Runella slithyformis gen. nov., sp. nov., a Curved, Nonflexible, Pink Bacterium

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Two strains of bacteria regarded as belonging to a new species were isolated from bodies of water near Baton Rouge, La. The cells of these strains were gram-negative, curved rods, the degree of curvature varying among cells in a single culture. A pink pigment was produced on glucose-peptone-yeast extract agar. The strains were nonmotile and nonfermentative, and the guanine-plus-cytosine contents of their deoxyribonucleic acids varied from 49.3 to 49.6 mol%. The species cannot be assigned to any known genus, and therefore a new genus, *Runella*, is proposed, with *R. slithyformis* as the type species. The type strain of this species is strain 4 (= ATCC 29530). At present, it is difficult to place the genus *Runella* in a family.

During examination of the bacteria that inhabit the bodies of fresh water in southern Louisiana, we repeatedly encountered bacteria that resembled those of the newly described genus *Flectobacillus* (2). The organisms were seen both on slides that had been suspended in water and in enrichments made by adding 0.1 g of peptonized milk to 100 ml of the water sample. We were successful in isolating two strains of these organisms, and subsequent characterization studies indicated that they are physiologically quite different from flectobacilli even though they are similar morphologically. These strains represent a new species and genus of bacteria, descriptions of which are presented.

MATERIALS AND METHODS

Bacterial strains. Two similar strains were isolated from water from the Baton Rouge, La., area by repeated streaking of water samples onto MS agar (0.1% each of glucose, peptone, and yeast extract, plus 1.5% agar) with incubation at room temperature. One strain, designated as strain 4, was isolated from University Lake, and the other, designated as strain 6, was isolated from Elbow Bayou. Cultures of these strains were maintained on MS agar, with transfers made at about 2-week intervals.

Utilization of carbohydrates. The production of acid from carbohydrates by aerobic or anaerobic means was determined by the method of Hugh and Leifson (1), in which MS agar was used but with the glucose replaced by 1% of the substrate to be tested and the agar concentration lowered to 0.3%. Incubation was continued for 8 weeks for cultures giving negative results.

Ability to grow on various media. The abilities of the organisms to grow on various media were investigated by making a single streak of the organisms from a fresh slant onto the surface of the medium and incubating at 25°C for a minimum of 3 weeks. The media tested were chocolate agar, blood agar,

eosin-methylene blue agar, phenol red-mannitol-salt agar, phenyl ethyl alcohol agar, nutrient agar, nutrient agar plus 5% sucrose, Trypticase soy agar, Trypticase soy agar plus 3% glucose, peptonized milk agar, MS agar, yeast extract-acetate-tryptone agar, McConkey agar, bismuth sulfide agar, and salmonella-shigella agar.

Additional characters. The techniques and methods used to determine the abilities of the strains to utilize sole carbon sources, to hydrolyze macromolecules, to produce specific enzymes or end products, and to determine their susceptibilities to antibiotics have been reported previously (2).

Two techniques were used to determine the guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNA) of the strains. One technique, used with strain 6, made use of the Marmur method (6) to purify the DNA, followed by determining the melting-point profile with a Gilford model 2400 spectrophotometer equipped with a temperature-controlled cuvette compartment (5). In the other technique, used with strain 4, DNA was partially purified by the method of Meyer and Schliefer (7), and the mole percent G+C was determined by the absorbance ratio technique of Ulitzer (9). Measurements of cell size were obtained with a Filar micrometer. Photomicrographs were obtained with a Gillet and Seibert microscope equipped with a Nikon model AFM camera.

