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Isolation and Characterization of *Shewanella alga* from Human Clinical Specimens and Emendation of the Description of *S. alga* Simidu et al., 1990, 335

HATSUMI NOZUE,[1]†* TETSUYA HAYASHI,[1] YASUHIRO HASHIMOTO,[2] TAKAYUKI EZAKI,[2] KOJI HAMASAKI,[3] KOUICHI OHWADA,[3] AND YOSHIRO TERAWAKI[1]

Department of Bacteriology, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto 390,[1] Department of Microbiology, Gifu University School of Medicine, 40 Tsukasamachi, Gifu 5002,[2] and Ocean Research Institute, University of Tokyo, Minamidai 1-15-1, Nakanoku, Tokyo 164,[3] Japan

Genetic and phenotypic studies on the strains biochemically identified as *Shewanella putrefaciens*, which had a G+C content ranging from 52 to 54 mol% were conducted. The moles percent G+C of the type strain of *S. putrefaciens* is 46. Surprisingly, DNA homology experiments revealed that all these strains are genetically related to *Shewanella alga* (which was reported to produce tetrodotoxin), not to the type strain of *S. putrefaciens*. In this study, we reidentified clinical strains of *S. putrefaciens* which have a high range of moles percent G+C, as does *S. alga*. We also characterized the reidentified strains and found that the original description of *S. alga* (U. Simidu, K. Kita-Tsukamoto, T. Yasumoto, and M. Yotsu, Int. J. Syst. Bacteriol. 40:331–336, 1990) is insufficient to identify this strain. An emended description of *S. alga* is given.

The organism now called *Shewanella putrefaciens* was first described in 1931 and classified as a member of the genus *Achromobacter* (4). In 1941, it was transferred to the genus *Pseudomonas* (16) on the basis of morphology. In 1972, it was transferred to the genus *Alteromonas* (1) on the basis of G+C content. Finally, in 1985, it was transferred to a new genus, *Shewanella*, on the basis of comparative 5S rRNA sequences (17). The type species of *Shewanella* is *S. putrefaciens* (17).

Many of the strains classified as *S. putrefaciens* were isolated from diverse sources, including environmental sources, such as spoilage flora of foods (12, 14, 18, 28), oil fields (24), and the ocean (1, 13), and diverse clinical sources, such as patients with otitis, bronchitis, pneumonia, and urinary tract infections (3, 6, 9, 14, 15, 21, 22, 29). However, the collected strains were heterogeneous and there were differences between environmental and clinical isolates (9, 14, 20, 21, 23, 24, 28). Owen et al. (20) divided the 10 environmental strains and 16 clinical strains into four groups. Group IV consisted of nine clinical strains. The moles percent G+C value for group IV (52.6) was clearly higher than those for the other three groups (43.9 to 46.9). All four groups retained the species identification of *S. putrefaciens*, despite the obvious heterogeneity and moles percent G+C values ranging from 43 to 55 (2).

Recently, we noticed that most strains isolated from human clinical specimens and identified as *S. putrefaciens* showed beta-hemolysis on sheep blood agar. However, environmental strains were nonhemolytic. These hemolytic strains had 52 to 54 mol% G+C. Although the hemolytic strains are biochemically identified as *S. putrefaciens* according to the description in the *Manual of Clinical Microbiology* (7), they exhibited high levels of DNA homology with the type strain of *S. alga*. In this study, we present evidence that these clinical strains of *S. putrefaciens* should be identified as *S. alga* and emend the description of *S. alga* on the basis of the physiological characterization of 35 additional strains.

S. alga is known to be a tetrodotoxin (TTX)-producing bacterium (25, 26). Production of TTX by some newly reidentified strains was examined. This is the first report of TTX production by strains isolated from human clinical specimens.

