PROPOSAL OF A NEOTYPE, ATCC 14216, FOR
PSEUDOMONAS AERUGINOSA (SCHROETER 1872)
MIGULA 1900 AND REQUEST FOR AN OPINION¹

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SUMMARY. *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 American Type Culture Collection strain 14216 is proposed as neotype strain (culture) of *Pseudomonas aeruginosa*, a description of which is presented. A request for an Opinion on the proposal of strain ATCC 14216 as the neotype has been submitted to the Judicial Commission of the International Committee on Bacteriological Nomenclature.

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In taxonomic studies, particularly those of a quantitative nature and with or without the use of computers, type strains serve as reference strains, becoming "markers" within a given analysis and between analyses of an investigator or several investigators. Thus, if the holotype for a species does not exist, it is important that a neotype strain be designated. The interpretation and integration of data analyses are greatly facilitated when common reference strains, holotype, paratype, cotype, lectotype or neotype (Sneath and Cowan, 1958), have been included. Sufficient data have been collected for the genus *Pseudomonas* by a number of workers (Viz. Haynes, 1951; Rhodes, 1959; Lysenko, 1961; Colwell and Liston, 1961) that compilation for a single quantitative and comparative study would be possible except that few strains were common to all the studies. It is hoped that designation of a neotype strain for the type species of the genus will be an aid in future work on the taxonomy of *Pseudomonas* by permitting inclusion of an accepted "marker," i.e. neotype, strain of *Pseudomonas aeruginosa*.

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A neotype culture, according to the Bacteriological Code (1958, Rule 9d, note C) is "one which has been accepted by international agreement to replace a type culture which is no longer in existence. It should agree with the diagnosis given by the original describer and should be recommended by those workers familiar with the species, and their agreed recommendation approved by the Judicial Commission" (quoted from Buchanan, 1962). Although it is well recognized that a type culture need not be typical in every respect, yet not based on an abnormality, it would be useful to select as neotype that culture which is as typical of the species as can be ascertained by available experimental methods.

In a mathematical approach to the study of bacterial species, an hypothetical Median Organism was derived for P. aeruginosa (Liston, Wiebe and Colwell, 1963). The description of the species was based on an Adansonian analysis of 78 coded features for each of 33 strains of P. aeruginosa. From this study, the strains ATCC 14216 and 14209 were recommended as candidates for neotype strain for the species. A further study was made of the 33 strains and an analysis of 172 coded features for each strain was carried out (Colwell, 1964). The Hypothetical Median Organism was recalculated and from the computed relationships of each individual strain to the Median Organism, the ATCC 14216 strain of P. aeruginosa was the most "typical" of the group of strains studied, sharing a 92% similarity to the Median Organism.

Schroeter (1872), in a paper concerning several pigments produced by bacteria, described a pigment which was bluish but became red on acidification, a reaction now generally recognized as characteristic of pyocyanin, the pigment produced by Pseudomonas aeruginosa.

Migula (1900) listed a description of P. aeruginosa and gave as synonyms, Bacterium aeruginosum Schroeter (1872), Bacillus aeruginosus Schroeter (1886) and Bacillus pyocyanus Gessard (1882). The organism was described by him as rodlike, small (averaging $0.6 \mu \times 2-3 \mu$), asporogenous, and very actively motile by a single polar flagellum. Other features given by Migula were: green fluorescent pigment produced in gelatin; gelatin liquefied; green fluorescent pigment on agar slopes, greenish fluorescence produced in milk; milk casein coagulated and gradually digested.

The American Type Culture Collection strain 14216 is

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the proposed neotype culture of Pseudomonas aeruginosa. This strain (strain D) was isolated from routine clinical material and was included in an investigation of the "metallic phenomenon" and bacteriophage typing of P. aeruginosa (Don and van Ende, 1950). Subsequent study of the antigenic structure of P. aeruginosa (van den Ende, 1952) also included this strain.

