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Description of a New Polymer-Secreting Bacterium from a Deep-Sea Hydrothermal Vent, *Alteromonas macleodii* subsp. *fijiensis*, and Preliminary Characterization of the Polymer†

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A deep-sea, aerobic, mesophilic, heterotrophic bacterium was isolated from fluid collected near an active hydrothermal vent. On the basis of phenotypic and phylogenetic analyses and DNA-DNA relatedness, strain ST716 could be assigned to the species *Alteromonas macleodii* as a new subspecies. This bacterium secreted an unusual high-molecular-weight polysaccharide in the presence of glucose in batch cultures. The viscosity of this exopolysaccharide is of the same order of magnitude as that of xanthan, another bacterial polysaccharide of industrial interest. This polysaccharide, produced during the stationary phase, contained glucose, mannose, pyruvated mannose, and galactose along with galacturonic acid and glucuronic acid.

Many studies have been conducted to understand the role and properties of exopolysaccharides (EPS) in the adhesion of bacteria to solid surfaces in marine and freshwater environments (4, 5, 22, 25, 26, 27, 30, 41, 44). Following the discovery of the first deep-sea hydrothermal vents in 1977 on the Galapagos Ridge, many sites were then explored, including the East Pacific Rise, the Southwestern Pacific back-arc basins, and the Mid-Atlantic Ridge. Deep-sea hydrothermal vents are characterized by high pressures, high concentrations of toxic elements (sulfides and heavy metals), high temperatures of hydrothermal fluids, and different gradients resulting from their mixing with cold oxygenated seawater. In such an environment, the presence of unusual microorganisms of biotechnological interest can be expected in terms of thermostable enzymes, biologically active molecules, hydrocarbon-degrading bacteria, and polysaccharide- and poly-β-hydroxyalkanoate-producing bacteria. As a result of preliminary studies, unusual eubacterial EPS of deep-sea origin were found and characterized (13, 39, 42, 43), and more polymer producers are expected, considering the large number of bacterial strains currently archived in collections and new hydrothermal sites to be explored. The objective of this work was to describe a new aerobic, mesophilic, heterotrophic strain isolated from a deep-sea hydrothermal vent. In medium supplemented with a suitable carbon substrate, this bacterium was shown to produce an acidic extracellular polysaccharide.

MATERIALS AND METHODS

Strain origin. During the French-Japanese cruise Starmer in July 1989 (7), samples of vent fluid, chimney rocks, invertebrate tissues, and seawater were collected by the French manned submersible *Nautile*. Bacterial strain ST716 was isolated from a diluted hydrothermal fluid (pH 6.2) collected with a titanium syringe near an active hydrothermal vent at a depth of 2,000 m in a rift system of the North Fiji Basin (16°59′S, 173°55′W).

Reference strains. *Alteromonas carrageenovora* (LMG 2154), *Alteromonas espejiana* (LMG 2866), *Alteromonas haloplanktis* (LMG 2852), *Alteromonas macleodii* (LMG 2843), *Alteromonas undina* (LMG 2880), and *Shewanella putrefaciens* (LMG 2268) were obtained from the LMG Collection, Ghent, Belgium.

Isolation procedure and cytological characterization. Strain ST716 was isolated on 2216E medium (28). Different samples were inoculated in liquid medium buffered with 50 mM MOPS (morpholinepropanesulfonic acid) at pH 7.5 and incubated at 25°C for 48 h. The successful cultures were subcultured on solid medium. This procedure was repeated several times until the presence of a pure monoculture was established by microscopic examination. Gram staining was carried out as described by Conn et al. (6). SpotTest Flagella Stain and India ink (Difco, Detroit, Mich.) were used to demonstrate the presence of flagella and capsule. The possibility of pigmentation was determined by the procedure of King et al. (18), modified by addition of NaCl (20 g/liter) to the medium. Polyhydroxybutyrate accumulation was tested by the method of Gauthier and Breittmayer (12) with *A. undina* as the control strain.

Physiological characteristics. Growth experiments were done with 20-ml tubes containing 5 ml of 2216E liquid medium. The tubes were inoculated at 10% (vol/vol) and incubated inclined on a table rotary shaker (Infors HT, Bottmingen, Switzerland) at 200 oscillations per min. Turbidity was measured directly on culture tubes by spectrophotometry at 520 nm (Spectronic 401; Milton Roy, Rochester, N.Y.) from 0.05 to 0.5 optical density units. In that range, the proportionality between turbidity and cell density is assumed to be constant. Growth rates were determined over temperatures ranging from 15 to 45°C. The effect of pH on the growth rate was determined from pH 5.5 to 8.5, using 50 mM MES (morpholineethanesulfonic acid, pH 5.5 to 6.5), 50 mM MOPS (pH 6.5 to 8), and 50 mM AMPSO {3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid, pH 8.5} buffers. Growth in the presence of different concentrations of Na^+ (between 10 and 70 g of NaCl per liter) was examined.