RESULTS

The results of the analysis of the two strains are summarized in Table 1. The cells were curved rods, with the degree of curvature of individual cells within a culture varying from nearly straight to curved in the shape of a ring. Coils of two to three turns were rarely formed, and filaments up to 14 μ m in length were produced. This morphological variation prompted us to determine whether the cells were flexible or capable of gliding motility, but observations

TABLE 1. *Characteristics of two strains of Runella slithyformis*^a

Character	Strain 4	Strain 6	Character	Strain 4	Strain 6
Gram reaction	—	—	Indole production	—	—
Pigmentation	Pale pink	Pale pink	Methyl red test	—	—
Fluorescence	—	—	Voges-Proskauer test	—	—
Formation of coils	Rare	Rare	NO ₃ reduction	—	—
Motility	—	—	H ₂ S production	—	—
Mol% G+C	49.6	49.3	Hydrolytic activity on:		
Cell size:			Cellulose	—	—
Length (μm)	2.5–4.0	2.0–4.5	Agar	—	—
Diameter (μm)	0.5–0.9	0.6–0.9	Chitin	—	—
Diameter of rings (μm)	2.0–3.0	2.0–2.5	Gelatin	—	—
Filament length (μm)	Up to 12.0	Up to 14.0	Starch	sl+	sl+
Acid produced aerobically from:			Casein	NG	NG
Pentoses			Esculin	—	—
Arabinose	—	—	Tributyrin	+	+
Ribose	—	—	Litmus milk	No change	No change
Xylose	NG	NG	Utilization of sole carbon sources:		
Methyl pentose			Acetate	—	—
Rhamnose	—	+	Benzoate	—	—
Hexoses			Citrate	—	—
Fructose	—	—	Formate	—	—
Galactose	+	—	Glycerol phosphate	—	—
Glucose	+	+	Methylamine	—	—
Mannose	sl+	NG	Propionate	—	—
Sorbose	—	—	Succinate	—	—
Glucosides			Tartrate	—	—
α-Methyl-D-glucoside	—	—	Malonate	—	—
Salicin	—	—	Methanol	—	—
Disaccharides			Growth on agar media:		
Cellobiose	—	—	Chocolate	1	1
Lactose	—	—	Blood	NG	NG
Maltose	+	+	EMB	NG	NG
Melibiose	—	—	NA	3	3
Sucrose	+	+	NAS	NG	NG
Trehalose	NG	NG	PRMS	NG	NG
Trisaccharide			PEA	NG	NG
Raffinose	—	+	TSA	NG	NG
Polysaccharides			TSAS	NG	NG
Dextrin	—	—	TSAG	NG	NG
Inulin	+	+	PMA	1	1
Alcohols			MS	4	4
Glycerol	—	—	YEAT	1	1
Erythritol	—	—	McConkey	NG	NG
Dulcitol	—	—	BS	NG	NG
Mannitol	—	—	SS	NG	NG
Sorbitol	—	—	Antibiotic susceptibility to:		
Production of enzymes			Actinomycin D (100 μg/ml)	S	S
Urease	—	—	Ampicillin (10 μg)	S	S
Lecithinase	—	—	Aureomycin (15 μg)	S	S
Lysine decarboxylase	—	—	Carbenicillin (50 μg)	S	S
Ornithine decarboxylase	—	—	Cephalothin (30 μg)	S	S
Phenylalanine deaminase	—	—	Colistin (10 μg)	R	R
ONPG	+	+	Erythromycin (15 μg)	S	S
Oxidase	+	+	Furadantin/macrodantin (300 μg)	S	S
Catalase	sl+	sl+	Gentamycin (10 μg)	S	S
Phosphatase	+	+	Kanamycin (30 μg)	R	S
Hemolysin	—	—			

TABLE 1—Continued

Character	Strain 4	Strain 6	Character	Strain 4	Strain 6
Mitomycin C (1 µg/ml)	S	S	Sulfamethoxazole/tri- methopterin (25 µg)	S	S
Neomycin (30 µg)	S	R	Tetracycline (30 µg)	S	S
Penicillin G (10 U)	S	S	Triple sulfa (1 mg)	R	R
Polymyxin B (300 U)	R	R			
Streptomycin (10 µg)	S	S			

" Symbols: +, positive, —, negative; NG, no growth. The growth responses on various media are graded from 1 to 4 for scant to luxurious growth, respectively. Abbreviations: S, susceptible; R, resistant; EMB, eosin-methylene blue; NA, nutrient agar; NAS, nutrient agar plus 5% sucrose; PRMS, phenol red-mannitol-salt agar; PEA, phenyl ethyl alcohol agar; TSA, Trypticase soy agar; TSAG, Trypticase soy agar plus 3% glucose; PMA, peptonized milk agar; YEAT, yeast extract-acetate-tryptone agar; BS, bismuth sulfide agar; SS, salmonella-shigella agar.

