Polymer-Producing Species of *Arthrobacter*

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**ABSTRACT**

GASDORF, HELEN J. (Northern Regional Research Laboratory, Peoria, Ill.), R. G. BENEDICT, M. C. CADMUS, R. F. ANDERSON, AND R. W. JACKSON. Polymer-producing species of *Arthrobacter*. J. Bacteriol. 90:147–150.1965.—Two slime-producing microorganisms, designated as NRRL B-1793 and NRRL B-1797, were isolated from a Guatemalan soil sample. Their morphological and physiological characteristics permit their assignment to the genus *Arthrobacter*. Both cultures produce a large amount of extracellular polysaccharide, the maximal amount being 1.4 g per 3 g of glucose. The carbohydrate constituents of B-1793 polysaccharide are galactose, glucose, and mannuronic acid; those of B-1797 are galactose, glucose, and glucuronic acid. The organisms are morphologically and physiologically alike. The differences between these two cultures and previously described species of *Arthrobacter* appear sufficient to designate a new species. The name *Arthrobacter viscous* sp. n. is proposed.

In the course of a survey to find polymer-producing microorganisms, two unusual cultures isolated from soil collected at the Guatemala City Airport in 1956 were selected for further study. Their morphological and physiological characteristics permit their assignment to the genus *Arthrobacter* (Conn and Dimmick, 1947), and it is proposed that they constitute a new species.

**MATERIALS AND METHODS**

The survey that revealed the new *Arthrobacter* forms was conducted on 34 samples of soil originating from Illinois; Indiana; New York; Arizona; Ontario, Canada; and Central and South America. The samples were plated on the synthetic basal medium of Pridham and Gottlieb (1948). This medium contained 0.26% (NH₄)₂SO₄ as the sole nitrogen source and was supplemented with various carbon sources (glucose, maltose, lactose, galactose, sucrose, arabinose, mannitol, glycerol, raffinose, α-methyl-d-glucoside). After an incubation period of 4 days at 30 °C, plates with viscous colonies were segregated for further processing. Isolates picked from the original plates and incubated in nutrient broth were then streaked on agar media containing various organic nitrogen sources.

The following *Arthrobacter* cultures from the Agricultural Research Service Culture Collection were selected for comparison by both routine and specific tests: *A. globiformis*, NRRL B-2880; *A. pascens*, NRRL B-1814; *A. pascens* (mucoid), NRRL B-2884; *A. aureus*, NRRL B-2879; *A. citreus*, NRRL B-1268 and NRRL B-2883; *A. tenuescens*, NRRL B-2861; *A. acrocyaneus*, NRRL B-2883; *A. simplex*, NRRL B-3157 and NRRL B-3158; and *A. ramulosus*, NRRL B-3159. All cultures were maintained on yeast-malt slants (Haynes, Wickerham, and Hesseltine, 1955) and were grown at 25 °C. Weekly transfers were made, and the cultures were stored at 10 °C.

The characteristics of B-1793 and B-1797 differed sufficiently from the published descriptions of *A. nicotianae* (Giovannoni-Sermanni, 1959), *A. duodecasis* (Lochhead, 1958), *A. flavescens* (Lochhead, 1958), *A. terregens* (Lochhead and Burton, 1953), *A. oxydans* (Siguros, 1956), *A. ureafaciens* (Krebs and Eggleston, 1939), and *A. helvolum* (Conn and Dimmick, 1947) that laboratory comparisons were not undertaken.

Carbohydrate fermentation and acid production were determined by use of phenol red broth containing 1% of various carbohydrates. The utilization of cellulose was tested by inoculating strips of filter paper immersed in a yeast-malt broth; cultures were grown in 300-ml flasks on a reciprocal shaker at 25 °C for 5 days. The standard media for other identification tests were prepared as described in the *Difco Manual*. Identification procedures as described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957) were used, with additional specific procedures as needed.

Soil extract for use in media to characterize members of *Arthrobacter* was made in the following way. Soil (500 g) was suspended in 1,300 ml of an

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aqueous 0.1% Na₂CO₃ solution and boiled for 1 hr. After the suspension was filtered through cheese-
cloth, the filtrate was centrifuged at 10,000 × g for 20 min. The supernatant fluid was decanted, and
the latter was separated into tryptone-glucose-yeast extract-agar.

Optimal pH for growth was determined as follows. Cells were grown in a medium containing
0.2% glucose, 0.25% peptone, 0.2% K₂HPO₄, 0.62% MgSO₄·7H₂O; and trace amounts of MnSO₄,
NaCl, and Fe₃(SO₄)₂ with pH values of 2 through 10 at 25°C on a rotary shaker. Samples were taken
at 4-hr intervals, and the amount of growth was estimated by measurement of light transmission
at 605 nm.

