ARTHROBACTER LUTEUS NOV. SP. ISOLATED FROM BREWERY SEWAGE

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(Received February 8, 1969)

A group of bacteria isolated from brewery sewage was studied taxonomically. They were gram-positive, facultatively anaerobic, pleomorphic, branching, non-motile, non-sporulating, non-acid-fast, and catalase-positive rods ($0.6\text{--}1.0 \times 0.8\text{--}10.0 \mu$). They formed cystites and showed bending-type cell division. They produced a yellow pigment and reduced nitrate, hydrolyzed starch, and liquefied gelatin. They produced acids from various carbohydrates.

These characteristics were compared with those of 18 strains of related microorganisms. The isolates seemed to belong to the genus *Arthrobacter*, but no corresponding species was found in the taxa appearing in Bergey's Manual (7th Ed.). The name *Arthrobacter luteus* was, therefore, newly proposed for these isolates.

While the new species was in accord with those of the genus *Arthrobacter* in basic characteristics, it also had similarities to some species of the genera *Nocardia*, *Cellulomonas*, *Microbacterium*, and *Corynebacterium*.

These observations suggested that the species occupied an intermediate position between the families *Corynebacteriaceae* and *Actinomycetaceae*.

During the course of a study on bacteria in brewery sewage, four strains of hitherto undescribed bacteria were isolated. This paper describes these new bacteria, which were identical with each other taxonomically and designated as *Arthrobacter luteus*.

MATERIALS AND METHODS

Microorganisms. Four strains, B-41, B-91, B-111-1, and B-621, were used. They were isolated from brewery sewage rich in discarded yeast. They resembled each other in their morphology, cultural characteristics, and gram-stain, and seemed to belong to the same group. Eighteen strains which had some similarities to the group were used for comparative tests (Table 1).

Determination methods. Determination tests were carried out mainly by the methods described in "Jikken Nogei Kagaku, Jo-Kan" (1) and "Manual

Table 1. List of microorganisms examined.

<i>Brevibacterium acetylicum</i> BREED IFO 3749
<i>Brevibacterium helvolum</i> LOCHHEAD IAM 1637
<i>Corynebacterium equi</i> MAGNUSSON IAM 1037
<i>Corynebacterium fascians</i> DOWSON IAM 1079
<i>Microbacterium lacticum</i> ORLA-JENSEN IAM 1640
<i>Microbacterium flavum</i> ORLA-JENSEN IAM 1642
<i>Cellulomonas flavigena</i> BERGEY <i>et al.</i> IFO 3754
<i>Cellulomonas gelida</i> BERGEY <i>et al.</i> IFO 3748
<i>Cellulomonas cellasea</i> BERGEY <i>et al.</i> IFO 3753
<i>Mycobacterium smegmatis</i> LEHMANN and NEUMANN IFO 3083
<i>Mycobacterium phlei</i> LEHMANN and NEUMANN IFO 3158
<i>Nocardia asteroides</i> BLACHARD IFO 3384
<i>Nocardia corallina</i> WAKSMAN and HENRICI IFO 3338
<i>Nocardia gardneri</i> WAKSMAN and HENRICI IFO 3385
<i>Arthrobacter aurescens</i> PHILIPS IFO 12136
<i>Arthrobacter simplex</i> LOCHHEAD IAM 1660
<i>Arthrobacter ureafaciens</i> CLARK IAM 1658
<i>Arthrobacter oxydans</i> SGUIROS IFO 12138

of Microbiological Methods" (2). The bacteria were identified according to "Bergey's Manual of Determinative Bacteriology" (3).

RESULTS AND DISCUSSION

General characteristics of the isolates

All four strains had the same morphological, cultural, and physiological characteristics which may be summarized as follows.

