 strains tested) are resistant to cephalothin (30-μg discs).

The DNA base composition varies between 28 and 29 mol%. The major fatty acids are 12:0, 14:0 3OH, 16:1 *cis* 9, 16:1 *trans* 9, 16:0, and 18:1 *cis* 11. Additional fatty acids that are present in smaller quantities are 14:1 *cis* 7 and 14:0.

Most *A. butzleri* strains have been isolated from diarrheic feces of humans and animals, from aborted fetuses, and from human blood. Although the association with diarrheal illness in humans and animals is striking, the clinical significance of *A. butzleri* remains to be proven.

The type strain is strain LMG 10828 (= CDC D2686 = ATCC 49616), which was isolated from human feces. Characteristics of the type strain are shown in Table 4.

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