Optimal pH for polymer production was also investigated. Cultures were grown in a medium
shown by Cadmus et al. (1965) to give optimal polymer production by B-173. The medium
consisted of 3% commercial corn sugar, 0.25% en-
yzme-hydrolyzed casein (EHC, Amber Labora-
tories, Inc., Milwaukee, Wis.), 0.4% K₂HPO₄,
0.08% MgSO₄·7H₂O, and 0.065% MnSO₄·4H₂O at
pH 5 through 10. The fermentations were carried
out on a rotary shaker at 25 C for 3 days. The
polymers were then precipitated from 10 ml of
crude centrifuged culture liquor with 2.5 volumes
of ethyl alcohol and 3 drops of saturated KCl.
Precipitated polymers were air-dried for 2 hr at
110 C and weighed. Yields were calculated as
grams per 100 ml of culture fluid.

Temperature relationships were determined at
10 and 37 C. Cultures were grown on yeast-malt
slant and then transferred daily for 3 days
while being maintained at 10 or 37 C. Growth was
not considered to take place at these temperatures
unless cultures survived and grew during at least
three serial transfers.

Gram staining was done according to the Hucker
modification and flagella staining by the Leifson
(1951) method.

Purified polymer from NRRL B-1973 and B-1797
was prepared for physical and chemical studies in
the following manner. The viscous culture liquor
was diluted 1:4 with water; 0.5 volume of ethyl
alcohol was added to expedite the centrifugation
of cells, and the mixture was centrifuged at 20,000 × g
for 30 min. The supernatant liquid was de-
canted, and the polysaccharide was precipitated
from this by addition of 1 g of KCl per 100 ml of
solution, and 2.5 volumes of ethyl alcohol. The
total precipitate was removed and dissolved in a
volume of water equal to the original volume of the
supernatant fluid, and then reprecipitated.

Finally, the purified precipitate was dissolved in
water and lyophillized to obtain a white, dry
product.

For chromatographic analysis, 2% solutions of
the polymers were hydrolyzed with 2 N HCl for
1 hr at 100 C. Hydrolysates were then spotted on
Whatman no. 1 paper, and the components were
separated by descending chromatography with the
solvent system, ethyl acetate-acetic acid-
pyridine-water, in the ratio of 5:1:5:3, v/v (Gee
and McCreary, 1957). This procedure chroma-
tographically separates galacturonic, glucuronic,
and mannuronic acids. The lactones corresponding
to the latter acids are also separated. Spots were
developed with the o-aminodiphenyl spray reagent
(Gordon, Thornburg, and Warum 1956).

RESULTS AND DISCUSSION

The two isolates, NRRL B-1973 and B-1797,
from Guatemalan soil produced extremely viscous
growth on media containing various carbon
spheres. Cells from an 18-hr culture on yeast-malt
agar were highly pleomorphic and gram-negative.
These cells changed from short-branching, curved
and straight rods at 8 to 12 hr to staphylococcal-
like forms at 24 to 48 hr. A definite life cycle
(Chaplin, 1957) seemed to take place. Many of
the cells exhibited uneven staining. The cultures
failed to initiate growth in synthetic medium with
phenol as a carbon source and also produced
nitrates from nitrates; consequently, Mycoplana
was eliminated as a generic possibility. Growth
did not occur on nitrogen-free synthetic media;
thus, the genus Azotobacter was eliminated.

Growth occurred on the manitol-calcium-glyc-
nero-phosphateagar of Riker et al. (1930) without
browning of the medium. Large circular colonies
were produced on nutrient gelatin. These charac-
teristics eliminated Agrobacterium radiobacter
(Hofer, 1941) as a possibility. Since cellulose was
not utilized, the genus Cellulomonas could be
excluded.

The occasional formation of "Y," "T," and
"U" forms with uneven staining appeared typical
of one of the soil diphtheroids, whose taxonomy
was reviewed by Clark (1952). Utilization of
inorganic nitrogen and the gram-negative staining
reaction ruled out Corynebacterium and pointed
toward Arthrobacter as the probable genus.

On the basis of morphological and physiological
comparisons, NRRL B-1973 and B-1797 do not
appear to fit the description of any of the known
species of Arthrobacter. They most closely re-
semble the nonchomogenic, vitamin non-re-
quiring species A. globiformis (Conn and
Dimmick, 1947), A. pascens (Loecher and Burton,
1953), A. simplex, (Jensen, 1934), and A. ramosus,
(Jensen, 1960), but are distinguished from each of
them by several characteristics. They differ from
A. globiformis and A. pascens by their urease
activity, lack of gelatin and starch hydrolysis, and
lack of hydrogen sulfide production; from A. s.
 simplex by their urease activity, lack of gelatin
hydrolysis, and lack of growth at 37 C; and from
A. ramosus in ability to reduce nitrates and lack of
gelatin hydrolysis. Furthermore, they produce
large amounts of extracellular polysaccharide in
medium consisting of various organic nitrogen
sources, potassium phosphate buffer, and inorganic salts. None of the comparison cultures produced extracellular polysaccharide under these conditions. Although Mulder and Antheniusse (1963) stated that some Arthrobacter strains are able to produce large amounts of extracellular polysaccharide, he gave data only on A. globiformis. The polymer produced by this organism contains glucose and glucuronic acid. Although B-1797 also produces a polymer containing glucuronic acid, it differs from A. globiformis in several respects; the strain B-1797, which elaborates a polymer containing mannuronic acid, also differs considerably otherwise from A. globiformis.