The shape and size of cells in young cultures (nutrient agar, 30°, 18 hr) were irregular, 0.6–1.0×2.0–10.0 μ . Club-shaped, curved, swollen, and occasionally branched cells were observed. In old cultures (after 72 hr), the cells became short rods or cocci with an average size of 0.6–1.0×0.8–1.0 μ . Cystitis (4) were observed when cultures were grown at a higher temperature (37°). The cells were non-motile, non-sporulating, and non-acid-fast. Cells were gram-positive in young cultures. In old cultures, gram-negative cells which sometimes included gram-positive granules were observed besides gram-positive cells. In young cultures on a nutrient agar plate (30°, 3 days), the edge of the colonies was entire, but deep colonies with filamentous edge were formed in old cultures (after 7 days). They produced a yellow pigment, reduced nitrate, hydrolyzed starch, but did not decompose cellulose. The catalase reaction was positive. Acids but no gas was formed from various carbohydrates. They grew in peptone broth containing up to 7% sodium chloride. They

were facultatively anaerobic.

From these characteristics, they seemed to belong to Family XII *Corynebacteriaceae* described in Bergey's Manual (7th Ed.). These characteristics of the isolates were compared with those of related organisms.

Comparison of the isolates with related organisms

1) Relation to *Brevibacterium* BREED

According to Bergey's Manual (7th Ed., 1957), bacteria of the genus *Brevibacterium* are characterized as being gram-positive, unbranched short rods. This genus was established by BREED for some bacteria whose taxonomical positions he found obscure with little phylogenic consideration. Consequently, as pointed out by SKERMAN (5), KOMAGATA *et al.* (6), and KINOSHITA *et al.* (7), the generic concept of *Brevibacterium* is not clear.

Brev. acetylicum IFO 3749 resembled the isolates in its morphological changes from rods to coccoid cells, tolerance for sodium chloride, ability to hydrolyze starch, and ferment sugars, but was different in its inability both to form branched cells and to reduce nitrate.

On the other hand, *Brev. helvolum* IAM 1637 resembled the isolates in forming branched cells in young cultures, but was different in the absence of morphological changes and inability to reduce nitrate, hydrolyze starch, and ferment sugars.

In view of the above facts, it may be reasonable not to include the isolates in the genus *Brevibacterium*.

2) Relation to *Corynebacterium* LEHMANN and NEUMANN

When LEHMANN and NEUMANN first distinguished the genus *Corynebacterium* in 1896, the genus was characterized by its pathogenic effect on animals, irregular cell shape, absence of endospores, immotility, and gram-positivity. The genus, however, now includes bacteria with a wide range of characteristics such as pathogenicity to plants and being motile (8).

From a historical viewpoint, pleomorphism seems to be a generic characteristic of the genus.

Recently, KOMAGATA *et al.* (9) found that most of the bacteria in this genus showed snapping-type cell division, and they proposed to regard this characteristic as a taxonomic one.

Coryn. fascians IAM 1079 was gram-positive, pleomorphic, and formed branched cells. Cell division of the organism was snapping-type unlike that of the isolates. The outline of the cells appeared sharper than that of the cells of the isolates. It did not have the physiological activities of the isolates.

Coryn. equi IAM 1037 was similar to the isolates morphologically except for the snapping-type cell division. It did not liquefy gelatin, ferment sugars except glucose, or hydrolyze starch.

On the basis of the above-mentioned differences, the isolates would not belong to the genus *Corynebacterium*.

3) Relation to *Cellulomonas* BERGEY *et al.* emend. CLARK

Bacteria of the genus *Cellulomonas* are characterized by gram variability, pleomorphism, and ability to decompose cellulose (3). However, three strains of *Cellulomonas* tested did not decompose cellulose. KOMAGATA *et al.* (6) have also reported that *C. uda* ATCC 491 and *C. gelida* ATCC 488 did not decompose cellulose. As many authentic strains have no cellulase activity, the generic concept of the genus may remain to be elucidated.