MATERIALS AND METHODS

Strains used and isolation technique. The type strain of *S. putrefaciens* IAM 12079 (= ATCC 8071^T [T = type strain]) and three IAM strains, previously registered in *S. putrefaciens* culture collections with accession numbers IAM 13595 (= NCTC 10737), IAM 13614 (= NCTC 10738), and IAM 13615 (= NCTC 10762), were supplied by the Microbial and Microalgal Research Center, Institute of Applied Microbiology, University of Tokyo. The type strain of *S. alga* (OK-1 = IAM 14159^T) was supplied by Ocean Research Institute, University of Tokyo. Forty clinical strains of *S. putrefaciens* isolated from different human clinical specimens were provided by several researchers in Japan (Table 1). We also isolated 32 strains of *S. putrefaciens* from pond water, sewage, foods, soil, and the skin of animals and used them to find physiological traits distinguishing *S. putrefaciens* from *S. alga*. Six of these strains were used for DNA-DNA hybridization experiments.

The primary step of isolation was performed in an aerobic environment by selective growth in peptone solution containing 1% peptone (Difco Laboratories, Detroit, Mich.), 1% NaCl, and 0.1% bile salts (Difco). Nonfermentative, hydrogen sulfide-producing colonies were selected on deoxycholate-hydrogen sulfide-lactose (DHL) agar (Eiken Chemical Co., Ltd., Tokyo, Japan). Isolates were finally identified as *S. putrefaciens* by additional biochemical tests described in the *Manual of Clinical Microbiology* (7).

Physiological tests. Physiological tests were performed by the methods described in the *Manual of Clinical Microbiology* (7), except for those tests described below. Lecithinase and lipase activities were detected by the methods of Stenstrom et al. (28), and β-galactosidase was detected as de-

* Corresponding author.
† Present address: Center for Molecular Biology, Wayne State University, Detroit, MI 48202.

TABLE 1. Characteristics of strains used[a]

Strain	G+C content (mol%)	Source	Hemolysis on blood agar
Received as *S. putrefaciens*			
IAM 12079^T (= ATCC 8071^T)	46	Butter	−
IAM 13595 (= NCTC 10735)	47	Cuttlefish	−
IAM 13614 (= NCTC 10738)	53	Feces	+
IAM 13615 (= NCTC 10762)	53	Pus	+
Received as *S. alga*			
IAM 14159^T (OK-1^T)	54		+
Tentatively identified as *S. putrefaciens*[b]			
SH 329	46	Clinical, human	−
SH 392	46	Clinical, human	−
SH 1190	47	Clinical, human	−
SH 2070	47	Clinical, human	−
SH 6190	46	Clinical, human	−
KM 776	47	Clinical, human	−
KM 773	47	Clinical, human	−
E 2	46	Soil	−
E 4	45	Soil	−
E 5	45	Soil	−
E 7	46	Soil	−
E 28	46	Food	−
E 37	46	Food	−
BH 278	53	Clinical, human, sputum	+
BH 582	53	Clinical, human, pus	+
KM 1109	52	Clinical, human	+
KM 1110	53	Clinical, human	+
GIFU 1187	53	Clinical, human	+
JH 116	53	Clinical, human	+
JH 120	54	Clinical, human, sputum	+
JH 121	54	Clinical, human, sputum	+
JH 122	53	Clinical, human, blood	+
JH 123	54	Clinical, human	+
JH 124	53	Clinical, human	+
SH 242	53	Clinical, human	+
SH 328	53	Clinical, human	+
SH 507	53	Clinical, human	+
SH 509	54	Clinical, human	+
SH 510	53	Clinical, human	+
SH 511	54	Clinical, human	+
SH 747	53	Clinical, human	+
SH 1109	53	Clinical, human	+
SH 1160	53	Clinical, human	+
SH 4236	53	Clinical, human	+
SH 5446	53	Clinical, human	+
SH 5563	53	Clinical, human	+
SH 6728	53	Clinical, human	+
SH 8764	53	Clinical, human, uterus	+
TPH 200	53	Clinical, human	+
TPH 218	54	Clinical, human	+
TPH 220	53	Clinical, human, urine	+
TPH 519	53	Clinical, human, otitis	+
TPH 535	53	Clinical, human	+
TPH 537	53	Clinical, human	+
TPH 5141	53	Clinical, human, gastritis	+
TPH 5142	53	Clinical, human	+

[a] All clinical, soil, and food strains were tentatively identified as *S. putrefaciens* by conventional biochemical tests.

