OXAMICETIN, A NEW ANTIBIOTIC OF BACTERIAL ORIGIN

III. TAXONOMY OF THE OXAMICETIN-PRODUCING ORGANISM

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The oxamicetin-producing organism designated as *Arthrobacter oxamicetus* TOMITA and KAWAGUCHI sp. nov. is described. The morphological, cultural and physiological characteristics of the organism as well as its taxonomic relationship to other species of *Arthrobacter* are also discussed. The type strain is No. B302-B75 by virtue of its being a single isolate.

A new amicetin-like antibiotic, oxamicetin[1,2], has been isolated from the culture broth of a bacterial strain, No. B302-B75, isolated from a soil sample collected at Kominato, Chiba, Japan. Strain No. B302-B75 is a non-sporulating, non-motile bacterium and the cells show morphological changes during growth. These features are characteristic of the family *Corynebacteriaceae*. Further taxonomic studies indicated that the organism represents a new species of the genus *Arthrobacter* and the name *Arthrobacter oxamicetus* TOMITA and KAWAGUCHI sp. nov. is proposed for strain No. B302-B75. The type strain is No. B302-B75 by virtue of its being a single isolate.

For the characterization of strain B302-B75, the procedures described by SKERMAN[6], GIBBS & SKINNER[19], and GIBBS & SHAPTON[20] were followed. For identification of the organism, the descriptions included in BREED *et al.*[4], CONN & DIMMICK[8], NIGEL DE SILVA & HOLT[9], and CUMMINS & HARRIS[10] were considered.

Morphological Characteristics

A conspicuous characteristic of strain B302-B75 is the pleomorphism of the cells in size and shape during the course of growth (Plates 1 and 2). Young cells (12~24 hours) are Gram-variable (generally positive) rods of various length with irregular shapes, being straight, bent, curved, filamentous, and occasionally with a rudimentary branch. These rods develop, after 32 hours or longer, into Gram-positive cocci in various arrangements. They may be single, paired, or chained; and if chained, the chains are straight, curved or zigzag.

Sizes of the cells are in the range of 0.5~0.8×0.6~4.0 microns. The organism is non-sporulating and non-motile, and acid-fast stains (ZIEHL-NIELSEN) are negative.

Cultural and Physiological Characteristics

Strain B302-B75 develops into two forms on YGA-agar* plates, a compact type and a diffuse type when grown at 28°C.

Compact type of YGA agar colonies: Small, 0.3~1.0 mm in diameter (3rd day). Compact,

* Yeast-glucose-asparagine agar: Yeast extract 0.05%, glucose 1%, sodium aspartate 0.1%, asparagine 0.05%, K_2HPO_4 0.1%, NaCl 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCl_2 \cdot 2H_2O$ 0.05%, agar 1.5%. pH 7.0~7.2 before autoclaving for 15 minutes at 120°C.

Table 1. Physiological reactions of strain B302-B75

Test	Response	Method and medium employed
Survival at 72°C	No survival	10 minutes in milk
Decomposition of cellulose	Negative	Inorganic salts agar plus 0.05 % yeast extract containing strip of filter-paper
Utilization of ammonium salt as a sole nitrogen source	Positive	Inorganic salts agar plus 1 % glucose
Utilization of citrate as a sole carbon source	Positive	SIMMONS' citrate agar
Pigment from nicotine	Negative (no growth)	Nicotine agar (SGUROS, 1955)[5]
Starch hydrolysis	Negative	HAYWARD's starch agar
Nitrite from nitrate	Negative	Peptone broth plus 0.1 % KNO_3
Gelatin liquefaction	Negative	Peptone broth plus 25 % gelatin (SKERMAN, 1967)[6]
Milk peptonization	Negative	Incubation at 22°C for 3 weeks
Milk coagulation	Positive (Slowly coagulated)	〃
Change of pH in milk	Slightly acidified	〃
Indole production	Negative	Peptone broth (KOVACS' reagent)
VOGES-PROSKAUER reaction	Negative	Peptone broth plus 1 % glucose
H_2S production from cysteine and thiosulfate	Positive	SKERMAN's (1967)[6] method, and lead acetate agar
Gas from carbohydrate	Negative	Glucose, sucrose and mannitol as carbohydrate in nutrient broth
Urease reaction	Positive	CHRISTENSEN's urea medium, determined after incubation at 28°C for 3~5 days
Catalase reaction	Positive	Reaction of hydrogen peroxide solution on the colonies incubated at 28°C for 2~3 days
Oxidase reaction	Negative	Reaction of *p*-aminodimethyl-aniline oxalate (KOVACS' oxidase reagent) on the colonies incubated at 28°C for 2~3 days
Oxidative *vs.* fermentative metabolism of carbohydrate	Oxidative (no acid produced)	Sucrose and mannitol as carbohydrate (HUGH & LEIFSON, 1953)[7]

raised, convex and circular. Smooth, rigid and dull. Opaque, whitish to cream-colored, later light-pinkish orange. No diffusible pigment.