API 20NE, API 50CH, API ZYM, and ATB 7 strips (BioMérieux SA, Marcy l'Etoile, France) were used for detection of metabolic properties. Biolog GN Microplates (Biolog Inc., Hayward, Calif.) were used to characterize the new strain. For all these tests, the suspension medium was added to a concentration of 20 g of NaCl per liter. Unfortunately, at this time, few marine bacteria appear in the Biolog GN database. Six marine, nonpigmented strains (mentioned before), selected for their phenotypic features were used as references and introduced into our database. A factor analysis in principal components connected with a hierarchical ascending classification was performed with the SPAD.N software package (CISIA, St. Mandé, France).

DNA base composition. DNA was isolated by the method of Sambrook et al. (33). The G+C mol% of the DNA was determined by thermodenaturation (21) with DNAs from *Escherichia coli*, *Clostridium perfringens*, and *Micrococcus luteus* (Sigma Chimie) as standards.

DNA reassociation. DNAs were extracted as mentioned above. The DNA hybridization from renaturation rates was determined by spectrophotometry (15).

Sequencing of the 16S rRNA genes (rDNAs). (i) DNA amplification. Preparation of bacterial DNA for PCR was based on the method of Sritharan and Barker (36). Bacteria were grown on marine agar. Colonies were suspended in 200 μl of lysis mixture (10 mM Tris [pH 8.0], 1 mM EDTA, 1% Triton X-100) and boiled for 5 min. After a single chloroform extraction, 5 μl of supernatant was used to amplify the small-subunit rRNA genes with two primers, correspond-

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ing to positions 8 to 28 and 1498 to 1509 of the *E. coli* small-subunit rRNA sequence. The initial denaturation step consisted of heating the reaction mixture at 95°C for 180 s, and this was followed by an annealing step (52°C for 60 s) and an extension step (72°C for 90 s). The thermal profile then consisted of 25 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 60 s, and extension at 72°C for 90 s. A final extension was carried out at 72°C for 5 min. This amplification reaction produced DNA molecules of 1.5 kb.

(ii) PCR product direct sequencing. The PCR products were analyzed on a 1% low-melting-point agarose gel that included a molecular weight standard for quantification of the PCR yield. PCR products were directly sequenced by a protocol described previously (1), with slight modifications. We determined almost the entire small-subunit rRNA sequence (corresponding to positions 29 to 1425 in the *E. coli* rRNA sequence). Eleven DNA primers were used in the sequencing reaction. These primers corresponded to the following positions in the *E. coli* sequence: primer S2, 99 to 119; primer S3, 242 to 262; primer S4, 342 to 356; primer S6, 518 to 534; primer S8, 684 to 702; primer S10, 906 to 925; primer S12, 1099 to 1114; primer S14, 1223 to 1240; primer S15, 1384 to 1400; and primer S17, 1493 to 1509. A reverse primer (8 to 28) was used in conjunction with primer S17 to amplify the small-subunit rDNAs.

(iii) Phylogenetic analysis and alignment: general procedure. The phylogenetic data described below were obtained (i) by using successive alignment and phylogeny procedures and (ii) by reinvestigating deep branching patterns after close relationships had been determined. In each phylogenetic analysis, we restricted the comparisons to nucleotide positions that were aligned without doubt. Some analyses were performed several times, with or without small domains that could have reached the point of saturation for mutations. Although this approach was probably not as efficient as carefully weighting each position independently, it was easier to use and was adopted as a reasonable compromise, considering the possible problems of crossing-over that sometimes affect rRNA sequences (35). In order to keep computation times within reasonable limits, it was not possible to include all representatives of outgroups and ingroups in maximum-parsimony and maximum-likelihood analyses for each phylogenetic analysis. This problem was alleviated by performing multiple analyses with different outgroups and different ingroups (as determined by neighbor-joining analysis). All sequence alignments and species selection were done with computer programs developed by us and available on request from R. Christen.