[b] Strain designations beginning with BH, KM, GIFU, JH, SH, and TPH were kindly provided by different hospitals in Japan. Strain designations beginning with E were isolated by us (see Materials and Methods).

scribed by Smibert and Krieg (27). The medium developed by Wood and Baird (30) was used to determine reduction of trimethylamine oxide. Trimethylamine production after 2 days of incubation at 30°C was examined by the method of Laycock and Reiger (12). Growth temperatures were tested on nutrient agar plates (Difco) supplemented with 1.5% NaCl for 7 days at 37 or 42°C and for 14 days at 4°C. Tolerance to NaCl was determined visually after 48 h of vigorous shaking in nutrient broth (Difco) supplemented with 6% NaCl at 30°C. Hemolysis was determined from sheep blood agar plates (Eiken Chemical Co., Ltd.) incubated at 30 or 37°C for 2 days. Flagellar staining was performed as described by Heimbrook et al. (8).

Determination of G+C content of DNA. Ten micrograms of the denatured DNA was hydrolyzed with P1 nuclease (50 μg/ml in 50 mM acetate buffer containing 0.1 mM $ZnCl_2$ [pH 5.3]; Yamasa Shoyu Co., Ltd., Chiba, Japan) for 2 h at 50°C. Then, 2.4×10^{-2} U of alkaline phosphatase (Takara Shuzo Co., Ltd., Tokyo, Japan) was added, and the mixture was incubated at 37°C for 1 h. Separation and quantification of nucleosides were performed with high-performance liquid chromatography (HPLC) system LC-6A (Shimadzu Co., Ltd., Kyoto, Japan) and a Cosmosil $5C_{18}$ column (Nakarai Tesque Co., Ltd., Kyoto, Japan). The nucleosides were eluted with a solvent system of 0.2 M $NH_4H_2PO_4$–acetonitrile (20:1 [vol/vol]).

DNA-DNA homology experiment. The method of fluorometric hybridization using biotin-labelled DNA was described previously (5). DNA-DNA hybridization was carried out in 50% formamide solution at 37°C for 180 min.

Analysis of TTX and its derivatives. Each strain was cultured in 400 ml of L medium, which contains 0.5% polypeptone (Nihon Pharmaceutical Co., Ltd.), 0.1% yeast extract (Difco Laboratories), 1% NaCl, 0.2% KCl, and 0.4% $MgSO_4 \cdot 7H_2O$. After incubation for 18 h at 20°C, cells were harvested by centrifugation at 16,000 × *g*, and then the toxins were extracted from cells and culture medium.

After the cells were washed with 0.3 M NaCl solution, they were suspended in 0.1% acetic acid, subsequently ultrasonicated, and boiled for 20 min. The extract was centrifuged at 25,000 × *g* for 20 min, and then the supernatant was purified using a SEP-PAK C_{18} cartridge (Waters Associates). The eluate was evaporated to dryness, and the residue was dissolved in a small amount of distilled water.

The culture medium was acidified to pH 3.5 with acetic acid, boiled for 20 min, and evaporated to dryness. The residue was extracted three times with methanol containing 1% acetic acid. After evaporation to dryness, the residue was dissolved in distilled water. The solution was further purified by passage through a charcoal column and a SEP-PAK C_{18} cartridge. The eluate was evaporated to dryness, and the residue was dissolved in a small amount of distilled water.

