THE PROPOSED NEOTYPE STRAINS OF PSEUDOMONAS
AERUGINOSA (SCHROETER 1872) MIGULA 1900

Request for an Opinion

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SUMMARY. An historical review of the name Pseudomonas aeruginosa (Schroeter) Migula is presented. The morphological and physiological characteristics, including electron micrographs and a photomicrograph of a stained preparation, are presented in Lysenko's proposed neotype strain (ATCC 10145, NCTC 10332, NCIB 8295, IBCAS 277, NRRL B-771, CCEB 481, RH 815) of Pseudomonas aeruginosa. This strain is compared with several other proposed neotype strains. The authors support the proposal by Lysenko and request an Opinion on this proposal by the Judicial Commission of the International Committee on Bacteriological Nomenclature.

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INTRODUCTION

The blue-green pus organism was first named Bacterium aeruginosum (Schroeter 1872, 126). The description given by Schroeter is as follows:

P.109: "Die kleinen Organismen, welche am häufigsten in ihren bewegten Formen als Bakterien, in ihren unbewegten als Bakteridien bezeichnet werden, stimmen darin überein, dass sie stickstoffhaltige, organische Stoffe (Protoplasma-massen) mit grosser Energie zur eigenen Ernährung verbrauchen, und dabei spezifische Stoffe mannigfacher Art bilden.

"Von diesen Produkten fallen eine Reihe von Pigmenten mit am meisten in die Augen. Es sind lebhaftere Färbungen der verschiedensten Art, die wir aus farblosen Protoplasma-Gebilden entstehen sehen, allein begleitet von massenhafter Entwicklung solcher kleiner Organismen, die wir demnach als alleinige Ursache der Farbenbildung ansehen müssen.

"Eine Reihe von Kulturen, die grösstenteils in den Wintermonaten 1868 bis 1870 im pflanzenphysiologischen Institute zu Breslau vorgenommen wurden, hatten den Zweck, Beobachtungen über derartige Pigmentbildungen zu sammeln. Im folgenden sollen kurz die erhaltenen Resultate nach der Reihe der Farben zusammengestellt werden. ..."

P.122: "Grün. Auf ausgelegten Stücken gekochter Kartoffeln sah ich einigemal eine fleckweise Grünfärbung eintreten. Die Farbe war ein dunkles Saftgrün, sie begann am Rande der Scheiben, breitete sich excentrisch aus und drang etwas in die Nährsubstanz ein. Eine Schleimauflagerung war nicht vorhanden, Bakterien vermochte ich nicht aufzufinden. So sehr ich auch geneigt bin, diese Pigment-Bildung bei Abwesenheit jeder anderen Ursache, auf Bakterien zurückzuführen, so kann ich sie doch nur als zweifelhaft in den Kreis dieser Betrachtungen ziehen.

"Eine genauer bekannte Produktion von grünem Farbstoff durch Bakterien findet in dem sogenannten grünen Eiter statt. Nach den von Anderen darüber vorgenommenen Untersuchungen, wird er durch bewegte Bakterien gebildet. Ich habe mehrfach grünen Eiter auftreten sehen, aber nicht in grosser Ausbreitung und unter Umständen, die mir eine mikroskopische und chemische Prüfung nicht möglich machten. Die Farbe war in diesen Fällen immer gleichmässig

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spangrün, etwas nach blau übergehend. Auffällig war die, schon andererseits hervorgehobene Tatsache, dass das Pigment nicht sowohl an dem Eiter haftet, der die Bakterien enthält, sondern in die Fäden der aufgelegten Charpie und Kompressen einzieht und diese in weiter Ausdehnung färbt. Es geht daraus hervor, dass auch hier nicht die Bakterien Träger des Farbstoffes sind, sondern dass er von diesen in die umgebende Flüssigkeit ausgeschieden wird...."

P.126: ... "Es ist vielleicht nicht unberechtigt bei jeder bestimmten Pigmentbildung einen spezifisch verschiedenen Organismus anzunehmen, und demgemäss neben einem Bacteridium prodigiosum (Ehrbg.) auch ein Bacteridium aurantiacum, luteum, cyaneum, violaceum, brunneum, neben Bacterium synxanthus und B. syncyanus (Ehrbg.) ein B. aeruginosum aufzustellen."

Schroeter (1872) reported the production of a water-soluble, blue-green pigment on sliced boiled potato and in pus. Schroeter acknowledged the work of others (without documentation) who observed that motile bacteria produce green pigment and green pus. It is clear that Schroeter (1872) neither microscopically observed nor isolated the organism which he named Bacterium aeruginosum. It might be said that the application of the name B. aeruginosum Schroeter is uncertain. However innumerable subsequent investigators have isolated, cultured, and observed the organism responsible for the production of blue-green pus and have characterized the causative organism and the water-soluble pigment (pyocyanine) it produces. Contemporary bacteriologists recognize only one species (Pseudomonas aeruginosa) which produces pyocyanine. Doubt concerning the application of the name Bacterium aeruginosum Schroeter is therefore quickly dispelled because no other organism has been implicated in the production of pyocyanine or blue-green pus. It is not clear why Cohn (1872, 157) referred to Bacterium aeruginosum Schroeter as Bacteridium aerugineum (sic).

The name Pseudomonas was proposed by Migula (1894, 237) for a genus which included a single species, P. violacea, which ordinarily would become the type species of the genus (by monotypy). There is no citation to a previously described species with this name nor is there any clue as to which of the nine or more previously described species

bearing the specific epithet violacea the name P. violacea Migula is to apply. The Editorial Board (Buchanan, Breed, and Cowan) of the Judicial Commission of the International Committee on Bacteriological Nomenclature (1951) concluded that under Rule 14 of the International Bacteriological Code of Nomenclature the species name Pseudomonas violacea Migula 1894 was not validly published, and under Rule 24 the name is illegitimate.