(iv) Phylogenetic methods. A neighbor-joining algorithm like that developed by Saitou and Nei (32) was used. The program was rewritten to include inputs and outputs compatible with the rRNA database and other programs developed in our laboratory (running on 386-compatible personal computers and better). For maximum-parsimony analyses, the PAUP program (37) for Macintosh computers was used. All topologies were first obtained by using the heuristic option. According to the time used, a branch-and-bound search was then undertaken by using the full data set or a restricted set of species selected on the basis of the results of the heuristic search. When several most-parsimonious trees were obtained, a 100% consensus tree was constructed and treated as the most parsimonious tree for constructing figures. Finally, a bootstrap analysis was performed (heuristic option) to check each topology for robustness. We favored a large number of analyses and 100 replications for each analysis rather than a high number (1,000) of bootstrap replications, since our experience has shown that increasing the number of bootstrap replications above 100 usually had only a small influence on the results compared with the choice of species. For maximum-likelihood analyses, the fDNAml program, derived from the DNAML program (9) and rewritten by G. J. Olsen (University of Illinois, Urbana), was recompiled on a Hewlett-Packard model 700 workstation. All analyses were performed by using the global option (in fact, the F Y G options). Finally, all trees were plotted by using a Macintosh computer and a program (njplot) developed by M. Gouy (URA 243 CNRS, Université Claude Bernard, Villeurbanne, France) that allows transformation of a formal tree representation (Newick's format) into MacDraw drawings. Only topologies that were found to be similar by all three methods were retained as "true trees." Recent theoretical works have indeed demonstrated that convergence of the results of all three methods is a very strong indication that the correct phylogeny has been determined (14, 17).

Production of ST716 polymer. While we were searching for marine exopolysaccharide-producing bacteria, ST716 was selected for its ability to show a mucoid phenotype on 2216E solid medium supplemented with glucose (30 g/liter) after 5 days at 25°C. Exopolymer production was performed at 25°C in a 2-liter bioreactor (SGI-Inceltech, Toulouse, France) containing 1 liter of 2216E-glucose broth. Batch cultures were inoculated at 10% (vol/vol) with a suspension of cells in exponential phase. The pH was adjusted and maintained at 7.2 by automatic addition of 0.25 N NaOH. Foaming was avoided by addition of Pluronic-PE6100 oil (BASF, Levallois/Perret, France) at 0.1% (vol/vol). The air flow was fixed at 30 liters/h, and the agitation rate was from 200 to 1,100 rpm. Bacterial growth was determined by measuring the culture turbidity at 520 nm. The viscosity of the culture broth was controlled with a Brookfield viscosimeter (model DV II) supplied with a small adapter (SC4-18/13R) at 25°C (Brookfield Engineering Laboratories, Stoughton, Mass.).

Isolation, purification, and characterization of ST716 polymer. EPS were isolated from the culture medium after 60 h. Cells were removed from the medium by high-speed centrifugation, and the polymer was precipitated from the supernatant by addition (1:1) of absolute cold ethanol. After sequential washes with increasing ratios of ethanol to water, the resulting polysaccharides were collected by low-speed centrifugation, desiccated under nitrogen, and stored at room temperature. Detailed procedures related to the extraction and purification procedures are reported elsewhere (39, 43).

Chemical analysis. The total neutral carbohydrate and hexuronic acid contents were determined by the orcinol-sulfuric method (31, 40) and the *meta*-hydroxydiphenyl method (3), respectively. Hexosamines were analyzed by the Elson-Morgan method (8). The molar ratio of monosaccharides was determined by the methods of Kamerling et al. (16) and Montreuil et al. (23). The monosaccharide residues were analyzed after either aqueous acid hydrolysis or acidic methanolysis of the polymers and subsequent gas chromatography (GC) analyses as peracetylated derivatives or trimethylsilyl derivatives, respectively. Protein and sulfate contents were determined by the method of Lowry et al. (20) with bovine serum albumin as the standard and Fourier transform-infrared spectroscopy (FTIR), respectively (19). Each value is a mean of three determinations.

Infrared spectroscopy. Pellets for infrared analysis were obtained by carefully grinding a mixture of 2 mg of polysaccharide with 200 mg of dry KBr and then pressing in a 16-mm-diameter mold. FTIR spectra were recorded on a BOMEM MB100 instrument with a resolution of 4 cm^{-1}. Spectra were run in the 4,000 to 400 cm^{-1} region.

Rheological analysis. The average molecular weight was determined by using a Chromatix KMX6 light-scattering detector. The measurements were carried out in 0.1 M NaCl solution. Viscosity measurements were performed at 20°C with a Contraves Low Shear 40 viscosimeter. Polymer solutions were obtained by dissolving the EPS in a 0.1 M NaCl solution.

Nucleotide sequence accession number. The small-subunit rRNA nucleotide sequence data realized for this study and used to build the tree shown in Fig. 2 have been deposited in the EMBL database and will appear under accession number X85174.

RESULTS

Morphology. Strain ST716 appeared as a motile, strictly aerobic, nonfermentative, nonluminescent, nonpigmented, encapsulated, gram-negative rod, 0.6 to 0.8 by 1.4 to 2 μm in size, with a single polar flagellum. Five-day-old colonies on 2216E-glucose were gummy, glistening, irregularly shaped, and about 0.9 cm in diameter (0.4 cm without sugar).

