Marine Star-Shaped-Aggregate-Forming Bacteria: *Agrobacterium atlanticum* sp. nov.; *Agrobacterium meteori* sp. nov.; *Agrobacterium ferrugineum* sp. nov., nom. rev.; *Agrobacterium gelatinovorum* sp. nov., nom. rev.; and *Agrobacterium stellulatum* sp. nov., nom. rev.†

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Two new species of aerobic, gram-negative, peritrichously flagellated or nonmotile marine bacteria usually forming star-shaped aggregates were isolated from northeastern Atlantic Ocean bottom sediments. These organisms resembled eight star-shaped-aggregate-forming bacterial species from the Baltic Sea originally ascribed to the genus *Agrobacterium* but not included on the *Approved Lists of Bacterial Names* because of their questionable relationships to true agrobacteria. These two sets of star-shaped-aggregate-forming bacteria were compared by means of phenotypic data, DNA base compositions, DNA-DNA relatedness, and one-dimensional electrophoretic analysis of low-molecular-weight RNAs (5S rRNA and tRNA). According to the results of genotyping, the northeastern Atlantic Ocean isolates and three of the Baltic Sea species formed a group of closely related bacteria that could not be excluded from the genus *Agrobacterium* with certainty. Until more genotypic data are available, these five marine species are regarded as a distinct subdivision of the genus *Agrobacterium* consisting of *Agrobacterium atlanticum* sp. nov. (type strain, 1480^T = DSM 5823^T), *A. meteori* sp. nov. (type strain, 1513^T = DSM 5824^T), *A. ferrugineum* sp. nov. nom. rev. emend. (type strain, ATCC 25652^T), *A. gelatinovorum* sp. nov. nom. rev. emend. (type strain, ATCC 25655^T), and *A. stellulatum* sp. nov. nom. rev. emend. (type strain, ATCC 15215^T). “*A. aggregatum*” proved to be a later subjective synonym of *A. stellulatum*, which had priority. The remaining four Baltic Sea species, “*A. agile*,” “*A. kieliense*,” “*A. luteum*,” and “*A. sanguineum*,” could not be placed in the new subdivision of *Agrobacterium*.

The formation of star-shaped aggregates is a phenomenon frequently occurring in bacteria, especially in members of the family *Rhizobiaceae* (22). Jennings (21), presenting some photomicrographs in 1898, was the first to observe star-shaped-aggregate formation in bacteria. In 1954, Stapp and Knösel (41) studied star-shaped-aggregate formation processes in some *Agrobacterium* and *Rhizobium* strains, and Knösel (25) reported star-shaped clusters in a culture of *Phyllobacterium rubiacearum*. A marine star-shaped-aggregate-forming bacterium described by Siebert and Schwartz in 1956 (37) was regarded by Stapp and Knösel (41) as a new species, *Agrobacterium stellulatum*. Additional marine- or brackish-water-type bacteria with star-shaped-aggregate-forming abilities were isolated by Ahrens (1) and Ahrens and Rheinheimer (2) from the Baltic Sea. These organisms resembled *A. stellulatum* and were therefore described as new *Agrobacterium* species, namely, *A. aggregatum*, *A. agile*, *A. ferrugineum*, *A. gelatinovorum*, *A. kieliense*, *A. luteum*, and *A. sanguineum*. In a personal communication with O. N. Allen, Ahrens later withdrew this proposal (3). Consequently, the species names of the Baltic Sea isolates, as well as of *A. stellulatum*, were not included on the *Approved Lists of Bacterial Names* (38) and subsequent validation lists and therefore have no standing in bacterial nomenclature.

From sediments of the northwestern African upwelling area, Rüger (31) isolated 74 strains of marine, mostly star-shaped-aggregate-forming bacteria that could be regarded as belonging to two new species of the same taxon as *A. stellulatum* and the marine *Agrobacterium* species described by Ahrens (1) and Ahrens and Rheinheimer (2). From DNA-rRNA hybridization experiments, De Smedt and De Ley (9) concluded that these species are not members of the genus *Agrobacterium*, and Kersters and De Ley (23) stated that these strains belong to one of the four rRNA superfamilies established by De Ley (7) which contains the genera *Agrobacterium*, *Rhizobium*, *Acetobacter*, *Gluconobacter*, *Spirillum*, and several other genera which are considered members of subclass alpha of the new class *Proteobacteria* proposed by Stackebrandt et al. (40).

Our phenotypic and genotypic data do not definitely support the exclusion of most of the marine strains from genus *Agrobacterium*. As a pro tempore measure, therefore, we propose a distinct subdivision of the genus *Agrobacterium* to accommodate those marine star-shaped-aggregate-forming bacteria which were originally placed in the genus *Agrobacterium* as *A. aggregatum*, *A. ferrugineum*, *A. gelatinovorum*, and *A. stellulatum* and the star-shaped-aggregate-forming isolates from the northwestern African upwelling area, which are described here as the new species *A. atlanticum* and *A. meteori*. Our studies revealed that the species name *A. aggregatum* is a later subjective synonym of *A. stellulatum*, which has priority. The Baltic Sea isolates “*Agrobacterium agile*,” “*A. kieliense*,” “*A. luteum*,” and

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TABLE 1. Selected strains from 55 *A. atlanticum* and 19 *A. meteori* isolates and culture collection strains used for substrate utilization tests and genotypic studies

Source of strain ^a	Sampling	
	Location	Depth (m)
Marine sediments		
<i>A. atlanticum</i>		
1373	21°37.6'N, 18°41.5'W	2,998
1474	21°25.2'N, 18°00.2'W	1,318
1480 ^T = DSM 5823 ^T	21°24.6'N, 17°53.6'W	1,023
1501	21°22.5'N, 17°34.3'W	417
1532	21°19.5'N, 17°29.2'W	191
1546	21°18.5'N, 17°22.5'W	85
1554	21°22.7'N, 17°46.9'W	738
<i>A. meteori</i>		
1479	21°24.6'N, 17°53.6'W	1,023
1496	21°24.6'N, 17°53.6'W	1,023
1503	21°22.5'N, 17°34.3'W	417
1504	21°22.5'N, 17°34.3'W	417
1513 ^T = DSM 5824 ^T	21°22.5'N, 17°34.3'W	417
ATCC		
<i>A. ferrugineum</i> ATCC 25652 ^T		
<i>A. gelatinovorum</i> ATCC 25655 ^T		
<i>A. stellulatum</i> ATCC 15215 ^T		
<i>A. stellulatum</i> ATCC 25650 ^b		
" <i>A. agile</i> " ATCC 25651 ^T		
" <i>A. kieliense</i> " ATCC 25656 ^T		
" <i>A. luteum</i> " ATCC 25657 ^T		
" <i>A. sanguineum</i> " ATCC 25659 ^T		
DSM		
<i>A. radiobacter</i> DSM 30147 ^T		
<i>A. rhizogenes</i> DSM 30148 ^T		
<i>A. tumefaciens</i> DSM 30205 ^T		

^a ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

^b Received as *A. aggregatum*.