Diffuse type of YGA agar colonies: Larger than compact type, 0.7~2.0 mm in diameter (3rd day). Diffused, circular and less raised. Smooth, soft and glistening. Opaque, cream-colored, later light-pinkish orange. No diffusible pigment.

YGA agar slant: Abundant growth. Smooth, soft, opaque, cream-colored, later light-pinkish orange. Not viscous. No diffusible pigment.

Nutrient agar slant: Abundant growth. Older colonies not colored. Others, same as on YGA agar.

Nutrient broth: Slight turbidity with sediment. No surface growth. No pigmentation.

Table 2. Carbohydrate utilization of strain B302-B75

Glycerol	+	Maltose	−
L-Arabinose	−	Raffinose	−
D-Xylose	−	Inositol	−
Rhamnose	−	D-Mannitol	+
D-Fructose	+	D-Sorbitol	+
D-Galactose	−	Dulcitol	−
D-Glucose	+	Starch	−
D-Mannose	−	Cellulose	−
Sucrose	+	Inulin	−
Lactose	−	Salicin	−

* Basal agar medium: PRIDHAM and GOTTLIEB medium
+: Utilization positive
−: Utilization negative

Growth temperature: Scant or no growth at 37°C. Restricted growth at 32~35°C. Good growth at 20~30°C.

Oxygen demand: Obligately aerobic.

NaCl tolerance in nutrient broth: No growth at 8 % NaCl. Restricted growth at 4~6 % NaCl. Good growth at 0.5~3 % NaCl.

Milk: Pale-orange ring-growth at surface. Sediment turns to pale-orange after 2~3 weeks. Slowly coagulated without peptonization.

Requirement for growth factors in chemically defined inorganic medium: None.

Results of the physiological tests and carbohydrate utilization tests with strain B302-B75 are shown in Tables 1 and 2, respectively.

Taxonomy

In the 7th Edition of "BERGEY's Manual of Determinative Bacteriology", six genera of Family *Corynebacteriaceae*[4] are described, among which three, *i. e.*, *Corynebacterium* LEHMANN and NEUMANN, 1896, *Listeria* PIRIE, 1940 and *Erysipelothrix* ROSENBACH, 1909 are clearly differentiated from strain B302-B75. Furthermore, the genus *Microbacterium* ORLA-JENSEN, 1919 is thermoduric (survives at 72°C for 15~30 minutes) and the genus *Cellulomonas* BERGEY *et al.* 1923; emend. CLARK, 1952 decomposes cellulose. Therefore these two genera also are different from the present organism. Genus *Arthrobacter* CONN and DIMMICK, 1947 contains soil organisms, which do not decompose cellulose, and have cells which are pleomorphic and generally non-motile. These properties are in accordance with those of strain B302-B75. Therefore we consider it a member of the genus *Arthrobacter*.

Strain B302-B75 utilizes nitrates or ammonium salts as a sole nitrogen source and citrates as a sole carbon source. Six species of genus *Arthrobacter* having such abilities are described in BERGEY's Manual. The characteristics of strain B302-B75 were compared with those of the six species as shown in Table 3. The results indicate that strain B302-B75 has some characteristics in common with *A. ureafaciens* (KREES and EGGLESTON, 1939) CLARK, 1955, but differences in chromogenicity, growth temperature, urease activity, gelatin liquefaction and milk coagulation are apparent.

SNEATH and SKERMAN[3] described five additional species of *Arthrobacter*. They are *A. atrocyaneus* KUHN and STARR, 1960, *A. duodecadis* LOCHHEAD, 1958, *A. flavescens* LOCHHEAD, 1958, *A. nicotianae* SGUROS, 1955 and *A. ramosus* JENSEN, 1960. Strain B302-B75 differs from *A. flavescens* and *A. duodecadis*[11] in its non-requirement for vitamin B_{12} and terregens factor; from *A.*

Plate 1. Photomicrograph of *A. oxamicetus*, rod-form cells (24-hour culture at 28°C, grown on YGA agar)

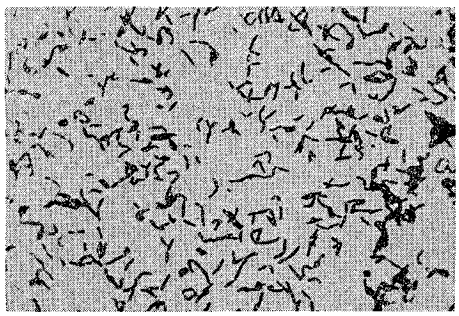

Plate 2. Photomicrograph of *A. oxamicetus*, coccoid cells (48-hour culture at 28°C, grown on YGA agar)