Cultural characteristics. The optimal temperature for growth was between 25 and 35°C (Fig. 1A), and the optimal pH was between 6 and 8 (Fig. 1B). The optimal ionic strength was between 25 and 40 g of NaCl per liter (Fig. 1C). The study of the growth rate at optimal conditions gave a doubling time of 26 to 33 min.

Metabolic properties. Positive responses were obtained for catalase and cytochrome oxidase. Neither denitrification nor accumulation of polyhydroxybutyrate was observed for strain ST716. The other biochemical and nutritional characteristics (Table 1) showed large utilization of carbohydrate substrates as the sole source of carbon. Antibiotic susceptibility tests showed that strain ST716 is bacitracin (2 mg/liter) resistant and cephalothin (8 to 32 mg/liter) intermediate.

Computer-based analysis of the results of Biolog GN Microplates indicated clearly that ST716 was an *Alteromonas macleodii*-like bacterium.

DNA base composition. The G+C content of strain ST716 was 47.6%.

DNA reassociation. The measurement of genomic DNA-DNA homology between *A. macleodii* and bacterium ST716 showed a homology value of 82%.

Small-subunit rRNA sequence and phylogenetic analyses. The sequence of strain ST716 was aligned by comparison with a database containing about 3,000 aligned eubacterial small-subunit rRNA sequences. Phylogenetic analyses including representatives of all eubacterial phyla and all proteobacterial subdivisions showed that the new bacterium belonged to the gamma subdivision of the phylum *Proteobacteria* (data not shown). The phylogenetic position of the new bacterium was then investigated in more detail by including representatives of gamma proteobacteria and representatives of the four other subdivisions of the phylum *Proteobacteria* (alpha, beta, delta, and epsilon) as outgroups. Analyses were performed by three

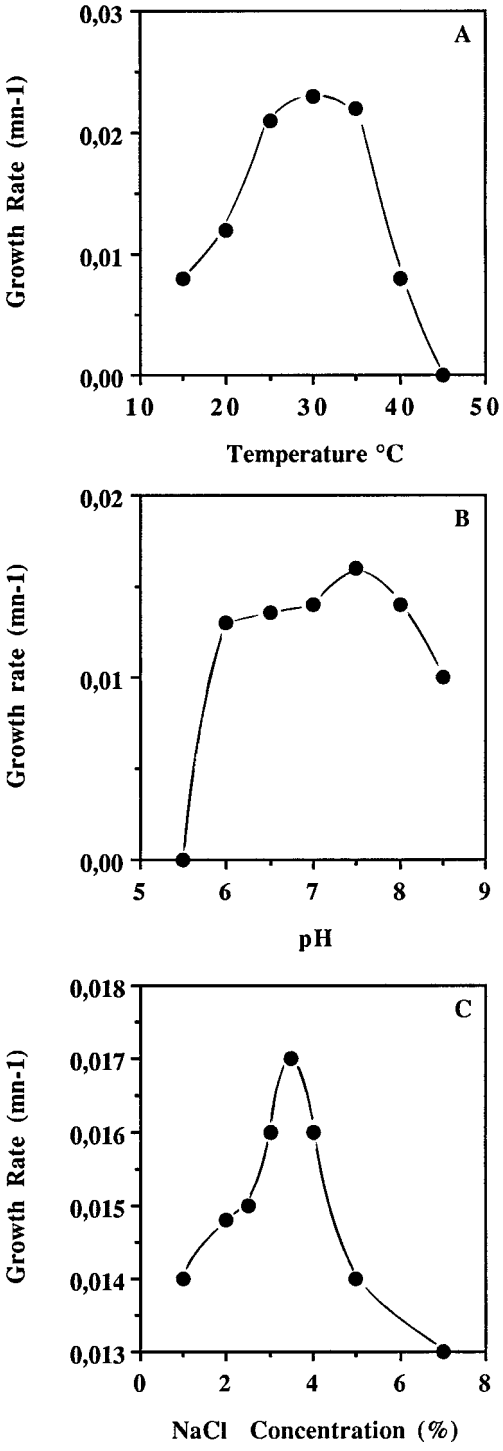

FIG. 1. Effects of temperature (A), pH (B), and NaCl concentration (C) on growth of strain ST716. Specific growth rates were calculated from the slopes of the growth curves.