"*A. sanguineum*" could not be assigned to the new subdivision of genus *Agrobacterium*.

MATERIALS AND METHODS

Bacterial strains. During the 36th cruise of the German research vessel *Meteor* in 1975, sediment bacteria were isolated at near-shore stations and from up to a depth of about 3,000 m in the northwestern African upwelling region at 21°N (31) (see Table 1). The strains were taken from seawater-agar spread plates which were prepared on board the ship from serial dilutions of the samples prepared immediately after subsampling of the sediments obtained by means of a box-grab sampler or a van Veen grab. The incubation temperature was 15°C, and the incubation time varied from 8 to 17 days, depending on the date of sampling. The seawater agar of ZoBell was modified and consisted of 5 g of peptone, 1 g of yeast extract, 0.01 g of FePO₄ · 4H₂O, 15 g of Difco Bacto-Agar, 750 ml of aged seawater, and 250 ml of distilled water (42). The pH was adjusted to 7.6. The star-shaped-aggregate-forming isolates consisted of 55 *A. atlanticum* and 19 *A. meteori* strains, as identified by phenotypic characteristics. Strains of both species from different depths were selected for additional phenotypic and genotypic studies and were compared with reference strains from the American Type Culture Collection, Rockville, Md., and the German Collection of Microorganisms, Braunschweig, Germany (Table 1).

Phenotypic tests. Common identification tests were performed in seawater media at 20°C as described previously (33). Nitrate reduction to nitrite and gas was determined in mineral media prepared with artificial seawater (34). The ability to grow under anaerobic conditions was tested with the OXOID Anaerobic System. In order to absorb the excess carbon dioxide which is developed in this anaerobic system, three test tubes with 10 ml of saturated KOH solutions were placed in the anaerobic jars. Susceptibility to antibiotics was tested on seawater-agar plates with OXOID sensitivity disks, and the tests for DNase activity were performed with OXOID DNase-agar.

The utilization of organic substrates as sole sources of carbon and energy was studied in microtiter plates by means of a multipoint inoculator; three wells were used for each strain. Turbidity was taken as an indicator for substrate utilization and measured with a microtiter plate photometer after 2, 4, 6, and 8 weeks of incubation at 20°C (32).

Isolation of DNA. DNA was isolated from cells grown in seawater broth containing the same concentrations of peptone, yeast extract, and FePO₄ · 4H₂O as the seawater agar and purified according to the method of Marmur (28). Because of copious slime production by the cells, the lysis procedure had to be modified. Depending on the strain, several milliliters of sodium lauryl sulfate solution (25%) was applied for about 1 h at 65°C. Then the cell suspensions were cooled to 25 to 30°C and subsequently incubated for an additional 1 to 3 h with 0.8 mg of lysozyme (Merck 5281) and 0.15 mg of proteinase K (Merck 24568) per ml. For strains 1373 and ATCC 25655^T, 0.8 mg of lysozyme, 0.8 mg of protease (Sigma P 6911), 0.4 mg of α-amylase (Merck 1329), and 0.5 mg of β-glucosidase (Sigma G 0395) were added per ml, and for strain 1474, 0.8 mg of lysozyme and 0.5 mg of β-glucosidase were added per ml.

DNA base composition. The G+C content of the DNA was determined from the midpoint value of the thermal denaturation profile (29) obtained with a Gilford model 250 spectrophotometer equipped with a Gilford model 2527 thermoprogrammer. The G+C content of the DNA was calculated from the melting temperature by using the equation of De Ley (6). DNA from *Escherichia coli* K-12, which has a melting temperature of 90.6°C in standard saline citrate (SSC) buffer (6), was used as a reference.

DNA-DNA hybridization. DNA hybridization was determined from renaturation rates according to the methods and conditions reported by De Ley et al. (8) and Gillis et al. (10). The DNA was isolated and purified as described above and then dialyzed against 2 liters of double-strength SSC for 3 days at 4°C. The buffer was replaced by fresh buffer solutions after 24 and again after 48 h. After dialysis, the DNA solutions (A₂₆₀, approximately 2.0) were passed three times through a French pressure cell at 11 × 10⁷ Pa. The DNA concentrations of every two samples to be hybridized were adjusted to an A₂₆₀ of 1.3 in 2× SSC, which corresponds to 65 µg of DNA per ml. The renaturation experiments were performed in a Gilford model 250 spectrophotometer equipped with a Gilford model 2527 thermoprogrammer. For the measurements, a Gilford thermocuvette containing the two DNA samples, a 1:1 mixture of both DNA solutions, and 2× SSC as a blank were used.

The DNA samples were denatured by raising the temperature to 105°C for 20 min. For renaturation, the temperature was then rapidly adjusted to 65°C. The decrease in absorbance, which reached linearity after a few minutes, was recorded for 40 min. Renaturation rates were determined from the renaturation curves. The degrees of DNA-DNA

binding were calculated from the renaturation rates with equation 20 of De Ley et al. (8). Two to five renaturation assays were performed for each combination of strains.

LMW-RNA profiles. This genotyping technique uses the stable low-molecular-weight (LMW) RNAs (5S rRNA and tRNA) of bacteria for taxonomic grouping of the organisms (16–18). The cells were grown on seawater-agar plates. Biomass for extraction of total RNA was harvested from single agar plates by scraping off all colonies with a soft rubber spatula and dissolving the material in 1 ml of buffer (50 mM sodium acetate, 10 mM EDTA, pH 5.1). The biomass was collected in microcentrifuge tubes by centrifugation, and the pellet was stored at -20°C before RNA extraction. Total RNA was extracted by hot phenol and subsequently precipitated with ethanol. This crude total RNA was purified and enriched for the LMW fraction by using Qiagen 100 pack according to the manufacturer's protocol (Diagen Corp., Düsseldorf, Germany). Purified RNA (2 to 3 μg) was subjected to high-power gel electrophoresis under denaturing conditions (11). The specific gel used consisted of 10% acrylamide with 7 M urea and a running buffer of 100 mM Tris, 83 mM boric acid, and 1 mM EDTA at pH 8.5. Running conditions were 3 h at 2,400 V and 60°C . After electrophoresis, RNA bands were visualized by silver staining according to Kolodny (26). More-technical information on the LMW-RNA profile method was given previously (16).