FIG. 1. Phase micrographs of cells of two strains of *Runella slithyformis*: (A) strain 4, and (B) strain 6. $\times 1,500$.

of the cells in microculture, in broth, and on agar media indicated that they were rigid and nonmotile.

In the Hugh and Leifson (1) oxidation/fermentation technique, the cells grew and produced acid only aerobically, thus indicating a respiratory metabolism. Acid was produced from less than one-third of the carbohydrates tested, and some carbohydrates (xylose, trehalose) appeared to prevent growth in three replicate sets

of tubes. Figure 1 shows the typical morphology of these two strains.

DISCUSSION

The organisms described above are sufficiently different from those of the known genera to merit their recognition as members of a new genus, which we propose to call *Runella*. In Table 2 are listed some of the characteristics that help to differentiate *Runella* from other genera that have some characteristics in common with the new genus.

Runella resembles *Vibrio* in DNA G+C content and in being curved, but it differs from *Vibrio* in being nonmotile, nonfermentative, incapable of anaerobic growth, and in producing a pink pigmentation. *Runella* differs from *Cytophaga* and *Flexibacter* in DNA G+C content and in being nonmotile; additionally, it differs from *Cytophaga* in being nonfermentative and unable to hydrolyze cellulose, agar, or chitin. *Runella* differs from *Spirosoma* in that the former is unable to produce coiled filaments readily or to hydrolyze gelatin and in that it produces a pink pigment rather than the yellow pigment produced by *Spirosoma*. Moreover, *Runella* produces acid from much fewer carbohydrates than does *Spirosoma* (2), and it is unable to utilize glycerol phosphate, succinate, tartrate, or malonate as the sole source of carbon.

In its cellular morphology and pigmentation, *Runella* most nearly resembles *Flectobacillus*, but it differs from that genus in that it has a higher DNA G+C content and is unable to produce urease, to hydrolyze esculin and gelatin, or to grow with succinate as a sole source of carbon.

These differences from the previously described genera indicate that the two isolates described in this paper should be considered as members of a new genus, *Runella*. The name is derived from the Middle English word 'rune,' which meant secret writing and which later became an alphabet used by ancient Scandinavians and Anglo-Saxons. The irregular curvature

of the cells resembles some of the early runic figures.

A description of the genus follows:

Runella gen. nov. (Run. el' la. M.E. n. *rune* an ancient alphabet; M.L. dim. ending *-ella*; M.L. fem.n. *runella* that which resembles figures of the runic alphabet).

Straight to curved rods, the degree of curvature varying among cells within a culture. The cells measure 0.5 to 0.9 by 2.0 to 4.5 μ m. The ends of a cell may overlap, producing a ring-shaped structure with an overall diameter of 2.0 to 3.0 μ m. Filaments up to 14 μ m long may be produced. On rare occasions, a coil of two to three turns may be produced. Gram negative. Nonmotile. Not flexible. Resting stages are not known. Colonies on MS agar contain a pale pink, non-water-soluble pigment.

Metabolism is respiratory; acids are produced aerobically from several carbohydrates. Strictly aerobic. Chemoorganotrophic.

Urease is not produced, and gelatin and esculin are not hydrolyzed.

Isolated from fresh water.

The G+C content of the DNA ranges from 49 to 50 mol%.

The type species is *R. slithyformis*.

