Reclassification of *Chromobacterium iodinum* (Davis) in a Redefined Genus *Brevibacterium* (Breed) as *Brevibacterium iodinum* nom.rev.; comb.nov.

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Chromobacterium iodinum (Davis) differs to such an extent from the type species of *Chromobacterium*, *Chr. violaceum* (Bergonzini), that it cannot be retained in this genus. Chemical, morphological and numerical phenetic data indicate a close relationship between *Chr. iodinum* and the species *Brevibacterium linens* (Wolff, Breed). It is suggested that *Chr. iodinum* be reclassified in a redefined genus *Brevibacterium* as *B. iodinum* (Davis) nom.rev.; comb.nov.

INTRODUCTION

The generic assignment of the bacteria named *Chromobacterium iodinum* (Davis, 1939) has always been controversial (Tobie, 1945; Gilman, 1953; Sneath, 1956, 1960; Steele, 1961; Gerber & Lechevalier, 1964; Colwell *et al.*, 1969). In the 8th edition of *Bergey's Manual of Determinative Bacteriology* the species is listed as *incertae sedis* (Sneath, 1974) and it does not appear in the Approved List of Bacterial Names (Skerman *et al.*, 1980).

Chromobacterium iodinum was isolated from milk during a routine bacteriological examination (Davis, 1939), but the original source could have been water, soil, human or animal. Davis (1939) described the organisms as Gram-negative, non-sporing, non-motile rods and stated that the most distinctive feature was the production of dark purple crystals on the surface of colonies and in the adjacent medium. He also noted the similarity between the pigment and crystals of 'ordinary iodine'. Chemical investigations (Clemo & McIlwain, 1938; Tobie, 1945; Clemo & Daglish, 1950) showed that the pigment, named iodinin, was a phenazine derivative quite unrelated to violacein, the pigment component of *Chromobacterium violaceum* (Tobie, 1938, 1939). Phenazines and their derivatives were thought to be fairly uncommon and for this reason Tobie (1945) suggested that all bacteria producing these compounds should be included in the genus *Pseudomonas*. He therefore proposed the reclassification of *Chr. iodinum* as *Pseudomonas iodina* and later *Pseudomonas iodinum* (Tobie, 1939, 1945). However, phenazines and their derivatives have subsequently been shown to be present in a variety of Gram-negative and Gram-positive bacteria (Gerber & Lechevalier, 1964; Lechevalier *et al.*, 1971).

Later studies of *Chr. iodinum* (*P. iodinum*), notably those of Gilman (1953), Steele (1961) and Sneath (1956, 1960), indicated that the organism was a Gram-positive rod with a coryneform morphology. Gilman (1953), however, thought the species should be retained in the genus *Pseudomonas*, despite its Gram-positive staining reactions. Sneath (1960)

suggested that it might well be classified in the genus *Corynebacterium* or in the genus *Brevibacterium* but did not pursue the matter further.

Colwell *et al.* (1969), as a result of a comprehensive study of three cultures named *Pseudomonas iodium*, proposed that the bacteria be reclassified in the genus *Arthrobacter*, as *Arthrobacter iodium*. However, in the numerical taxonomic study of Jones (1975), one strain of *Chr. iodium* (labelled 'coryneform species' NCIB 8179) was recovered in the same cluster as strains of *Brevibacterium linens* and showed no close relationship to any of the other coryneform taxa investigated, including *A. globiformis* and several other strains of the genus *Arthrobacter*. Nevertheless, Jones (1975) stated that the recovery of *P. iodium* in the same cluster as *B. linens* 'is not easily explained' because '... All attempts to demonstrate the presence of the *B. linens* carotenoid-like material failed. It is possible that it is a carotenoid-less mutant but it would additionally have had to develop the purple crystals of iodinin.' Further support for a relationship between the two species comes from the report that *Chr. iodium*, like *B. linens*, has a directly cross-linked meso-diaminopimelic acid-containing peptidoglycan (Fiedler *et al.*, 1970; Schleifer & Kandler, 1972), and that the guanine plus cytosine (G+C) content of the DNA is 63 mol % (Colwell *et al.*, 1969), similar to that of *B. linens* (Crombach, 1972; Yamada & Komagata, 1970, 1972).