RESULTS

The 74 marine star-shaped-aggregate-forming bacteria from sediments of the northwestern African upwelling area consisted of 55 *A. atlanticum* and 19 *A. meteori* strains. Seven *A. atlanticum* and five *A. meteori* strains from different depths were selected for further taxonomic examination and compared with star-shaped-aggregate-forming reference strains originally isolated from the Baltic Sea (1, 2) (obtained from culture collections) and with *A. stellulatum* (Table 1).

Phenotyping. (i) Morphology. The cells of *A. stellulatum* and of the isolates from the Baltic Sea and the northwestern African upwelling region were gram-negative, regular, club-shaped or dumb-bell-shaped rods or coccobacilli and sometimes straight or curved filaments. They occurred singly, in pairs and short chains, or in irregular clusters or starlike aggregates which developed predominantly at the end of the logarithmic growth phase in seawater broth. However, star-shaped-aggregate formation cannot always be observed; it seems to be dependent on the physiological state of the cells. Figure 1 shows star-shaped-aggregation formation in *A. atlanticum* 1480^T (Fig. 1a) and *A. meteori* 1513^T (Fig. 1b) after incubation in seawater broth for 4 days at 20°C .

(ii) Physiological and biochemical tests. The physiological and biochemical characteristics of all strains are presented in Table 2. Some strains were able to grow under reduced oxygen tensions and are therefore characterized as weakly positive in the test for anaerobic growth. Most of the northeastern Atlantic Ocean isolates required seawater media for growth; only 13 *A. atlanticum* and 10 *A. meteori* strains showed slight but significantly reduced growth in media prepared without seawater. The brackish-water-type Baltic Sea isolates were usually capable of weak growth in freshwater media, but their growth was stimulated in seawater broth. In contrast, terrestrial agrobacteria do not require seawater media, and some strains are even inhibited by sea salts (unpublished data).

FIG. 1. Star-shaped-aggregate formation by *A. atlanticum* 1480^T (a) and *A. meteori* 1513^T (b) after incubation in seawater broth for 4 days at 20°C . Bars = 10 μm (interference-contrast microscopy).

In contrast to terrestrial agrobacteria, the marine strains showed negative results in tests for aerobic acid production from carbohydrates when incubated as usual for a few days up to about 2 weeks. The marine strains may show positive or weakly positive results only after prolonged incubation and, in spite of good growth, not earlier than 4 to 6 weeks (Table 2). Ahrens and Rheinheimer (2) observed acid production from carbohydrates by their strains only after 8 to 10 weeks of incubation.

For the utilization of carbohydrates, carboxylic acids, alcohols, amino acids, and putrescine by the marine strains listed in Table 1, the test results obtained after 4 weeks of incubation at 20°C were used (Table 3). However, 86% of the positive reactions were already detected after 2 weeks. A few positive results occurring after 6 or 8 weeks of incubation were regarded as negative in Table 3. Strain *A. ferrugineum* ATCC 25652^T gave negative results in all substrate utilization tests at 20°C but was able to utilize propionate, fumarate, succinate, lactate, glycerol, and glutamate at 4°C within 4 to 6 weeks, thus showing behavior similar to that of some deep-sea bacteria described previously (32).

Genotyping. (i) DNA base compositions. The DNA base ratios of the northeastern Atlantic *Agrobacterium* strains and most of the Baltic Sea species were in the range of 54 to

TABLE 2. Phenotypic characteristics of marine *Agrobacterium* species and of some marine star-shaped-aggregate-forming bacteria of uncertain taxonomic position

Characteristic ^a	No. of strains with positive or slightly positive test results ^b											
	<i>A. atlanticum</i> (n = 55) ^c	<i>A. atlanticum</i> 1480 ^T (= DSM 5823 ^T)	<i>A. meteori</i> (n = 19) ^c	<i>A. meteori</i> 1513 ^T (= DSM 5824 ^T)	<i>A. ferrugineum</i> ATCC 25652 ^T	<i>A. gelatinovorum</i> ATCC 25655 ^T	<i>A. stellulatum</i> ATCC 15215 ^T	<i>A. stellulatum</i> ATCC 25650	" <i>A. agile</i> " ATCC 25651 ^T	" <i>A. kieliense</i> " ATCC 25656 ^T	" <i>A. luteum</i> " ATCC 25657 ^T	" <i>A. sanguineum</i> " ATCC 25659 ^T
Colony pigmentation												
Colorless, beige, yellowish	55	+	17	+	-	+	+	+	+	+	-	-
Brown, light brown, brown center	0	-	2	-	+	+	-	+	(+)	-	-	-
Yellow	0	-	0	-	-	-	-	-	-	-	+	-
Red	0	-	0	-	-	-	-	-	-	-	-	+
Motility	3	-	10	+	+	+	+	+	+	+	+	+
Facultatively anaerobic	0	-	1	-	-	-	(+)	(+)	(+)	-	-	-
Growth without seawater base	13	-	10	(+)	+	-	(+) ^d	(+)	(+)	(+)	-	(+)
Growth at 5°C, 7 days	28	-	13	(+)	+	(+)	(+)	(+)	ND	+	-	-
Growth at 37°C, 7 days	7	-	6	-	-	-	+	+	ND	-	-	(+)
Catalase	54	+	12	-	+	+	+	+	+	+	+	+
H ₂ S production from cysteine	0	-	2	-	-	-	ND	-	-	+	-	-
Susceptible to:												
Penicillin G, 2U	46	-	14	+	ND	ND	ND	ND	ND	ND	ND	ND
Oxytetracycline, 5 µg	55	+	16	+	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis of:												
Gelatin	0	-	2	-	-	+	-	-	-	-	-	-
Starch	0	-	1	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	7	-	6	-	ND	ND	ND	ND	ND	ND	ND	ND
Urease	0	-	8	-	-	ND	ND	ND	ND	ND	ND	ND
Lipase (tributyrin)	14	-	11	+	ND	ND	ND	ND	ND	ND	ND	ND
Nitrate reduction to nitrite	51	+	14	+	-	+	-	-	+	-	-	-
Nitrate reduction to gas	55	+	0	-	-	-	+	+	+	-	-	-
Aerobic acid production after 4-6 wk (not earlier) from:												
Glucose	52	+	10	-	+	-	-	-	-	-	-	-
Arabinose	46	+	2	-	ND	ND	ND	ND	ND	ND	ND	ND
Fructose	7	-	5	-	+	-	ND	(+)	-	(+)	-	-
Glycerol	29	+	5	-	+	+	ND	(+)	(+)	+	-	-
Lactose	1	-	1	-	ND	ND	ND	ND	ND	ND	ND	ND
Maltose	11	+	7	-	+	-	-	+	-	(+)	-	-
Mannitol	0	-	2	-	ND	ND	- ^d	ND	ND	ND	ND	ND
Sucrose	12	+	7	-	ND	ND	ND	ND	ND	ND	ND	ND
Xylose	32	+	3	-	+	-	ND	-	-	+	-	-