TABLE 1. Strain characteristics[a]

Substrate, reaction, or enzyme	Strain ST716	*A. macleodii*
Glycerol	+	+
Ribose	+	−
D-Xylose	−	+
Galactose	+	+
Glucose	+	+
Fructose	+	+
Mannitol	−	+
α-Methyl-D-glucoside	+	−
N-Acetylglucosamine	−	+
Amygdalin	+	+
Arbutin	+	+
Esculin	+	+
Salicin	+	−
Cellobiose	+	+
Maltose	+	+
Lactose	+	+
Melibiose	+	+
Sucrose	+	+
Trehalose	+	+
Melezitose	−	+
Raffinose	+	−
Starch	+	+
Glycogen	+	+
Gentobiose	+	+
D-Turanose	+	+
Gluconate	+	+
Esculin hydrolysis (β-glucosidase)	+	+
Gelatin hydrolysis (protease)	+	+
β-Galactosidase (PNPG)[b]	+	+
Phosphatase (alkaline)	+++	+++
Esterase (C 4)	+	+
Esterase lipase (C 8)	++	++
Leucine arylamidase	+++	+++
Valine arylamidase	+	++
Cystine arylamidase	+	+
Phosphatase (acid)	+	+

[a] The characteristics of strain ST716 and *A. macleodii* were determined with API 20NE strips (identification of nonenteric gram-negative rods), API 50CH strips (carbohydrate assimilation), and API ZYM strips (enzymatic activities). Tests that were negative for both strains are not included.
[b] PNPG, *p*-nitrophenyl-β-D-glucoside.

phylogenetic methods: neighbor joining, maximum parsimony, and maximum likelihood. All analyses were always congruent, regardless of which method was used or which representatives of the outgroups were chosen, and placed the new bacterium in the gamma class. More detailed analyses were finally performed with only sequences from bacteria of the gamma group. All methods led to the clustering of bacterium ST716 with the sequence of *A. macleodii*, with which it formed a distinct monophyletic cluster in all analyses. Maximum parsimony was run with the branch-and-bound search option and found one most parsimonious tree (consistency index, 0.689; homoplasy index, 0.311). Finally, the internal branch uniting the two species showed a significantly positive length at a P of <0.01 in maximum-likelihood analysis and was supported at the level of 100% of bootstrap replications (branch-and-bond option). These results are shown in Fig. 2 and Table 2.

Production of EPS. During batch fermentation of strain ST716, production of EPS began at the end of the exponential phase and continued throughout the stationary phase, reaching a value of 6.0 g (dry weight) per liter at the end of the experiment (60 h). Consequently, the broth became progressively more viscous, and the viscosity of the medium reached 10,000 cP at 0.3 rpm.

Characterization of EPS. FTIR of the EPS exhibited a broad O—H stretching band at 2,900 cm^{-1} (Fig. 3). A shoulder at the high-frequency side of the 1,630-cm^{-1} band could be assigned to the presence of carboxylic groups, which then absorb at

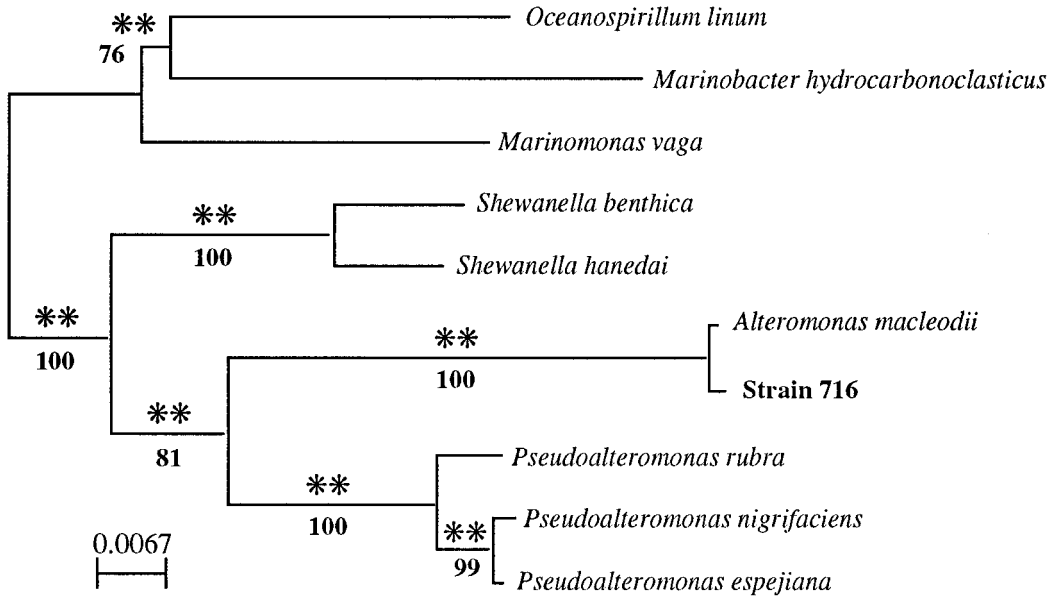

FIG. 2. Unrooted tree obtained by a neighbor-joining method. Branches that are significantly positive in a maximum-likelihood analysis are indicated by asterisks ($P < 0.01$). Branches that were retrieved in the most parsimonious tree are shown by percentages that also indicate how these branches are supported in a bootstrap analysis (only values above 50% are reported). *A. macleodii* and strain ST716 form a robust taxon. The level of branching of this taxon provides evidence that the two bacteria belong to a distinct genus, now recognized as the true *Alteromonas* genus.