Three strains used were very similar to the isolates in their pleomorphism, utilization of carbohydrates, and reduction of nitrate, but they were different from the isolates in their gram variability, slow growth, and intolerance to sodium chloride.

Though there are similarities between the isolates and some members of *Cellulomonas*, it is inappropriate at present to classify the isolates in the genus *Cellulomonas*, since cellulase activity is a fundamental characteristic of this genus in the current taxonomical system.

4) Relation to *Microbacterium* ORLA-JENSEN

In Bergey's Manual (7th Ed.), *Microbacterium* is characterized by its heat resistance in skimmed milk and its ability to produce lactic acid, but there has been a recent tendency to regard *Microbacterium* as a general term for thermotolerant strains of *Corynebacterium* rather than as an independent genus (7, 8, 10).

As *Microb. flavum* IAM 1642 and *Microb. lacticum* IAM 1640 were pleomorphic and formed branched cells, these two strains were not in accord with *Microbacterium* in the sense of SKERMAN's description (5), but had some similarities to *Arthrobacter* or *Corynebacterium*. Moreover, their heat tolerance estimated by both the capillary method and the test tube method (11) was found to be lower than SKERMAN's description.

These two strains were similar to the isolates in pleomorphism and branching, but they were distinctly different from the isolates in their cultural characteristics such as pigment production and appearance of the colonies.

Since the isolates did not conform to SKERMAN's description and they were also different from the two thermosensitive strains of this genus, the isolates do not belong to this genus.

5) Relation to *Mycobacterium* LEHMANN and NEUMANN

Mycobacterium is characterized as being saprophytic or parasitic, gram-positive, aerobic, acid-fast, and of rudimentary branching (3).

The isolates were, to some extent, similar to some strains of *Mycobacterium* described in Bergey's Manual (7th Ed.) in their pleomorphism, but they were distinctly different from the latter in their facultative anaerobic and non-acid-fast characteristics.

Mycob. smegmatis IFO 3083 and *Mycob. phlei* IFO 3158 were different from the isolates in morphology, and in aerobic and acid-fast characteristics. Therefore, the isolates do not belong to the genus *Mycobacterium*.

6) Relation to *Nocardia* TREVISAN

Similarities between the isolates and some strains of *Nocardia* in such characteristics as gram staining, pleomorphism, branching of cells, and filamentous deep colony, led us to investigate their relation to *Nocardia*.

Nocardia is characterized by being aerobic, gram-positive, and producing mycelia but some strains of *Nocardia* show morphological changes in their life cycle like *Corynebacterium* or *Arthrobacter* (3, 5, 10, 12).

Although the isolates seemed to be similar to some strains of *Nocardia* in their morphological changes during the life cycle and in their formation of colonies with filamentous edge on nutrient agar in old cultures, they did not seem to be a member of the *Nocardia* because they did not form such clear mycelia as *Nocardia*.

The three strains of *Nocardia* tested conformed to the description in Bergey's Manual (7th Ed.) in producing mycelia, forming clearly branching cells, and being physiologically inactive. They were definitely different from the isolates.

7) Relation to *Arthrobacter* CONN and DIMMICK

CONN and DIMMICK (13) studied a soil bacterium named *Bacterium globiforme* and found that it belonged to neither the *Mycobacterium* nor *Corynebacterium* and first proposed the genus *Arthrobacter*.

Bacteria of the genus *Arthrobacter* were, at that time, characterized as follows: "...strongly aerobic forms, showing rather complicated morphological life cycles, including rods, cocci, club, and branched forms, non-acid-fast, gram variable, able to live on inorganic nitrogen without added growth accessory substances, typically soil organisms" (13).

The genus *Arthrobacter* has been recognized generally (3, 14, 15) despite JENSEN's protest (8), and the concept of the genus has extended in respects to nutritional requirement (16, 17), gram stain (18, 19), and sources (15, 19).