Ion-paired reverse-phase HPLC was used to separate and detect TTX and its derivatives in the extracts (31). A JUSCO Tri Roter-VI chromatograph (Japan Spectroscopic Co., Ltd., Tokyo, Japan) with a Senshu Pak ODS-3251-D column (Senshu Scientific Co.) was used for HPLC. For detecting fluorescence, the excitation and emission wavelengths were set at 365 and 510 nm, respectively, on a Hitachi F1000 fluorescence spectrophotometer. The tissue culture bioassay (11) was performed for the determination and quantitative measurement of sodium channel-blocking toxin in the samples.

RESULTS

DNA base composition. The moles percent G+C values of the strains examined in this study are shown in Table 1. The G+C content of the *S. putrefaciens* type strain (IAM 12079^T) was 46 mol%. The moles percent G+C of IAM 13614, IAM 13615, and β-hemolytic clinical strains (which were biochemically identified as *S. putrefaciens*) were distinctly higher and ranged from 52 to 54. The results indicate that these clinical strains and two IAM strains are clearly different from IAM 12079^T but are quite similar to *S. alga* with regard to their G+C content, because the G+C content of the *S. alga* type strain (IAM 14159^T) was reported to be 54 mol% (26).

The G+C content of the nonhemolytic strains, including IAM 12079^T, IAM 13595, and some of the clinical strains and newly isolated environmental strains all ranged from 45 to 48 mol%.

DNA-DNA homology. DNA relatedness between *S. alga* IAM 14159^T and *S. putrefaciens* strains was examined (Table 2). Labelled DNAs from IAM 12079^T, SH 329, and IAM 13595 were used as DNA probes for *S. putrefaciens* strains that have a low moles percent G+C. Labelled DNAs from IAM 14159^T and IAM 13615 were used as DNA probes for *S. alga* and *S. putrefaciens* strains that have a high moles percent G+C. A high level of homology was found between IAM 13615 and IAM 14159^T (89 and 105%), but no significant homology was found between IAM 13615 and any *S. putrefaciens* strain that has a low moles percent G+C. DNA homology values between IAM 13615 and IAM 12079^T were 12 and 9%. The results mean that IAM 13615 belongs to the *S. alga* species and not to the *S. putrefaciens* species.

DNA-DNA hybridization experiments using clinical strains of *S. putrefaciens* which have a high moles percent G+C and the IAM 13615 DNA probe resulted in high DNA homology values ranging from 70 to 100% (Table 2). These results indicate that all of the clinical strains belong to the same species as IAM 13615; that is, they belong to *S. alga*. *S. putrefaciens* strains that have a low moles percent G+C were divided into three DNA homology groups, as shown by Owen et al. (20).

Physiological characteristics of *S. alga* strains. The physiological tests of *S. alga* strains, including IAM 14159^T, revealed several characteristics of *S. alga* that have not yet been described (Table 3), which are summarized as follows. (i) Growth occurred at 37 and 42°C, but not at 4°C. (ii) Growth occurred in nutrient broth containing 6% NaCl and on Salmonella-Shigella (SS) agar. (iii) All strains produced hydrogen sulfide. The reactions were very strong. The color of the Kligler iron agar slant changed to black within several hours of incubation at 37°C. (iv) The strains produced ornithine decarboxylase and lecithinase and reduced trimethylamine oxide to trimethylamine. (v) The strains exhibited clear hemolysis on sheep blood agar. Although hemolysis was obscure on the first day of incubation, it became clear on the second day at 37°C. (vi) Acids were oxidatively produced from D-ribose (100%) and D-glucose (60%) in the Hugh and Leifson O-F medium after 4 to 12 days of incubation. Acids were not produced from D-arabinose, L-arabinose, D-mannose, mannitol, *iso*-inositol, L-rhamnose, D-xylose, sucrose, maltose, and D-lactose after 14 days of incubation.