Runella slithyformis sp. nov. (slith.y.form' is *slithy* a nonsense word from Lewis Carroll's *Jabberwocky* for a fictional organism that is

"slithy" and can "gyre and gimble"; L. n. *forma* shape, form; M.L. adj. *slithyformis* slithy in form).

Rods vary from nearly straight to crescent shaped; filaments up to 14 μ m may occur. Rings with an outer diameter of 2.0 to 3.0 μ m may also occur. The cells vary in diameter from 0.5 to 0.9 μ m, and the usual length is 2.0 to 3.0 μ m. Coiled filaments are rarely produced. Gram negative. Nonmotile. Nonflexible. On MS agar, a pale pink, nondiffusible, nonfluorescent pigment is produced.

Metabolism is respiratory. No growth occurs under an anaerobic seal of pyrogallol-potassium hydroxide. Acid is not produced from arabinose, ribose, xylose, fructose, sorbose, α -methyl-D-glucoside, salicin, cellobiose, lactose, melibiose, trehalose, or dextrin. Acid is produced aerobically from glucose, maltose, sucrose, and inulin. Acid production from rhamnose, galactose, mannose, and raffinose is variable. Acids are not produced from the alcohols glycerol, erythritol, dulcitol, mannitol, and sorbitol.

Urease, lecithinase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, and hemolysin are not produced. Oxidase, β -galactosidase, and phosphatase are produced. Catalase activity is weak. Indole and H₂S are not produced. The methyl red and Voges-Proskauer tests are negative, and nitrate is not reduced.

TABLE 2. Comparison of *Runella* with other genera having some characteristics in common with it

Characteristic	Genus ^a					
	<i>Vibrio</i>	<i>Cytophaga</i>	<i>Flexi- bacter</i>	<i>Spiro- soma</i>	<i>Flecto- bacillus</i>	<i>Runella</i>
Motile (by means of)	+	+	+	—	—	—
	(Flagella)	(Gliding)	(Gliding)			
Respiratory metabolism	+	+	+	+	+	+
Fermentative metabolism	+	±	—	—	—	—
Anaerobic growth	+	±	—	—	—	—
Mol% G+C of DNA	40–50	33–42	31–43	51–53	39–41	49–50
Hydrolysis of cellulose, chitin, or agar	—	+	—	—	—	—
Urease	—	NA	NA	—	+	—
Esculin hydrolysis	NA	NA	NA	±	+	—
Gelatin hydrolysis	+	+ or NA	NA	+	+	—
Helical coils	—	—	—	+	Rare	Rare
Growth on:						
Glycerol-PO ₄	NA	NA	NA	+	—	—
Succinate	+	NA	NA	+	+	—
Tartrate	NA	NA	NA	+	—	—
Malonate	NA	NA	NA	+	—	—
Pigmentation	— or yellow	Yellow, or- ange, red	Yellow, or- ange, red, pink	Yellow	Pink	Pink

^a Data for *Vibrio*, *Cytophaga*, and *Flexibacter* are from references 3, 4, and 8; data on *Spirosoma* and *Flectobacillus* are from reference 2. Symbols: +, positive; —, negative; NA, data not available from the source used.

Starch and tributyrin are hydrolyzed, but cellulose, agar, chitin, gelatin, casein, and esculin are not. Litmus milk is unchanged.

Acetate, benzoate, citrate, formate, glycerol phosphate, methylamine, propionate, succinate, tartrate, malonate, and methanol cannot be utilized as sole sources of carbon.

The G+C content of the DNA ranges from 49.3 to 49.6 mol% (thermal denaturation or ratio absorbance).

Both strains have been deposited with the American Type Culture Collection (ATCC), Rockville, Md. under the accession numbers 29530 and 29531 for strains 4 and 6, respectively. Strain 4 is designated as the type strain of *R. slithyformis*. A description of this strain is given in Table 1.

At the present time, it is difficult to place the genus *Runella* in a family.

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REPRINT REQUESTS

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