Clostridium ramosum (Vuillemin) comb. nov.: Emended Description and Proposed Neotype Strain

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Seventeen strains of organisms which conform to the original and subsequent descriptions of the organism currently known as *Ramibacterium ramosum*, and which include Prévot's strain 113-I as well as recent isolates, have been shown to produce heat-resistant spores which are often difficult to detect. For this species we propose the name *Clostridium ramosum* (Vuillemin) comb. nov. (basonym: *Nocardia ramosa* Vuillemin) and Prévot's strain 113-I (ATCC 25582, VPI 0427) as the neotype. Recently isolated strains were from stitch abscesses, urinary infections, abdominal fluid, rectal abscesses and drainage, perineal drainage, vagina, feces, and sigmoid colon contents. Prévot-collection strains were from appendicitis, colitis, liver abscesses, and feces. Previously undescribed characteristics of this species are presented.

In 1898, Veillon and Zuber (8) described an anaerobic organism from pulmonary gangrene which they named *Bacillus ramosus*. However, this name is illegitimate because it is a later homonym of *Bacillus ramosus* Frankland and Frankland 1889. Later, Vuillemin (10) named this organism *Nocardia ramosa* and, according to the Note under Rule 26 of the International Code of Nomenclature of Bacteria (3), this represents a legitimate use of the specific epithet "*ramosus*," and Vuillemin, rather than Veillon and Zuber, is to be regarded as the author of this specific epithet.

Up to the present, this organism has been regarded as a nonsporeforming anaerobe, and in 1938 Prévot made it the type species of a new genus, *Ramibacterium*. Evidence is presented here, however, to show that this organism is a sporeforming anaerobe belonging to the genus *Clostridium*.

The original description of *Bacillus ramosus* by Veillon and Zuber (8) is minimal and presents only cellular and colonial morphology, gas production, odor, and pathogenicity. Nevertheless, we think the organisms described here are in all probability representative of the cultures studied by Veillon and Zuber. The strains we examined include the available strains labeled *Ramibacterium ramosum* from the Prévot collection. Of these, strain 113-I conforms closely to the original description by

Veillon and Zuber as well as to emended descriptions by Weinberg et al. (reference 11, photograph p. 521) and Prévot et al. (reference 6, photograph p. 461).

MATERIALS AND METHODS

Cultures. Cultures examined, and their sources, are listed in Table 1.

Methods. The cultures were examined according to the procedures given in the *Outline of Clinical Methods in Anaerobic Bacteriology* (9).

RESULTS AND DISCUSSION

Cellular morphology. Veillon and Zuber (8) described strains from pulmonary gangrene (p. 531) and from appendicitis (p. 537) as nonmotile, quite thin, gram-positive rods, straight or curved, with tapered ends, often of variable length, occurring in pairs, even short chains. Irregular rods and pseudofilaments made up of short rods are found. Cells occur singly, in pairs, entangled, or in masses, or in "V" and "Y" arrangement (which Veillon and Zuber, but not later authors, interpreted as true branching), often with swellings. In pus, the rods are usually short. "We have not demonstrated the formation of true spores" [translation]. In addition, Prévot et al. (6) reported cell measurements of 0.3 to 0.4 by 2 to 3 μ m and the presence of spherical swellings.

The strains described here conform to the

TABLE 1. *Sources of strains examined*

VPI no. ^a	Source
0123	Prévot Collection, Institute Pasteur (Paris) 91 Bis, appendix
0124	Beerens Collection, Institute Pasteur (Lille) 315
0127	Prévot Collection, one of two species isolated from 1659B, colitis
0427 ^b	Prévot Collection 113-I, no source indicated, record dated 1927
2932	Prévot Collection 4029, abscess
3090	Prévot Collection 3206A, feces
3206H	Isolated from culture of rectal abscess material received from A. Balows, Lexington Clinic, Lexington, Ky.
3515B	VPI isolate, feces
3526A	VPI isolate, feces
3550	VPI isolate, feces
4034D	Isolated from culture of stitch abscess pus received from A. Balows
4495H	Isolated from culture of urine received from John Poore, Corning Hospital, Corning, N.Y.
4616	From abdominal fluid, received for identification from John Poore
5027F	Isolated from culture of rectal drainage received from A. Balows
5606	From vagina, received for identification from D. W. Lambe, Jr., Emory University Hospital, Atlanta, Ga.
5607	From perineal drainage, received for identification from D. W. Lambe, Jr.
5614	From colon drainage, received for identification from Mary Fried, Tuscon Medical Center, Tuscon, Ariz.

^a Virginia Polytechnic Institute number.