^a The cells of all strains were gram-negative, regular, club-shaped or dumb-bell-shaped rods or coccobacilli 0.6 to 1.2 µm by 1.0 to 4.0 µm. Starlike aggregates were common but not always present. Motile strains were peritrichous or degenerately peritrichous, with 1 to 8 flagella; only "*A. agile*" ATCC 25651^T was polarly flagellated, with 1 to 3 flagella. Optimum growth temperatures were between 20 and 30°C. Oxidase positive. All strains were negative in the following tests: indole, methyl red, Voges-Proskauer, susceptibility to 10 µg of pteridine O/129, lysine and ornithine decarboxylase, and hydrolysis of casein, chitin, and alginate. Forty-five *A. atlanticum* and 18 *A. meteori* strains were tested for susceptibility to 50 µg of furazolidone and 10 µg of chloramphenicol (all strains positive) and for DNase activity (all but 2 strains negative). The following characteristics of strains ATCC 15215^T, ATCC 25650, ATCC 25651^T, ATCC 25652^T, ATCC 25655^T, ATCC 25656^T, ATCC 25657^T, and ATCC 25659^T were taken from Ahrens (1) and Ahrens and Rheinheimer (2): growth without seawater base, H₂S production from cysteine, gelatin hydrolysis, and acid production from glucose, fructose, glycerol, maltose, and xylose. For these reference strains, no results were reported for arginine dihydrolase, lysine and ornithine decarboxylase, and susceptibility to pteridine O/129.

^b Symbols: +, positive; (+), weakly positive; -, negative; ND, not tested or not reported.

^c Number of strains tested.

^d According to Stapp and Knösel (41).

59 mol% G+C. Quite different guanine-plus-cytosine ratios of 63 and 48 mol% were found for "*A. sanguineum*" and "*A. kieliense*," respectively (Table 4), indicating that these species are not closely related to the other strains.

(ii) **DNA-DNA relatedness.** The *A. atlanticum* strains are grouped together by their DNA-DNA homology values of 56 to 88% (Table 5). *A. meteori* 1513^T is distinct from all the other strains and represents a separate species. *A. stellulatum* ATCC 15215^T and *A. stellulatum* ATCC 25650 (received as *A. aggregatum*) have 106% DNA homology and are therefore identical at the species level. *A. gelatinovorum*

ATCC 25655^T showed DNA-DNA homology values of 37 and 42% with *A. atlanticum* strains, indicating closer relationships than were usually found between the marine *Agrobacterium* species (Table 5).

(iii) **LMW-RNA profiles.** High-resolution electrophoretic analysis of LMW RNAs (5S rRNA and tRNA) for genotypic identification of eubacteria has recently been developed (16-18). By using reference strains from culture collections, it was shown that bacterial strains with identical LMW-RNA band patterns are of the same genotype at the species level. Furthermore, it was possible to find genus-specific LMW-

TABLE 3. Utilization of organic substrates as sole carbon and energy sources by selected marine *Agrobacterium* species and some marine star-shaped-aggregate-forming bacteria of uncertain taxonomic position

Characteristic ^a	<i>A. atlanticum</i>			<i>A. meteori</i>										
	Species (<i>n</i> = 7) ^b	Strains with deviant results	Strain 1480 ^T (=DSM 5823 ^T)	Species (<i>n</i> = 5) ^b	Strains with deviant results	Strain 1513 ^T (=DSM 5824 ^T)	<i>A. ferrugineum</i> ATCC 25652 ^T	<i>A. gelatinovorum</i> ATCC 25655 ^T	<i>A. stellulatum</i> ATCC 15215 ^T	<i>A. stellulatum</i> ATCC 25650	" <i>A. agile</i> " ATCC 25651 ^T	" <i>A. kieliense</i> " ATCC 25656 ^T	" <i>A. luteum</i> " ATCC 25657 ^T	" <i>A. sanguineum</i> " ATCC 25659 ^T
Carbohydrate utilization														
L-Arabinose	—		—	—		—	—	—	+	+	+	—	—	—
Cellobiose	+	1554	+	—		—	—	—	+	+	+	—	—	+
D-Galactose	+	1554	+	—		—	—	—	+	+	+	—	—	—
Gluconate	+	1373, 1474, 1554	+	—		—	—	—	+	+	+	—	—	—
D-Glucose	+	1554	+	—		—	—	—	+	+	+	—	—	+
Maltose	+	1554	+	—		—	—	—	+	+	+	—	—	—
D-Mannose	+	1554	+	—		—	—	—	+	+	+	—	—	—
D-Ribose	—	1474, 1532, 1546	—	—		—	—	—	+	+	+	—	—	—
Sucrose	+	1554	+	—		—	—	—	+	+	+	—	—	+
Salicin	—		—	—		—	—	—	+	+	+	—	—	—
Trehalose	+	1554	+	—		—	—	—	+	+	+	—	—	—
D-Xylose	+	1373, 1554	+	—		—	—	—	+	+	+	—	—	—
Carboxylic acid utilization														
Na acetate	+	1554	+	—		—	—	+	+	+	+	—	—	+
Na propionate	+	1554	+	—		—	—	+	+	+	+	—	+	+
Fumarate	+		+	—		—	—	+	+	+	+	—	—	+
Succinate	+		+	—		—	—	+	+	+	+	—	—	—
Citrate	+		+	—		—	—	—	+	+	+	—	—	—
DL-3-Hydroxybutyrate	+	1554	+	—		—	—	+	+	+	+	—	+	+
Lactate	+	1554	+	—	1479, 1503	—	—	+	+	+	+	—	—	—
Pyruvate	+		+	—		—	—	+	+	+	+	—	—	+
Alcohol utilization														
Glycerol	+	1373	+	—		—	—	—	+	+	+	—	—	—
Mannitol	+	1474, 1554	+	—		—	—	—	+	+	+	—	—	—
Sorbitol	—		—	—		—	—	—	+	+	+	—	—	—
Amino acid utilization														
L-Alanine	+		+	—		—	—	+	+	+	+	—	—	—
L-Arginine	+	1373, 1554	+	—		—	—	+	+	+	+	—	—	—
L-Aspartate	+	1373, 1474, 1554	+	—		—	—	—	+	+	+	—	—	—
L-Glutamate	+		+	—		—	—	—	+	+	+	—	+	+
L-Histidine	—	1373	—	—		—	—	—	+	+	+	—	—	—
L-Lysine	+	1373, 1474	+	—		—	—	+	—	—	+	—	—	—
L-Ornithine	+	1373	+	—		—	—	+	+	+	+	—	—	—
Putrescine utilization	+	1373, 1554	+	—		—	—	+	+	+	+	—	—	—