1,730 cm^{-1} in acids. The band centered at 1,250 cm^{-1} consisted of two distinct bands, the first one located at 1,260 cm^{-1} and the other one at 1,230 cm^{-1}. The doublet has usually been referred to as the 1,250-cm^{-1} band and has been assigned to the antisymmetric O═S═O stretching vibrations. The absorption at 890 cm^{-1} indicated the β-glycosidic linkage of sugar.

Neutral sugars, as determined by the orcinol method with the mannose-galactose (1:1) ratio as the standard, and acidic sugars accounted for 42 and 38% of total sugars, respectively. Hexosamines, as analyzed by the Elson-Morgan method, were present at a very low concentration (2.3%). FTIR indicated a sulfate content of 5% (wt/wt). By using the cleaning procedures described above, the amount of protein was found to be as low as 4%.

Glucose and galactose as neutral sugars along with glucuronic acid were found in the same percentages (10 to 12%), while mannose, galacturonic acid, and pyruvated mannose were present in lower concentrations, 4.4, 6.3, and 5%, respectively (Table 3). The presence of pyruvate linked to the mannose was also determined by nuclear magnetic resonance and by comparison with xanthan (38). No mannuronic acid or guluronic acid was detected.

An average molecular weight (M_n) of 3.3×10^5 g/mol was found for this exopolymer. The intrinsic viscosity in 0.1 M NaCl solution reached 2,600 ml/g, with a Huggins constant of about 0.18. This value can be compared with that of a commercial xanthan tested in the same conditions, indicating an intrinsic viscosity of 2,200 ml/g, with a Huggins constant of 0.5.

DISCUSSION

Analyses of 16S rDNA as well as of whole genomic DNA-DNA relatedness recently demonstrated that the genus *Alteromonas* should be divided into two genera (11), and a new genus, *Pseudoalteromonas*, has been proposed to accommodate all the bacteria of the genus *Alteromonas* except *A. macleodii*. The genus *Alteromonas* (emended) should be restricted to this last single species. The deep-sea, aerobic, mesophilic, heterotrophic new bacterium that was isolated from a fluid sample collected in the vicinity of an active deep-sea hydrothermal

TABLE 2. rDNA sequence relatedness[a]

Strain	*A. macleodii*	ST716	*P. espejiana*	*P. nigrifaciens*	*P. rubra*	*S. benthica*	*S. hanedai*	*M. vaga*	*M. hydrocarbonoclasticus*	*O. linum*
A. macleodii		99.9	89.1	89.1	89.7	86.8	86.6	85.4	84.2	84.5
Strain ST716	0.001		88.9	88.9	89.3	86.4	86.2	84.8	83.9	84.1
P. espejiana	0.109	0.111		99.4	96.2	89.1	89.2	86.4	86.8	86.6
P. nigrifaciens	0.109	0.111	0.006		95.9	89.2	89.2	86.4	86.4	86.5
P. rubra	0.103	0.107	0.038	0.041		88.3	89.0	86.4	85.4	86.2
S. benthica	0.132	0.136	0.109	0.108	0.117		95.8	87.1	86.0	89.3
S. hanedai	0.134	0.138	0.108	0.108	0.110	0.042		87.2	85.1	88.0
M. vaga	0.146	0.152	0.136	0.136	0.136	0.129	0.128		87.7	89.0
M. hydrocarbonoclasticus	0.158	0.161	0.132	0.136	0.146	0.140	0.149	0.123		90.0
O. linum	0.155	0.159	0.134	0.135	0.138	0.107	0.120	0.110	0.100	

[a] Relatedness of rDNA sequences for the bacterial species shown in Fig. 2. Upper right, percent homology between any two sequences. Lower left, percent difference between any two sequences. All nucleotides available for rDNA sequences have been used for these calculations, in contrast to the phylogenetic analysis in Fig. 2, which included only positions that were clearly homologous. See Fig. 2 for complete species names.