Production of TTX and its derivatives. Production of TTX by four clinical strains which were newly reidentified as *S. alga*, IAM 13615, JH 122, TPH 519, and SH 8764, were examined (Table 4). A clear single peak corresponding to 4-*epi*-tetrodotoxin (4-*epi*-TTX) was detected in each cell

TABLE 2. DNA homology values of the strains tentatively identified as *S. putrefaciens*[a]

Strain	Mol% G+C	% DNA homology with the following probe:				
		IAM 12079^T	SH 329	IAM 13595	IAM 14159^T	IAM 13615
IAM 12079^T	46	**100**	53	54	0	12
E 5	45	95	50	56	ND[b]	ND
SH 329	46	43	**100**	40	14	10
SH 392	46	53	102	46	ND	ND
KM 773	47	36	99	28	ND	ND
KM 776	47	42	101	42	ND	ND
E 4	47	37	96	42	ND	ND
IAM 13595	47	28	23	**100**	10	3
E 2	46	42	24	76	ND	ND
E 7	46	50	42	77	ND	ND
E 28	46	55	36	96	ND	ND
E 37	46	52	43	93	ND	ND
IAM 14159^T	54	8	5	0	**100**	89
IAM 13615	53	9	2	5	105	**100**
IAM 13614	53	ND	ND	ND	ND	83
BH 278	53	ND	ND	ND	ND	106
BH 582	53	ND	ND	ND	ND	101
KM 1109	52	ND	ND	ND	ND	86
KM 1110	53	ND	ND	ND	ND	81
GIFU 1187	53	ND	ND	ND	ND	99
JH 120	54	ND	ND	ND	ND	77
JH 121	54	ND	ND	ND	ND	80
JH 122	53	ND	ND	ND	ND	82
JH 123	54	ND	ND	ND	ND	94
JH 124	53	ND	ND	ND	ND	79
SH 242	53	ND	ND	ND	ND	104
SH 328	53	ND	ND	ND	ND	70
SH 507	53	ND	ND	ND	ND	82
SH 509	54	ND	ND	ND	ND	88
SH 510	53	ND	ND	ND	ND	98
SH 511	54	ND	ND	ND	ND	91
SH 747	53	ND	ND	ND	ND	91
SH 1109	53	ND	ND	ND	ND	80
SH 1160	53	ND	ND	ND	ND	85
SH 4236	53	ND	ND	ND	ND	106
SH 5446	53	ND	ND	ND	ND	81
SH 5563	53	ND	ND	ND	ND	73
SH 6728	53	ND	ND	ND	ND	102
SH 8764	53	ND	ND	ND	ND	108
TPH 200	53	ND	ND	ND	ND	106
TPH 218	54	ND	ND	ND	ND	80
TPH 220	53	ND	ND	ND	ND	77
TPH 519	53	ND	ND	ND	ND	98
TPH 535	53	ND	ND	ND	ND	88
TPH 537	53	ND	ND	ND	ND	88
TPH 5141	53	ND	ND	ND	ND	96
TPH 5142	53	ND	ND	ND	ND	89

[a] IAM 12079 (= ATCC 8071^T) is the type strain of *S. putrefaciens*. IAM 14159 (= OK-1^T) is the type strain of *S. alga*.
[b] ND, not determined.

TABLE 3. Characteristics of *S. alga*

Property	% Positive clinical strains (n = 35[a])	Characteristic of *S. alga* IAM 14159[T]
Motility	100	+
Flagella, polar monotrichous	100	+
Pyoverdin	0	−
Hydrogen sulfide	100	+
Indophenol oxidase	100	+
Trimethylamine oxide	100	+
β-Galactosidase	6	−
Arginine dehydrolase	0	−
Lysine decarboxylase	0	−
Ornithine decarboxylase	100	+
Sodium citrate (Simmons)	0	−
Hydrolysis of:		
Urea	0	−
Lecithin	100	+
Lipid	100	+
Gelatin	100	+
DNA	100	+
Acid in O-F medium from:		
D-Glucose	(60)[b]	+
D-Arabinose	0	−
L-Arabinose	0	−
D-Mannose	0	−
Mannitol	0	−
iso-Inositol	0	−
L-Rhamnose	0	−
D-Ribose	(100)	+
D-Xylose	0	−
Sucrose	0	−
Maltose	0	−
D-Lactose	0	−
Growth under condition:		
SS agar	88	+
6.0% NaCl	100	+
4°C	0	−
37°C	100	+
42°C	97	+
Hemolysis on sheep blood agar	100	+