Lately, there has been a tendency to stress morphological characteristics such as pleomorphism and formation of cystites (3-5, 20). KOMAGATA *et al.* (9) found that most species of the genus *Arthrobacter* showed bending-type cell division, and they proposed to regard this characteristic as a taxonomic one.

All four authentic strains in this genus were similar to the isolates in their pleomorphism, cystite formation, and bending-type cell division. With gram stain, three strains except *A. simplex* IAM 1660 were positive like the isolates. On the other hand, these four strains were different from the isolates in their inability to ferment various carbohydrates and to form deep colonies.

Because the formation of branching cells and cystites, morphological changes in the life cycle, and bending-type cell division were considered to be more fundamental characteristics of the genus *Arthrobacter* in taxonomy than formation of deep colonies and ability to ferment sugars, the authors thought it reasonable to classify the isolates in this genus.

In the genus *Arthrobacter*, the isolates differed from *A. tumescens*, *A.*

Table 2. Differences between *A. luteus* nov. sp. and related bacteria.

	<i>A. luteus</i> nov. sp.	<i>A. oxydans</i> IFO 12138	<i>A. aurescens</i> IFO 12136
Nutrient broth	Moderate growth, yellow sediment	Abundant growth, ring, white sediment	Moderate growth, cream-colored sediment
Glutamate agar slant	Plumose, white	Filiform, white	Filiform, yellow
Nicotin agar slant	Scanty growth, white	Abundant growth, blue pigment	Growth slow and sparse
Litmus milk	Acid, coagulation	Slow peptonization, alkaline	Pale yellow ring, alkaline
Hydrogen sulfide	Not produced	Not produced	Produced
Fermentability	Acid Gas	Acid Gas	Acid Gas
Arabinose	+ —	— —	— —
Xylose	+ —	— —	— —
Glucose	+ —	+ —	± —
Mannose	+ —	— —	— —
Fructose	+ —	+ —	— —
Galactose	+ —	— —	— —
Lactose	+ —	— —	— —
Maltose	+ —	— —	— —
Saccharose	+ —	+ —	± —
Mannitol	— —	— —	— —
Glycerol	+ —	— —	— —
Inulin	+ —	— —	— —
Starch	+ —	— —	— —
Optimum temperature for growth (°)	30–37	25	20–32
Source	Brewery sewage	Tobacco leaves	Soil

+: Produced, ±: slight production, —: not produced.

citreus, and *A. terregens* in that they could utilize ammonium salts as a sole source of nitrogen. They differed from *A. globiformis*, *A. pascens*, and *A. simplex* in chromogenic character. They were also different from *A. ureafaciens* in that they could hydrolyze starch and reduce nitrate. Although the isolates were considered to be similar to *A. oxydans* and *A. aurescens* in that they utilized nitrate and ammonium salts, hydrolyzed starch, and reduced nitrate, they were found to be different from the two strains in the characteristics shown in Table 2.

The isolates were, therefore, regarded as a new species and the name *Arthrobacter luteus* was proposed for them.

Photomicrographs of the representative strain B-111-1 on nutrient agar slant are presented in Fig. 1.

Fig. 1. Photomicrographs of *Arthrobacter luteus* B-111-1. Grown on nutrient agar slant for (A) 14.5 hr, (B) 24 hr, and (C) 48 hr at 30°. Gram stain.

Fig. 2. Colonies of *Arthrobacter luteus* B-111-1.
Grown on nutrient agar plate for (A) 3 days and (B) 10 days at 30°.

The type culture of this species is *Arthrobacter luteus* nov. sp. B-111-1 deposited with the Fermentation Research Institute, the Ministry of International Trade and Industry, Inage, Chiba City.

The taxonomy of the family *Corynebacteriaceae* has been studied in more detail recently with increase in practical use of members of this family (7, 11, 21-24).