The maximal amount of polysaccharide produced by B-1973 in flasks was 1.24 g per 3 g of glucose; that of B-1797 was 1.30 g per 3 g of glucose. Final viscosity of cultures was 10,000 to 12,000 centipoises. Cadmus et al. (1963) showed that the maximal amount of polymer produced by B-1973 is 1.4 g per 3 g of glucose on a pilot-plant scale. The optimal pH for polymer production by both B-1797 and B-1797 was 7.0. Reduced production occurred at pH 5.0, 6.0, 8.0, and 9.0, with none below 4.0 or above 10.0.

The polymer from these organisms is gelatinous, fibrous, and somewhat cohesive when first precipitated from crude culture liquor. The lyophilized material is white and spongelike. Aqueous solutions of the polysaccharides are very viscous; a 1% solution gives a viscosity of 10,000 centipoises.

The carbohydrate constituents of the polysaccharide from B-1973 are galactose, glucose, and mannuronic acid; those of B-1797 are galactose, glucose, and glucuronic acid. The composition and structure of the polymer from B-1973 were determined by Sloneker et al. (1963). The polysaccharide was found to possess a linear structure composed of repeating units having equal proportions of D-mannuronic acid, D-glucose, and D-galactose. These three sugar residues make up 75% of the dry weight of the polysaccharide; the remaining 25% is in the form of O-acetyl groups. The structure of the B-1797 polysaccharide has not been determined.

In addition to the uronic acid components of their extracellular polysaccharide, B-1973 and B-1797 differ in that the latter produces much more mucoid growth on solid medium. However, these cultures are alike in all other morphological and physiological characteristics. They may, therefore, be considered to constitute a new species of the genus Arthrobacter, and B-1973 is designated as the type strain. Morphological and physiological descriptions follow.

**Morphology.** At 12 to 18 hr, short gram-negative rods were present. Some stained evenly, but the majority stained unevenly. Some "X," "Y," "U," and "T" forms present were also unevenly stained. The short rods exhibited bipolar staining. The longer rods appeared to have three to six heavily stained granules in a more lightly stained cell.

After 24 to 48 hr, micrococcus-like cell groupings were predominant. These cells were unevenly stained but appeared to be gram-negative.

Flagella staining of cells grown on yeast-malt slants and suspended in distilled water showed no flagella, but the highly pleomorphic "X," "Y," and "U" cell forms were made plainly visible by this technique.

**Colonial characteristics.** Agar streak: filiform, raised, glistening, viscous. Agar colonies: 3 mm, circular, raised, glistening, viscous, white, opaque. Soil extract-agar: filiform, moderate growth, glistening, soft, viscous, raised, cream-colored. Gelatin colonies: circular 2 to 3 mm, smooth, raised, glistening, opaque, no liquefaction. Asparagine-agar slant: growth moderately abundant, light cream, slightly mucoid, glistening, wrinkled edge. Asparagine-agar colonies: 1 mm, circular, translucent, white, raised, slightly mucoid. Potato: growth moderately abundant, glistening, soft, filiform, cream-buff. Yeast-malt slant: viscous, profuse white growth, slightly mucoid in 24 hr. Mannitol-calcium-glycerophosphate colonies: 3 to 3.5 mm, raised, glistening, mucoid, circular; CaCO₃ formed around colonies; no browning of medium.

**Physiology.** Aerobic, weakly catalase-positive. No acid production in glucose, sucrose, lactose, or mannitol. Final pH of fermentation of glucose was 7.7; sucrose and lactose, 7.5; and mannitol, 7.3. Slight acid production was noted in glycerol (pH 6.2). Basic products resulted from fermentations of arabinose, raffinose, and α-methyl glucoside (pH 8.3 to 8.4). No gelatin liquefaction shown after 3 months of incubation. Neither indole nor acetyl methylcarbinol was produced. Nitrites produced from nitrates. No starch hydrolysis. Lactus milk completely cleared in 1 month with no curd formation; reduction in 23 days and complete by 25 days; a hard pellicle formed on milk in about 21 days; reaction slightly basic. Urease produced. Citrate utilized as the sole source of carbon. H₂S not produced from either cysteine or thiosulfate. Inorganic nitrogen utilized as sole source of nitrogen. Optimal temperature, 25 to 28 C. No growth at 37 C and little growth after 3 to 4 days of incubation at 10 C. The optimal pH, 6.1; growth sharply reduced at pH values below 5.0 and above 7.5.

These organisms differ from any previously
described *Arthrobacter* species and constitute a new species, *Arthrobacter viscosus* sp. n.

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**Literature Cited**