Using the procedures described and employed by Colwell and Liston (1961) and Colwell (1964), the proposed neotype culture (ATCC 14216) of Pseudomonas aeruginosa has the following attributes: Gram-negative asporogenous rod-shaped organism. Cells straight. Curved cells, ovals or filaments not usually present. Soma size 0.2 to 0.6 μ x 1.2 μ . Motile by single polar flagellum. Cells occur as singles and in pairs. Moderate even turbidity with visible pellicle in yeast extract-peptone broth in 18-24 hours at temperatures between 25 and 37°C. Colonies on yeast extract-peptone agar 2-5 mm diam., gray, flat, translucent, with entire edge. Green diffusible, fluorescent, water soluble pigment produced. No growth obtained in broth or on agar at temperatures 0-5°C or above 44°C. Temperature range for growth 7°C to 44°C. Growth at pH 4.5-9.0; no growth at pH 4.0. Growth in peptone broth with added NaCl 0.5%; no growth with 10% NaCl. Growth on seawater medium but no requirement for seawater or for addition of the specific cations Na⁺ or Mg⁺⁺. Acid accumulating only under aerobic conditions in OF base medium (Hugh and Leifson, 1953 - Difco 0688) containing glucose. Neither acid nor gas accumulating in purple broth base medium containing the following carbon compounds; Dextrin, galactose, glycerol, lactose, maltose, mannitol, mannose, melibiose, melezitose, sorbitol, sucrose. Growth and production of acid in OF base medium containing glucose and iodoacetate. Agar not digested. Starch and aesculin not hydrolyzed. The indole, methyl red, Voges-Proskauer and trimethylamine oxide reduction tests negative. Koser's citrate test positive. Gluconate test positive. Abundant production of slime in gluconate medium (Haynes, 1951) and fluorescent pigment also detected (Paton, 1959). Pyocyanine and fluorescin produced in Burton's medium (Burton, Eagles and Campbell, 1947). Pyocyanine produced on Sabouraud maltose agar (Martineau and Forget, 1958). Pyorubin produced (King, Ward and Raney, 1954). Oxychlororaphin crystals not pro-

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Table 1. Characteristics of *Pseudomonas aeruginosa* ATCC strain number 10145* and 14216.

	10145	14216
<u>Morphology:</u>		
rod-shaped	+	+
curved rods	-	-
(oval) spheres	-	-
filaments	-	-
length (0.2-0.6 μ)	+	+
tapered end	+	+
single cells	+	+
pairs	+	+
chains	-	-
motile	+	+
polar flagellum	+	+
Gram negative	+	+
Gram variable	-	-
<u>Colony morphology (yeast extract-peptone agar):</u>		
medium colony (2-5 mm)	+	+
entire edge	-	+
convex	-	-
grey	+	+
opaque	-	-
translucent	-	+
<u>Liquid culture characteristics (yeast extract-peptone broth):</u>		
pellicle	+	+
ring	-	-
moderate turbidity	+	+
even turbidity	-	+
<u>Carbohydrate reactions:</u>		
glucose (Hugh and Leifson test) aerobic acidity	+	+
anaerobic acidity	-	-
growth in glucose + iodoacetate medium	+	+
acid produced in glucose + iodoacetate medium	+	+
glucose gas	-	-
lactose acid	-	-
lactose gas	-	-
maltose acid	-	-
maltose gas	-	-
sucrose acid	-	-
sucrose gas	-	-
galactose acid	+	-
galactose gas	-	-
mannitol acid	-	-
mannitol gas	-	-
mannose acid	-	-
ethanol $\rightarrow$ acetic acid	-	-

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Table 1. (continued)

	10145	14216
glycerol → dihydroxyacetone	-	-
melibiose acid	.	-
melezitose acid	.	-
dextrin acid	.	-
glycerol acid	.	-
sorbitol acid	.	-
agar digested	-	-
starch hydrolyzed	-	-
gluconate oxidized (Gaby)	+	+
gluconate → 2-keto gluconate (Paton)	+	+
gluconate → keto-gluconate (Haynes)	+	+
gluconate → slime or "oyster" (Haynes)	+	+
aesculin hydrolyzed	-	-
methyl red test	-	-
Voges-Proskauer test	-	-
<u>Pigmentation:</u>		
diffusible, water-soluble, green fluorescent		
pigment produced in yeast-extract +		
proteose-peptone agar	+	+
fluorescent pigment produced in Paton's medium	+	+
pyocyanine produced in Burton's medium	+	+
fluorescein produced in Burton's medium	-	+
green pigment produced on Sabouraud agar	+	+
pyocyanine, fluorescein and "pyorubin" produced		
in King, Ward, Rany agar	+	+
oxychlororaphine produced	-	-
L-tyrosine → melanins	-	-
L-phenylalanine → melanins	-	-
<u>Physiological tests:</u>		
growth at 0°C	-	-
growth at 37°C	+	+
growth range 7°C-44°C		
(minimum and maximum growth temperatures)	+	+
growth in NaCl concentration 0-5.0%	+	+
growth in 10% NaCl	-	-
growth - initial pH 4.0	-	-
growth - initial pH 4.5	-	+
growth pH 5.0-9.0	+	+
<u>Nutrition:</u>		
growth in vitamin-free casamino acids	+	+
growth in:		
0.3% malonate broth	+	+
0.1% sodium formate agar	-	-
L-arginine + basal salts medium	+	+
L-lysine + basal salts medium	-	+
DL-alanine + basal salts medium	+	+