^b Proposed neotype strain (ATCC 25582).

above descriptions and are nonmotile, quite thin, gram-positive rods, straight or slightly curved with rounded to slightly tapered ends, occurring singly, in pairs and short chains with "V" and "Y" arrangements, short winding chains with angular ("rail fence") appearance, or irregular masses. Central swellings and terminal or nearly terminal swellings are often seen. Cells of the proposed neotype strain are 0.6 to 0.9 μm wide by 2.3 to 10+ μm long; other strains range from 0.5 to 0.7 μm wide (with swellings up to 1.6 μm) by 2 to 12.8 μm long (with filaments up to 40+ μm).

Only in two morphological characteristics, branching and spore formation, do the present strains differ in any way from the entire original description. Veillon and Zuber described branching, and indeed cellular arrangement suggests branching (Fig. 1). Prévot concluded that this is false branching, and so it appears to us. Veillon and Zuber and later authors failed to detect spores. Indeed, we found that many cultures of the present strains fail to yield heat-resistant spores. However, if several tests are made in peptone-yeast extract-glucose broth or old chopped meat-agar slants, thin-walled, terminal to subterminal, nearly spherical spores can be observed in one in a thousand or more cells (Fig. 1). Such cultures are heat resistant, and outgrowth yields cultures

Fig. 1. Photomicrographs of Gram-stained cells of *Clostridium ramosum* ATCC 25582, proposed neotype strain. A and B, from 48-hr peptone-yeast extract-glucose culture. Note terminal swelling and "branching" in A and spore in B. C, from 24-hr chopped meat-glucose culture mixed with an equal volume of sterile distilled water and incubated at 37 C for 5 hr to demonstrate spore. Gram-stained on the under side of a cover slip (method of Robinow).

with the same properties as the original culture. One or more cultures of all strains resisted 80 C for 10 min. One strain (4616) tested resisted 90 C for 10 min and boiling for 10 min.

Colonial morphology. Veillon and Zuber described deep agar colonies in 48 hr as round to oval, pin point, fine, granular, brownish-yellow or grey. At first the edges of the

TABLE 2. Characteristics of strains of *Clostridium ramosum*

Characteristic	Test results		Reported characteristics	
	0427 (113-I Prévot) ^{a, b}	16 other strains ^b	0427 (113-I Prévot)	<i>R. ramosum</i> Prévot et al. (1967)
Hemolysis	— ^c	—		—
Egg yolk agar reactions	—	—		
Heat resistance 80 C, 10 min.	+ ⁻	+		—
Broth growth				
Turbid	+	+	+	+
Ropy sediment	v	v	+	
Smooth sediment . . .	v	v		
Growth in				
20% Bile-1% glucose .	+	+		
6.5% NaCl	— ⁺	— ⁺		
pH 9.6	+	+		
Gas (glucose agar) . . .	mod	sl-3+	sl	mod
Reduction of				
Nitrate	—	—		—
Neutral red	+	+	+	+ → —
Resazurin	+	+		
Acid from				
Amygdalin	A	A ^w		
L-Arabinose	—	— ^w		
Cellobiose	A	A		
Dextrin	— ^w	— ^w		
Dulcitol	—	— ^w		
DL-Erythritol	—	— ^w		
Esculin	w	wA		
D-Fructose	A	A	+	
D-Galactose	A	A	+	+
Glucose	A ^d	A ^e	+	+
Lactose	A	A	+	+
D(+)-Maltose	A	A		+
D-Mannitol	A ^w	v	+	+
D(+)-Mannose	A	A		
Melibiose	A ^w	A		
Pectin	A	A ^w		
Raffinose	A	A		
Rhamnose	—	v		
D(—)-Ribose	A ^w	v		
Salicin	A	A		
Starch	—	— ^w		
Sucrose	A	A		+
Trehalose	A	A		
D-Xylose	—	— ^w		

(continued)

TABLE 2. *Characteristics of strains of Clostridium ramosum* (Con't.)

Characteristic	Test results		Reported characteristics	
	0427 (113-I Prévot) ^{a,b}	16 other strains ^b	0427 (113-I Prévot)	<i>R. ramosum</i> Prévot et al. (1967)
Fermentation of				
Lactate	—	—		
Pyruvate	+	+		
Milk				
Curd	+	+	+	+
Digestion	—	—	—	—
Gelatin digestion	—	— ^{sl}	—	—
Chopped meat digestion	—	—	—	—
Hydrolysis of				
Esculin	+	+		
Starch	—	—		
Hippurate	—	—		
Production of				
Indole	—	—	—	—
Catalase	—	—		
Acetoin (AMC)	v	v		+
H ₂ S	—	—		
NH ₃	v	v		+
Acids from PY-glucose broth cultures (meq/100 ml)				
Acetic				
Average	1.7	1.2		+
Range	1.3–2.0	0.6–2.0		
Formic				
Average	2.4	2.5		+
Range	1.8–3.2	1.3–5.6		
Lactic				
Average	0.9	1.2		
Range	0.5–1.4	0.3–3.0		
Succinic				
Average	0.3	0.3		
Range	0.2–0.6	0.0–0.8		

^a Results from five replicate test sets.