It is now recognized that the results of lipid analyses are of value in the taxonomy of the coryneform bacteria (Collins *et al.*, 1977, 1979*a*, *b*, 1980*a*; Minnikin *et al.*, 1978, 1979). The cellular morphology and lipid composition of *Chr. iodium* were therefore investigated in an attempt to clarify its taxonomic position especially with regard to its apparent taxonomic relationship with *B. linens*.

METHODS

Strains. *Brevibacterium linens* strains ATCC 9172, ATCC 9174 and NCIB 8546 and *Chromobacterium iodium* strains NCDO 613 and NCIB 8179 were obtained from the relevant culture collections.

Morphological examination. Colonial and cellular morphology were examined and Gram-stains were done as described by Cure & Keddie (1973) and Jones (1975).

Cultivation. Cultures for lipid analyses were grown in shake flasks in nutrient broth no. 2 (Oxoid) supplemented with glucose (2 mg ml⁻¹) for 2 to 3 d at 30 °C. Cultures were checked for purity at maximum growth, collected by centrifugation (6000 *g* for 10 min), washed with distilled water and freeze-dried.

Analysis of fatty acid methyl esters. Dry organisms (50 mg) were examined using the acid methanolysis and thin-layer chromatography (t.l.c.) procedure described by Minnikin *et al.* (1975). Fatty acid methyl esters were purified and examined by gas-liquid chromatography as previously described (Collins *et al.*, 1980*b*).

Analysis of polar lipids. Free lipids were extracted from dry organisms (50 mg) using a modification (Kates, 1972; Card, 1973; Minnikin *et al.*, 1979) of the procedure of Bligh & Dyer (1959). Polar lipid patterns were obtained by two-dimensional t.l.c. of lipid extracts using HPTLC Kieselgel 60 F₂₅₄ (Merck Art. 5628) plates (10 × 10 cm). Chromatograms were developed in the first dimension with chloroform/methanol/water (65:25:4, by vol.), and in the second with chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) Spraying with 10 % molybdophosphoric acid in ethanol followed by heating at 125 °C for 15 min revealed the presence of all lipids. Specific spray reagents for lipid phosphate (Dittmer & Lester, 1964), α -glycols (periodate-Schiff) (Shaw, 1968), sugars (α -naphthol and anisaldehyde/H₂SO₄) (Jacin & Mishkin, 1965; Stahl & Kaltenbach, 1961) and free amino groups (ninhydrin in water-saturated butanol) were also used.

Analysis of isoprenoid quinones. Isoprenoid quinones were extracted and purified as described by Collins *et al.* (1977) and were further examined by reverse phase partition chromatography using Merck RP-18F₂₅₄ reverse phase thin-layer plates (10 × 10 cm) and a polar developing mixture of acetone/water (99:1, v/v) (Collins *et al.*, 1980*a*). Mass spectra of the isoprenoid quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 200 to 220 °C.

RESULTS

Phase contrast micrographs indicated that *Chromobacterium iodium* exhibited a coryneform morphology and a rod/coccus cycle (see Fig. 3), similar to that described for *Brevibacterium linens* (see Cure & Keddie, 1973).

Fig. 1. Gas-liquid chromatographic analysis of representative fatty acid methyl esters of *Chromobacterium iodinum* (a) and *Brevibacterium linens* (b). The column (6.5 m) containing 7 % ethylene-glycol adipate on 80 to 100 mesh Chromosorb G AW-DMCS was operated isothermally at 200 °C. Abbreviations: i, iso acid; ai, anteiso acid; s, straight-chain acid.

Whole-organism methanolysates of the test strains showed the presence on thin-layer chromatographs of single spots corresponding to non-hydroxylated fatty acid methyl esters (Goodfellow *et al.*, 1976; Minnikin *et al.*, 1978). All strains produced closely related fatty acid profiles; representative profiles are illustrated in Fig. 1 and quantitative data are shown in Table 1. The fatty acids were composed primarily of anteiso- and iso-methyl-branched acids (98.4 to 98.8 %) although straight-chain acids were also present in small amounts (1.2 to 1.6 %). The major fatty acids in all strains were 14-methylhexadecanoic (anteiso C₁₇) and 12-methyltetradecanoic (anteiso C₁₅) acids (Table 1).