Table 1. (continued)

	10145	14216
B-alanine + basal salts medium	+	+
L-proline + basal salts medium	+	+
L-phenylalanine + basal salts medium	-	-
<u>Other biochemical tests:</u>		
nitrate reduced to nitrite	+	+
nitrite reduced	+	+
trimethylamine oxide reduced to trimethylamine	-	-
urea hydrolyzed	-	+
oxidase (Kovacs)	+	+
cytochrome oxidase (Gaby)	+	+
H ₂ S produced on lead acetate agar	-	-
cystine → H ₂ S	-	-
sodium thiosulfate → H ₂ S	-	-
arginine dihydrolase (Thornley)	+	+
tryptophan → indole	-	-
gelatin liquefied	+	+
litmus milk → acid, peptonization	+	+
litmus (milk) reduced	-	-
ammonia produced in peptone water	+	+
L-phenylalanine → phenylpyruvic acid	-	-
NH ₄ H ₂ PO ₄ utilized as N source	+	+
casein hydrolyzed	+	+
lecithinase	+	+
lipase (tweens 20, 40, 60, 80)	+	+
acetamide reaction	+	+
calcium lactate → acetate → carbonate	+	+
seawater required for growth	-	-
catalase	+	+
growth in Koser's citrate	+	+
<u>Antibacterials:</u>		
sensitive to:		
0/129 vibriostat	-	-
penicillin, high concentration disc	-	-
dihydrostreptomycin, 10 µgm	-	+
chloromycetin, 30 µgm	-	-
erythromycin, 15 µgm	-	-
novobiocin, 30 µgm	-	-
kanamycin, 30 µgm	-	+
aureomycin, 30 µgm	+	-
terramycin, 30 µgm	+	+
polymyxin B, 300 units	+	+
tetracyclin, 30 µgm	-	+

. = not tested.

* neotype strain proposed by Hugh and Leifson (1964, Intl. Bull. Bacteriol. Nomen. Tax. 14(2):69-84).

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duced. Gelatin liquefied. Casein hydrolyzed. Lecithinase and lipase tests positive. Acid peptonization of litmus milk with a soft casein coagulum formed. Ammonia produced in peptone water. Nitrate reduction to nitrogen gas. Phenylalanine deaminase negative. Arginine dihydrolase positive. Acetamide positive. Christensen's urea, catalase and cytochrome oxidase positive. Hydrogen sulfide not produced from peptone (lead acetate agar, Difco 0088), cystine or sodium thiosulfate (Colwell and Quadling, 1962). Growth on casamino acids-inorganic salts agar, on inorganic-N agar ($\text{NH}_4\text{H}_2\text{PO}_4$), and on inorganic salts agar with the following amino acids added as single source of N and C: l-arginine, l-lysine, DL-alanine, B-alanine and l-proline. No growth with l-phenylalanine, l-tyrosine, or formate as N and C source. Dihydroxyacetone not accumulated from glycerol. Ethanol not converted to acetic acid. Calcium lactate converted to carbonate through acetate. Growth in malonate broth (Difco 0395) and lysine broth (Difco 0215). Not sensitive to penicillin (high concentration sensi-disc, Baltimore Biological Laboratories), vibriostat 0/129 (Shewan, Hodgkiss and Liston, 1954), chloromycetin (30 μgm), erythromycin (15 μgm), novobiocin (30 μgm), or aureomycin (30 μgm), sensitive to dihydrostreptomycin (10 μgm), kanamycin (30 μgm), terramycin (30 μgm), polymyxin B (300 units) and tetracyclin (30 μgm).

The attributes presented are in agreement with those reported for Pseudomonas aeruginosa by Schroeter (1872), Migula (1900), and Haynes and Burkholder (1957).

A request for an Opinion from the Judicial Commission of the International Committee on Bacteriological Nomenclature, that the ATCC 14216 strain of Pseudomonas aeruginosa be designated as neotype has been submitted to Professor R. E. Buchanan, Chairman of the Judicial Commission.

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