[a] Number of strains examined, including IAM 13614, IAM 13615, and 33 clinical strains.
[b] Parentheses indicate that the reaction was delayed.

extract (Fig. 1). TTX and anhydrotetrodotoxin (anh-TTX) were detected in culture medium, but, the peaks corresponding to these two compounds were very small and unclear. Additional experiments using tissue culture assay (Table 4) showed the presence of significant levels of sodium channel-blocking toxin in both cells and culture medium for two strains (TPH 519 and SH 8764) or in either the cells or culture medium for two strains (IAM 13615 and JH 122).

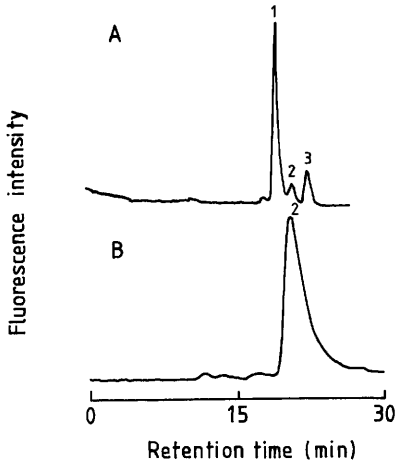

FIG. 1. HPLC chromatogram of TTX and its derivatives. (A) Extract from strain JH 122 of *S. alga*; (B) authentic TTX and its derivatives. Peaks: 1, TTX; 2, 4-*epi*-TTX; 3, anh-TTX.

DISCUSSION

Since the first report by King on clinical isolates of *S. putrefaciens* (10), the strains were conventionally identified by the following characteristics: nonfermentative, gram-negative, rod shaped, oxidase positive, hydrogen sulfide production. However, heterogeneity of the species was pointed out by many researchers (2, 7, 13, 14, 18, 20, 21, 23, 24, 28), especially the remarkable differences between clinical and environmental isolates. One of the reasons why taxonomic study of these isolates had not been done was the difficulty in collecting strains from human clinical specimens, because *S. putrefaciens* infections in humans are rare.

We obtained 40 strains individually isolated from human clinical specimens during the period of 1978 to 1991 in Japan. Studies of the characteristics of these strains led us to the findings that 33 of these strains were β-hemolytic and had apparently higher moles percent G+C values than that of the type strain of *S. putrefaciens*. Taxonomic studies on *S. putrefaciens* strains, including the type strain, some IAM strains, and newly isolated clinical and environmental strains, were then performed. The results showed that all clinical strains that have 52 to 54 mol% G+C belong to *S. alga*, not to *S. putrefaciens*.

The type strain of *S. alga* (IAM 14159[T]), which was isolated from red algae is reported to be a TTX-producing marine bacterium (25, 26). We showed that four clinical strains identified as *S. alga* in this study produced 4-*epi*-TTX

TABLE 4. Production of TTX and its derivatives by *S. alga* strains isolated from human clinical specimens

Bacterial strain	Source	Compound detected by HPLC			Quantitative measurement of TTX by tissue culture assay (ng/g of cells)
		TTX	4-*epi*-TTX[a]	Anh-TTX	
IAM 13615	Cell extract	−	+	−	<142
	Culture medium	+	−	+	1,302
JH 122	Cell extract	−	+	−	335
	Culture medium	+	−	+	<223
TPH 519	Cell extract	−	+	−	341
	Culture medium	+	+	+	341
SH 8764	Cell extract	−	+	−	279
	Culture medium	+	−	+	2,440

[a] 4-*epi*-TTX and anh-TTX are TTX derivatives with lower lethal toxicities to mice than that of TTX (19).