Strains possessed remarkably similar, characteristic polar lipid patterns, representatives of which are shown in Fig. 2. Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) were readily identified in all the extracts by their chromatographic behaviour and staining properties. In addition, all strains produced a characteristic lipid which gave negative reactions to the lipid phosphorus and ninhydrin sprays but positive reactions to the periodate-Schiff and α -naphthol reagents. These glycolipids (G) gave a green coloration with the anisaldehyde/H₂SO₄ reagent indicating that they may contain mannose residues. This possibility was supported by co-chromatography of the unidentified glycolipid with dimannosyl diacylglycerol from *Corynebacterium aquaticum* (Khuller & Brennan, 1972; Minnikin *et al.*, 1978).

No evidence was found for the presence of ubiquinones (coenzyme Q) in the extracts of any strains; components that co-chromatographed with vitamin K were the only isoprenoid quinones detected. Ultraviolet absorption spectra of the purified quinones showed absorption maxima at 242, 248, 260, 270 and 326 nm, in accord with published data for menaquinones (Dunphy & Brodie, 1971). The mass spectra of the menaquinone samples

Table 1. *Percentage fatty acid composition of test strains*

Equivalent chain lengths on respective g.l.c. columns*	OV-1	12:0	12:8	13:7	14:0	14:8		15:0	15:7	16:0	16:8		17:0
	EGA	12:0	12:7	13:5	14:0	14:5	14:7	15:0	15:5	16:0	16:5	16:7	17:0
Assignment†		12:0	<i>ai</i> -13	<i>i</i> -14	14:0	<i>i</i> -15	<i>ai</i> -15	15:0	<i>i</i> -16	16:0	<i>i</i> -17	<i>ai</i> -17	17:0
<i>Brevibacterium</i> <i>linens</i> ATCC 9172		tr	tr	0.8	0.1	8.7	33.5	tr	11.5	1.1	4.3	40.0	tr
<i>Brevibacterium</i> <i>linens</i> NCIB 8546		—	tr	0.9	0.1	8.0	34.2	0.1	11.0	1.0	4.2	40.5	tr
<i>Brevibacterium</i> <i>linens</i> ATCC 9174		—	tr	0.8	0.2	8.0	32.9	tr	11.4	1.2	1.4	40.9	0.2
<i>Chromobacterium</i> <i>iodinum</i> NCDO 613		tr	0.2	1.0	0.3	7.5	32.5	tr	9.7	1.3	4.3	43.2	tr
<i>Chromobacterium</i> <i>iodinum</i> NCIB 8179		tr	0.1	0.9	0.2	7.9	33.0	0.1	10.1	1.2	4.3	42.0	0.2

tr, Trace amount.

* According to Miwa *et al.* (1960); EGA refers to ethyleneglycol adipate column.

† Abbreviations for fatty acids are explained by the following examples: 15:0 for the straight-chain pentadecanoic acid; *i*-15 for the *iso* acid 13-methyltetradecanoic acid; *ai*-15 for the *anteiso* acid 12-methyltetradecanoic acid.

Fig. 2. Two-dimensional thin-layer chromatograms of polar lipids from *Chromobacterium iodinum* (a) and *Brevibacterium linens* (b). Chloroform/methanol/water (65:24:4, by vol.) was used in the first dimension, and chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) was used in the second dimension. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; G, glycolipid.

showed intense peaks at m/e 187 and 225 derived from the naphthoquinone nucleus, as expected from published data (Azerad & Cyrot-Pelletier, 1973). Strong peaks corresponding to molecular ions (M^+) were also observed. Details of the mass spectral analyses are shown in Table 2. Strains contained major amounts of dihydrogenated menaquinones with eight isoprene units [abbreviated as MK-8(H_2)], although small amounts of MK-8 and MK-7(H_2) were also detected in the mass spectra and on reverse phase partition chromatography.