As mentioned above, *A. luteus* has a slight similarity to the genus *Nocardia* in that it produces rigid deep colonies with rudimentary filamentous edge under the surface colonies when cultured on nutrient agar plate at 30° for more than 7 days (Fig. 2). It also has similarity to some species of the genera *Cellulomonas*, *Microbacterium*, or *Corynebacterium* in that it produces acids from various carbohydrates fermentatively on the test proposed by HUGH and LEIFSON (25).

From these characteristics, *A. luteus* may be an intermediate form between the families *Corynebacteriaceae* and *Actinomycetaceae*.

Description of *Arthrobacter luteus* nov. sp.

Strains: B-41, B-91, B-111-1 and B-621.

Type culture: B-111-1.

Rods, 0.6 to 1.0 by 0.8 to 10.0 micra. Pleomorphic. Curved, swollen, club-shaped cells and rudimentary branching of cells are observed. In old cultures, the cells become short rod form or coccoid form, measuring 0.6 to 0.8 by 0.8 to 1.0 micra and sometimes irregular in shape. Cystites are observed when cultured at 37°. Spore not formed. Gram-positive in young cultures. In old

cultures, gram-negative cells which sometimes include gram-positive granules are observed besides gram-positive cells. Non-acid-fast.

Nutrient agar colonies: Circular, smooth, entire, raised, glistening, yellow, butyrous. In old cultures, filamentous deep colonies are observed.

Glutamate agar colonies (26): Growth scant, circular, smooth, filamentous, flat, white, opaque. The colonies develop little better in the medium than on the surface of the medium.

Nutrient agar slant: Growth moderate, filiform, glistening, smooth, yellow. In old cultures, the edge becomes filamentous.

Glutamate agar slant: Growth scant, plumose, white, opaque, medium unchanged.

Nutrient broth: Moderate turbidity with yellow sediment, no pellicle.

Nutrient gelatin stab: Slow liquefaction.

B.C.P. milk: Acid, coagulation.

Nitrite produced from nitrate.

Indole not produced.

Hydrogen sulfide not produced.

Starch hydrolyzed.

Methyl Red test: Weakly positive.

Voges-Proskauer test: Negative.

Ammonia not produced from peptone.

Potato: Moderate growth, yellow.

Acid but no gas from arabinose, xylose, glucose, mannose, fructose, galactose, lactose, maltose, saccharose, trehalose, sorbitol, glycerol, salicin, α -methylglucoside, inulin, dextrin, and starch. No acid and no gas from mannitol, raffinose, rhamnose, and inositol.

Succinate is utilized as a sole carbon source but citrate is not utilized.

Paraffin is utilized but phenol or *m*-cresol is not utilized.

Urease slightly produced.

Cellulose not attacked.

Blue pigment is not produced on nicotin agar slant.

NaCl broth: Good growth up to 7% NaCl, growth in a few cases in 8%.

Optimum temperature for growth: 30 to 37°. No growth at 52°.

Optimum pH for growth: Between 7.0 and 8.0.

Catalase: Positive.

Did not survive for 10 min at 72° in skimmed milk.

Facultatively anaerobic.

Source: Brewery sewage.

The authors wish to express their sincere thanks to Dr. Y. Okuda, member of the Japan Academy, Prof. Dr. T. Uemura, the Institute of Applied Microbiology, the University of Tokyo, Dr. K. Sudo, the National Institute of Animal Health, Prof. Dr. T. Arai, the Institute of Food Microbiology, Chiba University, Dr. S. Takahashi, the Institute of Tuberculosis, and Dr. K. Komagata, the Central Research Laboratories, Ajinomoto Co.,

Inc., for their helpful suggestions throughout this study. They wish to express their thanks to the Institute for Fermentation, Osaka, for the kind supply of microorganisms. They would like to thank Mr. M. Tokikuni, President, and Mr. T. Suzuki, the Managing Director, of Kirin Brewery Co., Ltd., for giving them the opportunity to publish this paper. They are also grateful to Dr. Y. Kuroiwa, Director of this Research Laboratories, for his kind guidance and encouragement.

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