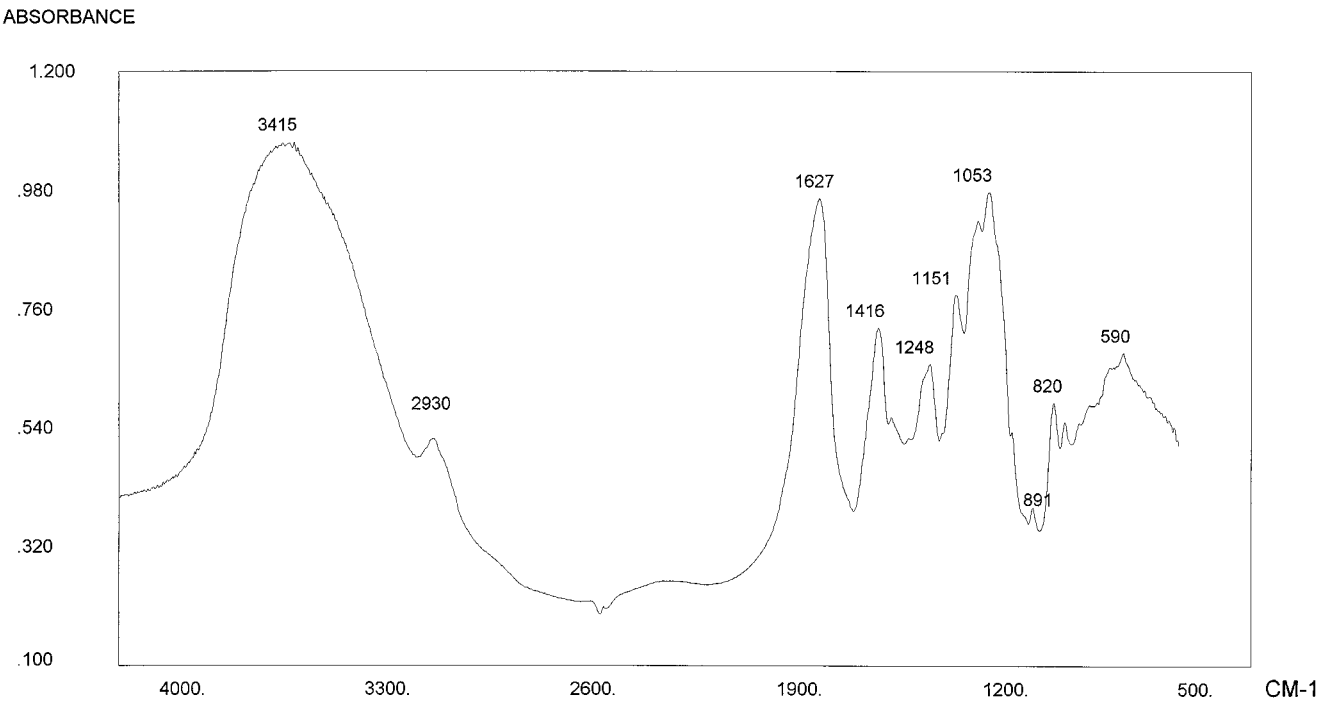

FIG. 3. FTIR spectrum of the EPS produced by strain ST716.

vent clearly belongs to the species *A. macleodii*. It is similar to *A. macleodii* according to morphological and biochemical data (including Biolog GN Microplates) and G+C content (2). Phylogenetic analyses of 16S rDNA clearly demonstrated that this bacterium formed, with *A. macleodii*, a well-defined taxon that deserves the rank of a genus. Since the measurement of genomic DNA-DNA homology revealed a relatedness of 82% between the two bacteria, it is appropriate to conclude that they belong to the same species (10). Because this homology is less than 90% and because the two bacteria have (i) different metabolic patterns, (ii) different EPS chemical properties, and (iii) different origins (2,000 m depth for the new bacterium), we propose that they should be described as separate subspecies, and the name *Alteromonas macleodii* subspecies *fijiensis* is proposed for the new bacterium.

These results reinforce the conclusion advanced by P. Vincent et al. (43) about strain HYD1545, which is also similar to *A. macleodii*, and indicate that subspecies of *A. macleodii* are present in very different deep-sea hydrothermal ecosystems, such as 13°N on the East Pacific Rise for strain HYD1545 and White Lady in the North Fiji Basin for the new bacterium.

The chemical composition of the polymer produced extracellularly by *A. macleodii* subsp. *fijiensis* shows that it is a heteropolysaccharide composed mainly of galactose, glucose, and glucuronic acid. Galacturonic acid and mannose were also found, along with pyruvated mannose. These sugars are quite common to many microbial EPS preparations (29). Glucuronic acid and galacturonic acid were the only hexuronic acids found in this microbial EPS of deep-sea origin. A considerable part of the polymer was not accounted for by the analytical procedures followed. However, this is not unusual and happens with other bacterial EPS preparations. This could be due either to a failure in the analytical methods used to determine the correct amounts of components or to the existence of unusual compounds undetectable by the methods used. Because of the structural complexity of EPS, with a variety of monomeric units and linkages having different stabilities in the presence of acid, a single hydrolysis procedure will often not be sufficient to cleave every linkage quantitatively. Some EPS fractions are very resistant to acid hydrolysis, and the various stabilities of the liberated monosaccharides can require different conditions of hydrolysis (34).