^a Incubation was for 4 weeks at 20°C. None of the strains utilized adipate, *p*-hydroxybenzoate, or quinate.^b Number of strains tested.

RNA bands if the different species were phylogenetically closely enough related (17).

All LMW-RNA profiles of the strains given in Table 1 have been studied, and representative results are shown in Fig. 2. The following groups of strains showed identical LMW-RNA profiles in every band: (i) *A. stellulatum* ATCC 15215^T and ATCC 25652^T (Fig. 2, lanes a and b, respectively); (ii) *A. atlanticum* 1480^T, 1474, 1532, 1546 (Fig. 2, lanes e through h, respectively), 1373, 1501, and 1554 (data not shown); and (iii) *A. meteori* 1513^T, 1479, 1503 (Fig. 2, lanes i through k, respectively), 1496, and 1504 (data not shown). These findings indicate that these three groups of strains are all identical at the species level. This identity was confirmed by comparing gel scans of the single strains comparable to the ones given for the individual species in Fig. 3A. The five *Agrobacterium* species *A. atlanticum*, *A. ferrugineum*, *A. gelatinovorum*, *A. meteori*, and *A. stellulatum* have individual LMW-RNA band patterns which significantly differed from each other by at least one band position (Fig. 2 and 3A). Bands of specific discriminative value between species are

labeled in Fig. 2 and 3A to show the differences between *A. atlanticum* and *A. gelatinovorum* on the one side (arrow at the 5S rRNA band at 117 nucleotides in Fig. 2 and 3A, lane d) and between *A. atlanticum* and *A. meteori* on the other side (arrow at the different class 2 tRNA band in Fig. 2 and 3A, lane i).

For the genotypic grouping of a set of strains as a taxon above the rank of species, identical LMW-RNA bands or banding patterns have to be found in all the individual species of that taxon (18). This can best be done with the help of normalized gel scans that indicate the precise position of the bands and are also able to resolve the very strong bands in the class 1 tRNA. As can be seen in Fig. 3A, there are two major bands, connected by dashed lines, in each of the tRNA classes that occur in all five species. The 5S rRNA region is characterized by two bands at 127 and 117 nucleotides (nt) except in *A. stellulatum*, which displays only the 117-nt band. The second 5S rRNA band of *A. atlanticum* and *A. meteori* has a size of 116 nt, indicating a loss of one nucleotide at one of the molecule's ends or some other

TABLE 4. DNA base compositions of marine *Agrobacterium* strains and of some other marine star-shaped-aggregate-forming bacteria

Strain ^a	Mol% G+C
<i>A. atlanticum</i>	
1373	55
1474	56
1480 ^T	55
1501	54
1532	55
1546	54
1554	57
<i>A. meteori</i> 1513 ^T	58
<i>A. ferrugineum</i> ATCC 25652 ^T	58
<i>A. gelatinovorum</i> ATCC 25655 ^T	59
<i>A. stellulatum</i> ATCC 15215 ^T	59
<i>A. stellulatum</i> ATCC 25650	59
" <i>A. agile</i> " ATCC 25651 ^T	58
" <i>A. kieliense</i> " ATCC 25656 ^T	48
" <i>A. luteum</i> " ATCC 25657 ^T	55
" <i>A. sanguineum</i> " ATCC 25659 ^T	63

^a G+C ratios as reported in the literature: *A. ferrugineum* ATCC 25652^T, 60 mol% (1); *A. gelatinovorum* ATCC 25655^T, 57.6 mol% (9); *A. stellulatum* ATCC 25650, 58.7 mol% (9); "*A. agile*" ATCC 25651^T, 57.1 (9) and 59 (1) mol%; "*A. kieliense*" ATCC 25656^T, 50.6 mol% (9); "*A. luteum*" ATCC 25657^T, 57 mol% (1); and "*A. sanguineum*" ATCC 25659^T, 64 mol% (1).

size-disturbing effects like hairpin formation. The greatest resemblance between the gel scans can be seen with *A. gelatinovorum* and *A. atlanticum*. These two species can be distinguished only by the difference in the second 5S rRNA position (arrow, Fig. 3A, lane d). The least similar to all the other of the five species is *A. stellulatum* because it lacks the first 5S rRNA band and because of the differences in the second part of the class 1 tRNA. Overall, the gel scans of the five different marine *Agrobacterium* species share enough bands and band patterns to group them into one taxon above species rank. This high degree of similarity among these five species can also be judged from all the differences seen in the gel scans of the second five species, shown in Fig. 3B. Here,

FIG. 2. LMW-RNA profiles of the investigated strains. Arrows indicate bands of specific discriminative value for different marine species. Lanes: a through k, marine *Agrobacterium* spp.; a, *A. stellulatum* ATCC 15215^T; b, *A. stellulatum* ATCC 25652^T; c, *A. ferrugineum* ATCC 25652^T; d, *A. gelatinovorum* ATCC 25655^T; e, *A. atlanticum* 1480^T; f, *A. atlanticum* 1474; g, *A. atlanticum* 1532; h, *A. atlanticum* 1546; i, *A. meteori* 1513^T; j, *A. meteori* 1479; k, *A. meteori* 1503; l, molecular weight standards (5S rRNA hydrolysate, tRNA^{Tyr}, and tRNA^{Phe} from *E. coli*); m through p, marine species incertae sedis; m, "*A. kieliense*" ATCC 25656^T; n, "*A. sanguineum*" ATCC 25659^T; o, "*A. luteum*" ATCC 25657^T; p, "*A. agile*" ATCC 25651^T; q through s, terrestrial *Agrobacterium* species; q, *A. tumefaciens* DSM 30205^T; r, *A. radiobacter* DSM 30147^T; s, *A. rhizogenes* DSM 30148^T. Abbreviations at bottoms of lanes are of species names.