^b Strains grow well at 25 to 45 C. Most strains produce a noticeable odor (musty, sour, or fetid) when grown on Brain Heart Infusion agar. No acid from adonitol, glycerol, glycogen, inositol, inulin, melezitose, D-sorbitol, L-sorbose. No propionic, isobutyric, *n*-butyric, isovaleric, *n*-valeric, isocaproic, *n*-caproic, heptanoic, or caprylic acids detected.

^c Superscripts indicate variation of 10 to 30% of the strains or test sets. Symbols: +, 90 to 100% of the strains or test sets positive; —, 90 to 100% of the strains or test sets negative; v, variable; A, pH below 5.5; w, pH 5.5 to 6.0; sl, slight; mod, moderate.

^d pH 4.8.

^e pH 4.5 to 5.0.

colonies are diffuse or bristled like the fuzz of chicks. Later, the colonies develop more well-defined edges. Surface colonies are small, grey-white-brownish, transparent. On continued incubation, they enlarge slightly, become yellowish, and have a well-defined edge.

Surface colonies of the proposed neotype strain are 0.5 to 2 mm in diameter, circular to slightly irregular, entire to slightly scalloped, convex, translucent, smooth, colorless to grey-white.

Surface colonies of other strains are similar

to those of the proposed neotype strain. However, colonies of some strains are raised or slightly umbonate, some are semiopaque, some have diffuse edges, and some (by obliquely transmitted light) have a slightly mottled or finely granular appearance.

Other characteristics originally described. Veillon and Zuber further stated that the organism was obligately anaerobic, did not grow at room temperature, and produced only a small amount of gas and a characteristic fetid odor. Broth cultures were turbid with a fine white sediment in 3 to 4 days and were still viable after 1 month. Subcutaneous abscesses were produced in guinea pigs and also in rabbits. In rabbits, the infection was fatal in 8 to 10 days. Intravenous inoculation in rabbits was fatal in several days with "intoxication et cachexie."

Although no other information was given in the original description, the species was recognized by many subsequent authors, and some additional information was provided in descriptions by Hauduroy et al. (2) and Weinberg, Nativelle, and Prévot (11), for examples. The characteristics reported by these authors [as summarized by Prévot et al. (6)] are included in Table 2, in which also are listed the characteristics of the neotype strain and of 16 other strains of the species.

According to Prévot's records of the proposed neotype strain, 2 cc inoculated into a guinea pig produced shock, shaking, crying, weight loss, cachexia, and death in 18 days with no macroscopic lesions; 5 cc of culture produced shock, shaking, slight swelling, emaciation, and death in 11 days; 5 cc of cells inoculated intraperitoneally produced cachexia and death in 25 days with no lesions. According to Prévot et al. (6), laboratory cultures lose pathogenicity in 4 to 6 weeks. Pathogenicity cannot be maintained by animal passage. Many references concerning isolations from human infections are given.

Since we have shown that the organisms are anaerobic, sporeforming rods, the species is a member of the genus *Clostridium*. John L. Johnson of our laboratory has demonstrated for these strains a guanine-cytosine ratio of 27, which is similar to the value for many other species of the genus *Clostridium*. Cell walls contain meso-diaminopimelic acid (C. S. Cummins, our laboratory).

We propose the organism be designated *Clostridium ramosum* (Vuillemin) comb. nov.

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Objective synonyms: *Bacillus ramosus* Veillon and Zuber 1898, p. 531 and 537. (Not *Bacillus ramosus* Frankland and Frankland 1889, p. 388.) *Nocardia ramosa* Vuillemin 1931, p. 132. *Actinomyces ramosus* (Vuillemin) Nannizzi 1934, p. 42. *Fusiformis ramosus* (Vuillemin) Topley and Wilson 1936, p. 358. *Bacteroides ramosus* (Vuillemin) Hauduroy et al. 1937, p. 71. *Ramibacterium ramosum*, [Veillon and Zuber(sic)] Prévot 1938, p. 296.

ra.mo'sum. L. adj. *ramosum* much-branched.

Proposed neotype strain: ATCC 25582 ← V.P.I. 0427 ← Prévot 113-I.

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