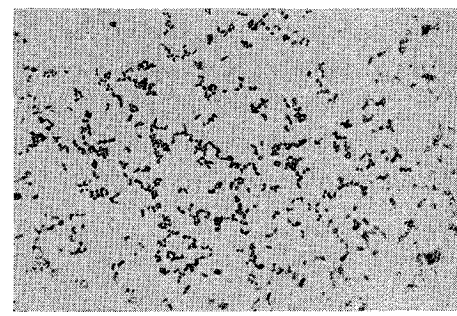

Table 3. Comparisons of eleven *Arthrobacter* species* and strain

	Chromogenicity	Utilization of NH_4 salts	Utilization of citrate	Pigment from nicotine	Starch hydrolysis	Nitrite from nitrate	Growth at 37°C
A. globiformis	none	+	+		+	+	– or scant
A. pascens	none	+	+		+	+	– or scant
A. simplex	none	+	+		–	+	+
A. oxydans	yellow or pearl gray	+	+	+ (deep blue)	+	+	
A. aurescens	yellow	+	+	– (+)**	+	+	slight
A. ureafaciens	yellow	+	+	–	–	–	+
A. crystallopoietes	none	+	–	– (–)	–	+	slow
A. polychromogenes	deep blue, later yellow green	+	+	– (±)	+	+	
A. viscosus	none	+	+	–	–	+	–
A. marinus	none	+	+	– (+)	–	–	+
Strain NRRL B3381	none or yellow	– or ±	+		+	+	+
Strain B302-B75	pink orange	+	+	– (–)	–	–	– or scant

* Based on information supplied in literature descriptions. Blanks: no information available in reaction

** + or – in parentheses means presence or absence of growth on the test medium

atrocyaneus[12] in its lack of blue-pigmentation, its optimum growth temperature, its positive urease activity and its lack of ability to hydrolyze starch; from *A. nicotianae* in its pigmentation, and from *A. ramosus*[13] in its inability to liquefy gelatin and its chromogenicity.

The characteristics of strain B302-B75 were further compared with those of five *Arthrobacter* species and strains described in recent literature. They are *A. crystallopoietes* ENSIGN and RITTENBERG, 1963[14], *A. polychromogenes* SCHIPPERS-LAMMERTSE, 1963[15], *A. viscosus* GASDORF *et al.*, 1965[16], *A. marinus* COBET *et al.*, 1970[17] and *Arthrobacter* sp. NRRL B-3381 (erythromycin-producer); FRENCH *et al.*, 1970[18]. The comparisons with strain B302-B75 are also shown in Table 3. Based on the literature descriptions, *A. viscosus* shares several common features with strain B302-B75 but differs from it in the lack of pigment on sugar media, in its peptonization but non-coagulation of milk, in its reduction of nitrate and in its production of viscous polysaccharide.

The various morphological, cultural and physiological characteristics of strain B302-B75 described above lead us to conclude that strain B302-B75 is a new species of *Arthrobacter*, for which the name *Arthrobacter oxamicetus* sp. nov. is proposed. The epithet *oxamicetus* is derived from the name of the antibiotic oxamicetin. The type strain is No. B302-B75 by virtue of its being a single isolate. The type strain has been deposited in the collection of the American Type Culture Collection, Rockville, Maryland, and the Fermentation Research Institute, Chiba, Japan, where it has been assigned the designations ATCC 21788 and FERM-P No. 2088, respectively.

Discussion

Genus *Arthrobacter* is reported to show some resemblance to the genera *Brevibacterium* BREED,

B302-B75

Gelatin liquefaction	Milk peptonization	H_2S production	Urease reaction	Catalase reaction
+	+	+	−	+
+	+	+	−	+
+	+	+	−	+
+	+	−		+
+	+	+	−	+
+	+	+	−	+
+	+	−	+	
+	− (−)	−	−	+
−	+	−	+	+
−	+	+	−	+
+	+	+		
−	− (+)	+	+	+

description. +: positive reaction, −: negative

1953 and *Kurthia* TREVISAN, 1885 of family *Brevibacteriaceae* and also to genus *Mycobacterium* LEHMANN and NEUMANN, 1896 of family *Mycobacteriaceae*. Strain B302-B75 would not be placed in any of these genera because of the unique pleomorphism of its cells. Furthermore, strain B302-B75 would not be placed in genus *Kurthia* which shows facultatively anaerobic growth, or in genus *Mycobacterium* which is acid-fast and grows slowly.

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