TABLE 5. DNA-DNA homology between pairs of marine star-shaped-aggregate-forming bacteria

Strain	% Homology ^a												
	1480 ^T	1373	1474	1532	1546	1554	1513 ^T	ATCC 15215 ^T	ATCC 25650	ATCC 25655 ^T	ATCC 25652 ^T	ATCC 25657 ^T	ATCC 25656 ^T
<i>A. atlanticum</i>													
1480 ^T	103												
1373	72	98											
1474	71	77	ND										
1532	62	79	72	ND									
1546	67	56	88	82	ND								
1554	72	59	68	70	68	ND							
<i>A. meteori</i> 1513 ^T	27	22	29	34	24	22	100						
<i>A. stellulatum</i> ATCC 15215 ^T	32	25					24	ND					
<i>A. stellulatum</i> ATCC 25650	29	21					30	106	97				
<i>A. gelatinovorum</i> ATCC 25655 ^T	37	42					34	69	31	98			
<i>A. ferrugineum</i> ATCC 25652 ^T	28	34					25	30	24	33	100		
“ <i>A. luteum</i> ” ATCC 25657 ^T	23	21					21	20	24	33	20	101	
“ <i>A. kieliense</i> ” ATCC 25656 ^T	27	24					21	34	24	20	30	26	98

^a ND, not determined.

FIG. 3. Baseline-corrected gel scans of LMW-RNA profiles from all marine *Agrobacterium* species (A) and from *A. tumefaciens* and all other star-shaped-aggregate-forming species that do not belong to the marine *Agrobacterium* subdivision (B). Lettering of the single gel scans is as in Fig. 2 for corresponding lanes. Characteristic genus-specific peaks are connected by dashed lines. Arrows indicate bands of specific discriminative value for different species.

the 5S rRNA for the first band ranges from 128 to 131 nt, and the second band, mostly the major 5S rRNA band, is at 120 nt. The differences in the tRNA patterns are quite obvious, especially among the class 1 tRNAs, where always more bands were detected than just the three major bands detected for the first five marine *Agrobacterium* species.

DISCUSSION

Phenotypic characteristics. On the basis of phenotypic characteristics (Table 2), the marine star-shaped-aggregate-forming bacteria from the northwestern African upwelling area could be regarded as members of the same taxon as *A. stellulatum* and the isolates from the Baltic Sea described by Ahrens (1) and Ahrens and Rheinheimer (2). To reexamine the uncertain taxonomic position of the marine star-shaped-aggregate forming bacteria, additional phenotypic and geno-

typic studies were performed with selected marine isolates and with reference strains from culture collections (Table 1).

On the basis of polyamine determinations, Hamana und Matsuzaki (13) grouped the very different strains "*A. agile*" ATCC 25651^T, *A. stellulatum* ATCC 25650 (ex *A. aggregatum*), *A. gelatinovorum* ATCC 25655^T, and "*A. kieliense*" ATCC 25656^T into one type but separated *A. stellulatum* ATCC 15215^T from the nearly identical strain *A. stellulatum* ATCC 25650. Thus, grouping of the marine star-shaped-aggregate-forming bacteria into different types by means of their polyamine contents cannot be supported by genotypic data (Tables 4 and 5 and Fig. 2 and 3).

Except for the number and arrangement of flagella, "*A. agile*" is phenotypically identical to *A. stellulatum* (Tables 2 and 3) but genotypically distinct from it (Fig. 2 and 3B) and probably belongs in the vicinity of the genus *Pseudomonas* (9). This example shows again that phenotypic characteris-

tics alone are not sufficient for the separation of bacterial taxa. Different genotyping methods were therefore applied for further taxonomic examination of the marine star-shaped-aggregate-forming bacteria.

Genotyping. (i) **DNA base compositions.** The DNA base compositions shown in Table 4 indicate that "*A. sanguineum*" and "*A. kieliense*" are not closely related to the other marine star-shaped-aggregate-forming strains. In general, DNA base analysis results for the reference strains are in good accord with data in the literature, which are also listed in Table 4.

(ii) **DNA-DNA relatedness.** With an average of 71%, the DNA homology values between pairs of *A. atlanticum* strains are in the range defining a species, although strain 1373 showed only 56 or 59% reassociation with two of the other strains (Table 5). In the literature, the limit set for species varies between 60 and 80% homology (5, 12, 36). The relatively low homology values between some *A. atlanticum* isolates are possibly due to interactions between extracellular slime produced by the strains and DNA (15). Homology values greater than 100%, as found between *A. stellulatum* ATCC 15215^T and *A. stellulatum* ATCC 25650, should theoretically not exist but are in the range of experimental error associated with the photometrical or every other DNA-pairing method (8, 12, 15, 19). Because of the high background of about 30% in the optical method (12, 19, 27), DNA-DNA homology values below 30% in Table 5 should be interpreted as unspecific hybridization. The two *A. stellulatum* strains are identical at species level, but they showed different homology values of 69 and 31% with *A. gelatinovorum* ATCC 25655^T (Table 5). Although two identical strains should have the same percentage values when both are compared with a third strain (14), there are some other examples in the literature when this is not true (14, 20).

(iii) **LMW-RNA profiles.** High-resolution electrophoretic analysis of LMW RNAs (5S rRNA and tRNA) is useful for the genotypic identification of eubacteria at the species and genus levels (17, 18). Recently, the utility of LMW-RNA profiles was also demonstrated for the taxonomic differentiation of an extended set of gram-positive genera, including *Listeria*, *Lactobacillus*, *Clostridium*, and *Streptococcus* (39). The LMW-RNA profiles presented in Fig. 2 and 3A indicate that the five marine *Agrobacterium* species have enough similarities to be grouped as a separate taxon above species rank (see dashed lines in Fig. 3A). On the other hand, there are specific differences in at least one band position among the five species (see arrows in Fig. 2 and 3A), while strains of the same species show no differences at all (compare lanes e through h with lanes i through k in Fig. 2). The formation of a separate taxon for the five strains of marine agrobacteria is further supported by their major differences from all other marine star-shaped-aggregate-forming species, the true agrobacteria (compare lanes a through k with lanes m through s in Fig. 2 and Fig. 3A with 3B), and other phenotypically related genera like *Alcaligenes* and *Deleya* (17). Furthermore, "*A. agile*," "*A. kieliense*," "*A. luteum*," and "*A. sanguineum*" have LMW-RNA profiles so different from those of the five other marine star-shaped-aggregate formers and of "true" agrobacteria (Fig. 2 and 3) that they cannot be included in either of these taxonomic groups.