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from *Brevibacterium linens* and *Chromobacterium iodinum*

The main component of each series is denoted by + + +, any components greater than 50 % of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue	MK-7	MK-8	
		—	(H ₂)
Degree of hydrogenation <i>m/e</i>	(H ₂) 650	716	718
<i>Brevibacterium linens</i> ATCC 9172	+	+	+++
<i>Brevibacterium linens</i> ATCC 9174	+	+	+++
<i>Brevibacterium linens</i> NCIB 8546	+	+	+++
<i>Chromobacterium iodinum</i> NCDO 613	+	+	+++
<i>Chromobacterium iodinum</i> NCIB 8179	+	+	+++

DISCUSSION

The recovery of menaquinones as the sole isoprenoid quinones from the *Chr. iodinum* strains confirms the views of several workers that this organism is Gram-positive (Gilman, 1953; Sneath, 1956, 1960; Jones, 1975; Weitzman & Jones, 1975). The Gram-negative genera *Chromobacterium* and *Pseudomonas* contain ubiquinones (coenzyme Q) as their sole isoprenoid quinones (Bishop *et al.*, 1962; Lester & Crane, 1959; Page *et al.*, 1960; Whistance *et al.*, 1969).

The presence of MK-8(H₂) as the major menaquinone isoprenologue in strains of *Chr. iodinum* (Table 2) together with their coryneform morphology, fatty acid (Table 1) and polar lipid profiles (Fig. 2) support the results of numerical taxonomic (Jones, 1975), peptidoglycan (Fielder *et al.*, 1970) and DNA base composition (Colwell *et al.*, 1969; Yamada & Komagata, 1970) studies, in indicating a taxonomic relationship between *Chr. iodinum* (Davis) and *B. linens* (Wolff) Breed. However, our results and those of Fielder *et al.* (1970) and Jones (1975) do not support the suggestion of Colwell *et al.* (1969) that *Chr. iodinum* should be reclassified in the genus *Arthrobacter*. *Arthrobacter globiformis* (the type species of the genus) and related species contain MK-9(H₂) as their major menaquinone isoprenologue (Yamada *et al.*, 1976; Collins *et al.*, 1979a) and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as their major phospholipids, together with a complex mixture of glycolipids consisting of mono- and digalactosyl diacylglycerols and dimannosyl diacylglycerol (Kostiwi *et al.*, 1972; Minnikin *et al.*, 1978). In addition, the peptidoglycan of *A. globiformis* and related species contains L-lysine (Fiedler *et al.*, 1970; Schliefer & Kandler, 1972).

On the basis of the results presented here and the results of the earlier numerical taxonomic (Jones, 1975), cell wall (Fiedler *et al.*, 1970; Schleifer & Kandler, 1972) and DNA base composition (Colwell *et al.*, 1969; Yamada & Komagata, 1970) studies, we consider that the bacteria now designated *Chr. iodinum* should be reclassified in the same genus as *B. linens*.

Brevibacterium linens is the type species of the genus *Brevibacterium* Breed 1953. This genus is presently considered to be *incertae sedis*, and therefore *B. linens*, the type species, is also so designated (Rogosa & Keddie, 1974). However, there is much evidence that *B. linens* strains form a distinct group which could form the nucleus of a redefined genus *Brevibacterium*, as first suggested by Yamada & Komagata (1972) and later by Jones (1975), Keddie & Cure (1977) and Sharpe *et al.* (1978). We therefore consider that the bacteria now referred to as *B. linens* (Wolff) Breed and *Chr. iodinum* (Davis) should be classified as two separate species in a redefined genus *Brevibacterium*.

Chromobacterium iodinum differs from *B. linens* in the production of a characteristic phenazine derivative (iodinin) and also lacks the carotenoid characteristic of *B. linens*

Table 3. *Some chemotaxonomic features of coryneform taxa*

Data from Schleifer & Kandler (1972), Minnikin *et al.* (1978), Keddle & Jones (1980) and M. D. Collins (unpublished results).