TABLE 5. Characteristics distinguishing *S. alga* from *S. putrefaciens*[a]

Species	G+C content (mol%)	Hemolysis on sheep blood agar	Growth under condition:				Acid produced from:	
			6% NaCl	4°C	42°C	SS agar	Maltose	L-Arabinose
S. alga	52–54	+ (100)	+ (100)	− (0)	+ (97)	+ (88)	− (0)	− (0)
S. putrefaciens	45–48	− (0)	− (0)	+ (65)	− (0)	− (8)	+ (88)	+ (100)

[a] Thirty-six strains of *S. alga* were used, including IAM 14159^T (the type strain of *S. alga*), IAM 13614 and IAM 13615 (which were originally classified as *S. putrefaciens*), and 33 clinical strains. Forty-one strains of *S. putrefaciens* were used, including IAM 12079^T (the type strain of *S. putrefaciens*), IAM 13595, and seven clinical strains and 32 environmental strains isolated in this study. Values in parentheses are the percentages of positive strains.

and might release TTX or anh-TTX into the culture medium. The results of tissue culture assay which is used for detection of the sodium-channel blocking activity of TTX also support the production of TTX and its derivatives by these strains.

On the basis of 35 strains genetically identified as *S. alga*, we clarified the physiological differences between *S. alga* and *S. putrefaciens* as presented in Table 5. Thus, we propose the emended description of *S. alga* given below.

Emended description of *Shewanella alga* (Simidu, Kita-Tsukamoto, Yasumoto, and Yotsu 1990). Cells are gram-negative, straight short rods and have polar flagella. They grow in 6% NaCl, on Salmonella-Shigella agar, and at 42°C, but not at 4°C. Colonies become yellow-orange or brown after 2 days of incubation at 37°C. Reduction of trimethylamine oxide reaction is positive. All strains produce hydrogen sulfide, indophenol oxidase, ornithine decarboxylase, gelatinase, DNase, lecithinase, and lipase. Acids are produced by oxidation from D-ribose, but not from D-arabinose, L-arabinose, D-mannose, mannitol, *iso*-inositol, L-rhamnose, D-xylose, sucrose, maltose, and D-lactose. Acid production from D-glucose is variable. They exhibit hemolysis on sheep blood agar and are pathogenic to humans. The G+C content of the DNA is 52 to 54 mol%. Production of tetrodotoxin and its derivatives is confirmed by the type strain (IAM 14159^T) (25, 26) and some clinical strains.

The species can be differentiated from *S. putrefaciens* by the following characteristics: growth at 42°C, in 6% NaCl, and on Salmonella-Shigella agar, hemolysis on sheep blood agar, acid production from D-ribose but not from L-arabinose.

The type strain is IAM 14159 (OK-1; Simidu et al., 1990).

The moles percent G+C of the type strain is 54 (Simidu et al., 1990).

Conclusions. Thus, we reidentified the strains of *S. putrefaciens* which have 52 to 54 mol% G+C as *S. alga*. However, the question remains as to whether or not *S. alga* belongs to the genus *Shewanella*, because *S. alga* is the only species in the genus that has a high moles percent G+C. Analysis of part of the sequence of 16S rRNA of *S. alga* showed that this strain is more closely related to *S. putrefaciens* than to species in the *Pseudomonas* or *Alteromonas* genus (26). Comparative study of the full sequence of 16S rRNA with other strains in the *Shewanella* genus is needed to confirm the taxonomic situation of *S. alga*.

At the same time, we found at least three DNA homology groups within the strains which have 45 to 48 mol% G+C. Similar findings for the *S. putrefaciens* strains have been reported by several researchers (14, 20, 23, 28). At present, however, we could not find significant traits to differentiate these homology groups. A new classification of *S. putrefaciens* should be proposed in the near future after significant characteristics distinguishing between the different DNA homology groups are found.

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