The DNA-DNA homology data (Table 5) are consistent with the LMW-RNA profile results at the species level. All *A. atlanticum* strains have homology values of >56%, with an average of 71% $\pm$ 8% (standard deviation), clearly indicating the formation of a species; the high DNA-DNA

homology between the two *A. stellulatum* strains also provides evidence for identity at the species level. At the genus level, the close relationship of *A. gelatinovorum* with *A. atlanticum* (Fig. 2 and 3A) is supported by DNA-DNA homologies of 37 and 42% (Table 5).

Taxonomy. The marine star-shaped-aggregate-forming bacteria isolated from the Baltic Sea by Ahrens (1) and Ahrens and Rheinheimer (2) resembled *A. stellulatum* and were therefore described as new *Agrobacterium* species. Because of the questionable relationships of these isolates to terrestrial agrobacteria, Ahrens withdrew this proposal (3). The species names of the marine isolates were therefore not included on the *Approved Lists of Bacterial Names* (38) and have no standing in bacterial nomenclature. The exclusion of these species from the genus *Agrobacterium* was further supported by DNA-rRNA hybridization experiments between true agrobacteria and the marine strains except *A. stellulatum* and "*A. sanguineum*" (9).

The genotypic data presented in Fig. 2 and 3 have shown that five of the marine star-shaped-aggregate-forming species have enough similarities to be grouped as a distinct taxon apart from genus *Agrobacterium*. For the present, however, it does not seem appropriate to create a new genus for this group of bacteria because there are not enough distinctive phenotypic characters to separate the marine strains from genus *Agrobacterium* (Table 6). The only distinguishing characteristics that could be found for these two taxonomic groups of bacteria were that the marine strains required seawater base or showed preferential growth in seawater media and that acid production from carbohydrates was negative or, only after incubations longer than 4 to 6 weeks, weakly positive.

Until more taxonomic data are available, we therefore propose to subdivide the genus *Agrobacterium* to accommodate both the terrestrial and plant pathogenic species in subdivision 1 and the marine star-shaped-aggregate-forming species in subdivision 2. An example for subdividing a genus to include different rRNA homology groups is the genus *Pseudomonas* (30). The marine subdivision of *Agrobacterium* comprises the new species *A. atlanticum* and *A. meteori* and the revived species *A. ferrugineum*, *A. gelatinovorum*, and *A. stellulatum*. The species name *A. aggregatum* is a later subjective synonym of *A. stellulatum*, which has priority.

Characteristics common to all species of the marine subdivision of *Agrobacterium* are as follows. The cells are gram-negative, regular, club-shaped or dumb-bell-shaped rods or coccobacilli 0.6 to 1.2 μ m wide and 1.0 to 4.0 μ m long. Straight or curved filaments possible. Occurring singly, in pairs and short chains, or in irregular clusters or starlike aggregates developing predominantly at the end of the logarithmic growth phase in seawater broth. Motile strains peritrichous or degenerately peritrichous, with 1 to 8 flagella. Endospores not formed. Aerobic, but some strains are able to grow under reduced oxygen tensions. Most species require media based on seawater or show preferential growth in seawater media. Optimum growth temperatures between 20 and 30°C. Oxidase positive. Indole, methyl red, Voges-Proskauer, lysine and ornithine decarboxylase negative. Hydrolysis of casein, chitin, and alginate negative. Aerobic acid production from carbohydrates negative or, only after incubations longer than 4 to 6 weeks, weakly positive. The cells utilize many carbohydrates but not adipate, *p*-hydroxybenzoate, or quinate and usually produce considerable extracellular slime. G+C content of the DNA ranges from 54

TABLE 6. Differentiation of marine and terrestrial agrobacteria from other colorless gram-negative, peritrichously flagellated genera frequently occurring in marine environments

Characteristic	<i>Agrobacterium</i> spp. ^a		<i>Alcaligenes</i> spp. ^b	<i>Deleya</i> spp. ^c
	Subdivision 1 (terrestrial ^d)	Subdivision 2 (marine)		
DNA base composition (mol% G+C)	57–63	54–59	56–70	52–68
Rods, coccoid rods, or cocci	+	+	+	+
Regular, club-shaped or dumb-bell-shaped rods or coccobacilli	+	+	–	–
Starlike aggregates	+	+	–	–
Seawater base requirement or preferential growth in seawater media	–	+	–	+
Growth under reduced oxygen tensions	+	D	–	–
Nitrate or nitrite reduction to gas	D	D	+	–
Urease	+	–*	–	D
Acid from:				
Glucose	+	— ^e	D	D
Arabinose	+	— ^e	ND	D
Fructose	+	— ^e	ND	D
Maltose	+	— ^e	ND	D
Xylose	+	— ^e	D	D
Habitat	Soil, infected plant material	Marine environments	Soil, water, feces, clinical specimens	Marine environments

^a Symbols: +, positive; –, negative; ND, not determined or not reported; *, 67 of 75 tested strains were negative; D, positive and negative strains are known.

^b According to Kersters and De Ley (24).

^c According to Baumann et al. (4) and Rüger et al. (35).

^d According to Kersters and De Ley (23).

^e —, Negative or weakly positive only after incubation for more than 4 to 6 weeks.

to 59 mol%, as determined from thermal denaturation profiles.

Description of the marine *Agrobacterium* species: the physiological and nutritional properties of the species and their type strains are as presented above for the whole group. Additional characteristics are listed in Tables 2 and 3. Differential traits of the species and of the species incertae sedis are presented in Table 7 and below.

Agrobacterium atlanticum sp. nov. (at.lan'ti.cum. M.L. adj. *atlanticum*, pertaining to the Atlantic Ocean). Colonies are circular; flat to raised; translucent; colorless, beige, or yellowish; with mean diameter of 2 to 3 mm. Nitrate is reduced to nitrite and gas. After 4 to 6 weeks of incubation, usually acid production from glucose, arabinose, glycerol, and xylose but not from fructose, lactose, maltose, mannitol, or sucrose. Twenty-six of 34 organic substrates are utilized by the majority of the strains. Arabinose, ribose, salicin, sorbitol, histidine, adipate, *p*-hydroxybenzoate, and quinate usually not utilized. Isolated from marine sediments of the eastern Atlantic Ocean. The type strain is strain 1480 (=DSM 5823). The G+C content of its DNA is 55 mol%.