Taxon	Major wall diamino acid	Mol % G + C	Mycolic acids	Fatty acid types*	Major menaquinone isoprenologue(s)	Polar lipids†
<i>Arthrobacter globiformis</i> group	L-Lysine	59-66	absent	S, A, I, U	MK-9(H ₂)	DPG, PG, PI, MGDG, DGDG, DMDG
<i>Arthrobacter simplex</i>	LL-DAP	72-74	absent	S, A, I, U, T	MK-8(H ₄)	DPG, PG, OH-PG
<i>Brevibacterium emend.</i>	meso-DAP	60-64	absent	S, A, I	MK-8(H ₂)	DPG, PG, DMDG
<i>Caseobacter</i>	meso-DAP	60-67	present	S, U, T	MK-8(H ₂), MK-9(H ₂)	DPG, PI, PIDM
<i>Cellulomonas</i>	L-Ornithine	71-73	absent	S, A, I	MK-9(H ₄)	DPG, PI, PGL
<i>Corynebacterium sensu stricto</i>	meso-DAP	51-59	present	S, U	MK-8(H ₂), MK-9(H ₂)	DPG, PI, PIDM
<i>Curtobacterium</i>	D-Ornithine	67-71	absent	S, A, I	MK-9	DPG, PG, G
<i>Microbacterium lacticum</i>	L-Lysine	69-70	absent	S, A, I	MK-10 MK-11, MK-12	DPG, PG, DMDG

* S, Straight-chain acids; A, *anteiso* acids, I, *iso* acids; U, monounsaturated acids; T, tuberculostearic acid.

† DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannosides; OH-PG, phosphatidylglycerol containing hydroxylated fatty acids; PGL, unknown phosphoglycolipids; G, unknown glycolipids; MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol; DMDG, dimannosyl diacylglycerol.

(Jones, 1975). In addition, they can be differentiated on the basis of certain physiological tests (see species description). A problem arises because the name *Chromobacterium iodinum* (Davis) is not included in the Approved List of Bacterial Names (Skerman *et al.*, 1980). Therefore we propose the revival of the species epithet '*iodinum*' and at the same time the transfer of bacteria so designated to the genus *Brevibacterium* as *Brevibacterium iodinum* nom.rev.; comb.nov. (see Rules 28a and 41b; Lapage *et al.*, 1975).

Emended description of the genus Brevibacterium (Breed, 1953; Skerman *et al.*, 1980) – *Brevibacterium emend.*

The following description is based on the literature descriptions of *Brevibacterium linens* (Wolff) Breed and *Chromobacterium iodinum* (Davis) (Davis, 1939; Gilman, 1953; Sneath, 1956, 1960, 1974; Steele, 1961; Mulder *et al.*, 1966; Colwell *et al.*, 1969; Yamada & Komagata, 1970; Fiedler *et al.*, 1970; Crombach, 1972; Jones, 1975; Collins *et al.*, 1977, 1979a; Keddle & Jones, 1980; the present work).

The cells show a marked change of form during the growth cycle in complex media. Older cultures (about 3 to 7 d at 25 °C) are composed entirely or largely of coccoid cells which, on transfer to suitable fresh complex medium, grow out to give the slender irregular rods characteristic of exponential phase cultures. Many cells are arranged at an angle to each other to give V-formations; primary branching may occur. As growth proceeds, the rods become shorter and are eventually replaced by the coccoid cells (or short rods of occasional strains) characteristic of stationary phase cultures. Both rods and coccoid cells are Gram-positive, non-acid fast and non-motile; endospores are not formed. They are obligate aerobes, the mode of metabolism is respiratory, never fermentative; acids are not

formed from glucose and other sugars in peptone media. They are catalase positive. The cell wall peptidoglycan is based on *meso*-diaminopimelic acid (variation A1 γ); the cell walls do not contain arabinose. The bacteria contain dihydrogenated menaquinones with eight isoprene units, predominantly *anteiso*- and *iso*-methyl-branched fatty acids (*anteiso* C₁₅ and *anteiso* C₁₇) as major components, polar lipids comprising diphosphatidylglycerol, phosphatidylglycerol and dimannosyl diacylglycerol, but lack mycolic acids. The DNA base composition is in the range 60 to 64 mol % G + C.

The main distinguishing features between the genus *Brevibacterium* and other coryneform taxa are listed in Table 3. The type species is *B. linens*.

Emended description of Brevibacterium linens (Wolff) Breed

This description is based mainly on studies of the type strain ATCC 9172 (Skerman *et al.*, 1980) and strains ATCC 9174 and NCIB 8546 (Fiedler *et al.*, 1970; Schliefer & Kandler, 1972; Rogosa & Keddie, 1974; Jones, 1975; Keddie & Cure, 1977; Collins *et al.*, 1979a; Keddie & Jones, 1980; the present work).