Seventeen other polysaccharide-producing bacteria have been isolated from hydrothermal deep-sea vents. They all be-

TABLE 3. Monosaccharide ratios[a]

Strain	% of total									
	Rha	Glc	Gal	Man	Man X[b]	GalX 6-4	Glc UA	Gal UA	GlcNAc	Sum
ST716	—	10.5	11.1	4.4	5	—	12.2	6.3	—	49.5
HYD1545	0.3	17	9.4	0.6	—	NQ[c]	4	2.4	ND[d]	33.7
HYD1644	0.9	9.1	12.4	1.6	—	—	4.9	2.4	—	31.3

[a] Monosaccharide ratios (grams per 100 g [dry weight]) in the EPS produced by *A. macleodii* subsp. *fijiensis* ST716 and other *A. macleodii* strains isolated from deep-sea hydrothermal vents. Monosaccharides were analyzed as trimethylsilyl glycosides following methanolysis.
[b] X, as pyruvate.
[c] NQ, not quantified.
[d] ND, not determined.

long to the true *Alteromonas* genus (42). The EPS were analyzed and classified in five groups according to their chemical and rheological properties (13). The carbohydrate composition of the polymer secreted by *A. macleodii* subsp. *fijiensis* is different from that found in other bacteria from deep-sea vents. *Alteromonas* sp. strain HYD1644 (13, 42) (CNCM I-1282; Institut Pasteur, Paris, France; patent no. 047 D PCT 191), phenotypically close to *A. macleodii* subsp. *fijiensis*, produces an acidic polysaccharide consisting of the same neutral and hexuronic sugars but also contains a new one identified as an diacidic hexose. Another *Alteromonas* sp., strain HYD1545 (13, 39, 42, 43) (CNCM I-1284; Institut Pasteur; patent no. 047 D PCT 191), isolated from the same deep-sea origin, was shown to produce an acidic polysaccharide made of neutral sugars (50%), uronic acids (40%), and 4,6-*O*-(1-carboxyethylidene)-galactose (GalX; X indicates pyruvate) (10%) and composed of galactose, glucose, glucuronic acid, galacturonic acid, and GalX in a molar ratio of 2.5:3:2:2:1.

Many marine bacteria, including *Vibrio*, *Pseudomonas*, and *Pseudoalteromonas* spp., can produce extracellular polysaccharides under similar conditions. *Pseudoalteromonas atlantica* and *Shewanella colwelliana* are known to produce acidic polysaccharides. Until now, only a few *Alteromonas* spp. have been described as EPS producers. *A. macleodii* 2MM6 (24) was shown to produce an acidic polysaccharide consisting of tetrasaccharide repeating units containing D-galactose, 3-*O*-acetyl-2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-L-guluronic acid, and 3,6-dideoxy-3-(4-hydroxybutyramido)-D-galactose residues. The polymer produced by *A. macleodii* subsp. *fijiensis*, in a medium supplemented with glucose, had a different carbohydrate composition. The viscosity of this high-molecular-weight polymer (2,600 ml/g) was compared with that of a commercial xanthan and found to be of the same order of magnitude. Additional rheological studies are in progress to evaluate possible applications of this new polymer. In addition, this uronic-rich exopolymer could be expected to have some heavy-metal-binding ability and applications in the fields of biodetoxification and wastewater treatment.

Description of *A. macleodii* subsp. *fijiensis* subsp. nov. *Alteromonas macleodii* subsp. *fijiensis* (fi.ji.en′sis. N.L. adj. *fijiensis*, from Fiji, Pacific Ocean). Cells are gram-negative straight rods that are 0.6 to 0.8 μm in diameter and 1.4 to 2 μm in length. Motile by means of a single polar flagellum. Not luminescent and not pigmented. Strictly aerobic. Chemoorganotrophs with respiratory but not fermentative metabolism. Oxidase and catalase positive. Growth occurs at 20 to 35°C but not at 4°C. Seawater base obligate for growth. Does not denitrify. No constitutive arginine dihydrolase system. Does not accumulate poly-β-hydroxybutyrate. The G+C content of the DNA is 47.6 mol%. DNA-DNA homology with the *A. macleodii* type strain is 82%. Isolated from a deep-sea hydrothermal field on a rift system of the North Fiji Basin. The type strain ST716 has been deposited in the Collection Nationale de Culture de Microorganismes (Institut Pasteur) as strain CNCM I-1627.

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