Description of *Agrobacterium meteori* sp. nov. *Agrobacterium meteori* (me'te.ori. M.L. gen. n. *meteori*, of meteor; after the German research vessel *Meteor*). Colonies are circular; flat to raised; translucent; colorless, beige, or yellowish; with mean diameter of 2 to 3 mm. Nitrate is reduced to nitrite but not to gas. Except from glucose after 4 to 6 weeks of incubation, acid usually not produced from carbohydrates. None of 34 organic substrates utilized. Isolated from marine sediments of the eastern Atlantic Ocean. The type strain is strain 1513 (=DSM 5824). The G+C content of its DNA is 58 mol%.

Agrobacterium ferrugineum (ex Ahrens and Rheinheimer 1967) nom. rev. emend. Colonies are circular; flat to raised; initially translucent, colorless, or light brown; later dark brown or with brown center; with mean diameter of 2 mm.

Nitrate not reduced to nitrite or gas. Acid produced from glucose, fructose, glycerol, maltose, and xylose after 4 to 6 weeks of incubation. None of 34 organic substrates utilized at 20°C, but the type strain was able to utilize propionate, fumarate, succinate, lactate, glycerol, and glutamate at 4°C within 4 to 6 weeks. Isolated from seawater of the Baltic Sea. The type strain is strain ATCC 25652. The G+C content of its DNA is 58 mol%.

Agrobacterium gelatinovorum (ex Ahrens 1968) nom. rev. emend. Colonies are circular, flat to raised, initially colorless, later with brown center, mean diameter of 1 to 2 mm. The only species capable of gelatin hydrolysis. Nitrate reduced to nitrite but not to gas. Acid produced from glycerol after 4 to 6 weeks of incubation but not from glucose, fructose, maltose, or xylose. Only 12 of 34 organic substrates utilized. Isolated from seawater of the Baltic Sea. The type strain is strain ATCC 25655. The G+C content of its DNA is 59 mol%.

Agrobacterium stellulatum (ex Stapp and Knösel 1954) nom. rev. emend. Colonies are circular, flat to raised, translucent, initially colorless or beige, later with brown center, mean diameter of 2 mm. Growth under reduced oxygen tensions. Nitrate reduced to nitrite and gas. No acid produced from glucose or xylose; weak or variable acid production from fructose, glycerol, and maltose after 4 to 6 weeks of incubation. Thirty of 34 organic substrates utilized but not lysine, adipate, *p*-hydroxybenzoate, or quinate. Isolated from marine sediment and from seawater. The type strain is strain ATCC 15215. The G+C content of its DNA is 59 mol%.

Species incertae sedis: the taxonomic position of "*A. agile*" is probably in the vicinity of the genus *Pseudomonas* (9). The remaining three marine star-shaped-aggregate-forming species, "*A. sanguineum*," "*A. kieliense*," and "*A. luteum*," were originally thought to be members of the marine subdivision of genus *Agrobacterium* because of their

TABLE 7. Differentiation between marine *Agrobacterium* species and some other marine star-shaped-aggregate-forming bacteria of uncertain taxonomic position

Characteristic	Test result ^a								
	<i>Agrobacterium atlanticum</i>	<i>Agrobacterium meteori</i>	<i>Agrobacterium ferrugineum</i>	<i>Agrobacterium gelatinovorum</i>	<i>Agrobacterium stellulatum</i>	<i>Agrobacterium agile</i>	<i>Agrobacterium kieltense</i>	<i>Agrobacterium luteum</i>	<i>Agrobacterium sanguineum</i>
DNA base composition (mol% G+C)	54–57	58	58	59	59	58	48	55	63
Colony pigmentation									
Colorless, beige, yellowish	+	+	–	+	+	+	+	–	–
Brown, light brown, brown center	–	–	+	+	–	(+)	–	–	–
Yellow	–	–	–	–	–	–	–	+	–
Red	–	–	–	–	–	–	–	–	+
Flagellation, if motile									
Peritrichous, degenerately peritrichous	+	+	+	+	+	–	+	+	+
Polar, 1–3 flagella	–	–	–	–	–	+	–	–	–
Facultatively anaerobic	–	–	–	–	(+)	(+)	–	–	–
Catalase	+	D	+	+	+	+	+	+	+
H ₂ S production from cysteine	–	–	–	–	–	–	+	–	–
Gelatin hydrolysis	–	–	–	+	–	–	–	–	–
Nitrate reduction to nitrite	+	D	–	+	–	+	–	–	–
Nitrite reduction to gas	+	–	–	–	+	+	–	–	–
Aerobic acid production after 4–6 wk (not earlier) from:									
Glucose	+	D	+	–	–	–	–	–	–
Fructose	–	D	+	–	(+)	–	(+)	–	–
Glycerol	D	D	+	+	(+)	(+)	+	–	–
Maltose	–	D	+	–	D	–	(+)	–	–
Xylose	D	–	+	–	–	–	+	–	–
Utilization of:									
Arabinose	–	–	–	–	+	+	–	–	–
Cellobiose	+	–	–	–	+	+	–	–	+
D-Glucose	+	–	–	–	+	+	–	–	+
Salicin	–	–	–	–	+	+	–	–	–
Trehalose	+	–	–	–	+	+	–	–	–
Succinate	+	–	–	+	+	+	–	–	–
Glycerol	+	–	–	–	+	+	–	–	–
Sorbitol	–	–	–	–	+	+	–	–	–
L-Alanine	+	–	–	+	+	+	–	–	–
L-Glutamate	+	–	–	–	+	+	–	+	+
L-Histidine	–	–	–	–	+	+	–	–	–
L-Ornithine	+	–	–	+	+	+	–	–	–

^a Data for H₂S production, gelatin hydrolysis, and aerobic acid production from carbohydrates for all species except *A. atlanticum* and *A. meteori* are derived from Ahrens (1). Symbols: +, >80% of strains positive; (+), >80% of strains weakly positive; D, 21 to 79% of strains positive; –, >80% of strains negative.

phenotypic characteristics. However, their LMW-RNA profiles were so different from those of the other five species (Fig. 2 and 3) that they cannot be included in the genus *Agrobacterium*. According to their overall characteristics, presented in Tables 2 and 4, these species may be regarded as separate taxa in the family *Rhizobiaceae* as described by Jordan (22).

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