Surface colonies on nutrient agar are small (0.1 to 0.2 mm diam.) after 1 to 2 d, becoming larger (2.5 mm) on extended incubation; convex with entire margin, shiny; pale yellow to deep orange pigment produced. Pigment often produced only in the presence of light. Pigmented growth gives characteristic colour reactions with various acids and bases (Jones, 1975). Cultures on suitable media show the rod/coccus growth cycle and staining reactions described for the genus. Optimum growth temperature 20 to 25 °C; growth poor or absent at 5 and 37 °C. Salt tolerant, grows in the presence of NaCl (10 %, w/v). Aerobic; mode of metabolism respiratory, never fermentative. Acids are not formed from glucose and other sugars in peptone media. Catalase positive, weakly oxidase positive. Gelatin liquefied, casein hydrolysed, hippurate hydrolysed, extracellular DNAase produced. The cell wall peptidoglycan contains *meso*-diaminopimelic acid (Fiedler *et al.*, 1970); arabinose is not present but the wall polysaccharide contains a glycerol teichoic acid (Fiedler & Stackebrandt, 1978). Mycolic acids are not present (Goodfellow *et al.*, 1976). The principal isoprenoid quinones are dihydrogenated menaquinones with eight isoprene units. The fatty acid composition is mainly *anteiso*- and *iso*-methyl-branched acids with small amounts of straight-chain acids. The major fatty acids are 14-methylhexadecanoic (*anteiso* C₁₇) and 12-methyltetradecanoic (*anteiso* C₁₅) acids. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol and dimannosyl diacylglycerol. The G + C content of the DNA, determined by estimation of the melting point, is 60 to 64 mol % (Yamada & Komagata, 1970; Crombach, 1972; Bousfield, 1972).

Description of Brevibacterium iodinum nom.rev.; comb.nov.

This description is based on studies of strains NCDO 613, NCIB 8179, ATCC 15728, and ATCC 15729 (Clemo & Daglish, 1950; Gilman, 1953; Sneath, 1956, 1960; Steele, 1961; Colwell *et al.*, 1969; Fiedler *et al.*, 1970; Jones, 1975; the present work).

Surface colonies on nutrient agar are 0.75 to 1 mm diam. after 1 to 2 d, becoming larger (2 to 5 mm) on extended incubation; convex with entire margin, shiny. Blue to purple extracellular crystals of iodinin visible within 2 d in areas of heavy growth. Iodinin production not influenced by light but better production on certain media (Colwell *et al.*, 1969). Colour reactions typical of *B. linens* growth absent (Jones, 1975). Cultures on suitable media show the rod/coccus growth cycle and staining reactions described for the genus (Fig. 3). Gram-positive, but may appear Gram-negative (Jones, 1975). In very young cultures (about 8 h) cells frequently stain one-half Gram-positive, the other Gram-negative (Colwell *et al.*, 1969). Optimum growth temperature 25 °C; growth at 30 °C, but poor or absent at 5 and 37 °C. Aerobic; mode of metabolism respiratory. Acids not formed from glucose and other sugars in peptone media. Catalase positive, strongly oxidase

Fig. 3. *Chromobacterium iodinum* (NCIB 8179) grown in medium EYGA (Cure & Keddle, 1973) at 25 °C: (a) after 6 h; (b) after 3 d. Inoculum, coccoid cells from 7 d EYGA slope culture at 25 °C. Bar markers represent 5 μ m.

positive. Gelatin hydrolysed, extracellular DNAase produced, weak hydrolysis of casein, hippurate not hydrolysed (Jones, 1975). The cell wall peptidoglycan contains *meso*-diaminopimelic acid (Fiedler *et al.*, 1970). Mycolic acids are not present. The principal isoprenoid quinones are dihydrogenated menaquinones with eight isoprene units. The fatty acid composition is mainly *anteiso*- and *iso*-methyl-branched acids with small amounts of straight-chain acids. The major fatty acids are 14-methylhexadecanoic (*anteiso* C₁₇) and 12-methyltetradecanoic (*anteiso* C₁₅). Diphosphatidylglycerol, phosphatidylglycerol and dimannosyl diacylglycerol comprise the polar lipids. The G+C content of the DNA, determined by estimation of the melting point is, 63 mol % (Colwell *et al.*, 1969). The type strain is NCDO 613.

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