Migula (1895 p. 29) described the genus Pseudomonas in greater detail and listed 11 previously named species, the first of which was Pseudomonas pyocyanea (Gessard) Migula 1895 with the synonym Bacillus pyocyaneus Gessard.

Migula (1900 p. 884) published the new combination Pseudomonas aeruginosa (Schroeter 1872) Migula 1900 with the following synonyms: Bacterium aeruginosum Schroeter 1872, Bacillus aeruginosus Schroeter 1886, Bacillus pyocyaneus Gessard 1882b. Migula incorrectly ascribed the binary combination Bacillus aeruginosus to Schroeter 1886 p. 157. Bacillus aeruginosus (Schroeter 1872) should be attributed to Trevisan 1885 p. 101.

The Judicial Commission of the International Committee on Bacteriological Nomenclature (1952 Opinion 5) conserved the generic name Pseudomonas Migula 1894 and designated Pseudomonas aeruginosa (Schroeter 1872) Migula 1900 as the type species with the following synonyms: Bacterium aeruginosum Schroeter 1872, Bacillus pyocyaneus Gessard 1882a, Pseudomonas pyocyanea (Gessard) Migula 1895 p. 29. The citations for the last two synonyms are incorrect and should be Pseudomonas pyocyanea (Zopf) Migula 1895.

Zopf (1884 p. 83) ascribes Micrococcus pyocyaneus to Gessard 1882b. Migula (1895 p. 29) and Matzuschita (1902 p. 132) ascribe Bacillus pyocyaneus to Gessard. Macé (1889 p. 495), Migula (1900 p. 884), and Macé (1901 p. 852) ascribe Bacillus pyocyaneus to Gessard 1882b. Bergey et al. (1923 p. 123) and Breed et al. (1957 p. 99) ascribe Bacillus pyocyaneus to Gessard 1882a. The Judicial Commission of the International Committee on Bacteriological Nomenclature (1952, Opinion 5 p. 121), ascribe Bacillus pyocyaneus to Gessard 1882a. Examination of the Gessard 1882a and 1882b references clearly show that the binary combination Micrococcus pyocyaneus and Bacillus pyocyaneus were not used by Gessard. The term Bac-pyocyaneus α was used by Gessard (1890), and Ernst (1887) used the name Bacillus pyocyaneus (Zopf) Flügge 1886.

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Seven synonyms of Pseudomonas aeruginosa (Schroeter 1892) Migula 1900, 884 are:

Bacterium aeruginosum Schroeter 1872, 126
Bacteridium aerugineum (sic) Cohn 1872, 157
Micrococcus pyocyaneus Zopf 1884, 83
Bacillus aeruginosus (Schroeter) Trevisan 1885, 101
Bacillus pyocyaneus (Zopf) Flügge 1886, 286
Pseudomonas pyocyanea (Zopf) Migula 1895, 29
Bacterium pyocyaneum (Zopf) Lehmann and Neumann
 1896, 267

Inasmuch as Schroeter 1872 did not isolate Bacterium aeruginosum, the existence of a type strain is impossible. The alternative is to establish, according to prescribed procedure, a neotype strain. In this instance the neotype strain must: 1) produce pyocyanin, and 2) be a motile rod (Bacterium). Schroeter (1872) observed the water-soluble and blue-green pigment of Bacterium aeruginosum on boiled potato and in pus. P. aeruginosa is the only species of bacteria known to produce water-soluble blue-green pigment (pyocyanine). Subsequent work has demonstrated that P. aeruginosa is Gram-negative, rod-shaped, and motile by means of a polar flagellum. There is general agreement that P. aeruginosa has these characteristics.

During the past 5 years, several strains have been suggested in the literature as proposed neotype strains or temporary "working type strains" of P. aeruginosa. Sneath and Cowan (1958) designated NCTC 6750 a temporary working type strain for P. aeruginosa pending the establishment of a neotype strain by the International Committee on Bacteriological Nomenclature. This strain probably need not be considered seriously as a suitable neotype strain since it is atrichous. Sneath and Cowan (1958) also suggested NCTC 2000 as a reference strain for P. aeruginosa. Lysenko (1961) proposed strain CCEB 481 as the neotype strain. On the basis of median organism hypothesis studies, Liston et al. (1963) recommended either ATCC 14209 or ATCC 14216 as a suitable neotype strain. Others (personal communications) are of the opinion that old stock strains might be more suitable than recent isolates as candidates for a neotype strain and suggest that the following P. aeruginosa strains, all of which were isolated before 1925, be con-

sidered: ATCC 97, 256, 260 and 262. There are no rules or recommendations in the International Bacteriological Code of Nomenclature to indicate that an old strain is to be preferred over a recent isolate as a neotype strain. The singular advantage of an older strain is that it might be more extensively documented than a recent isolate. In the case at hand there appears to be no real advantage to the selection of an older strain as the neotype; it is our opinion that ability to produce pyocyanine is a more important consideration. This point is discussed below. However the only requirement of a neotype is that it agree with the diagnosis given by the original describer.

The purpose of this paper is to compare the characteristics of the above eight Pseudomonas aeruginosa strains (ATCC 97, 256, 260, 262, 10145, 14149, 14209 and 14216) and to suggest which one of the eight candidate strains would most adequately serve as the neotype strain.

MATERIALS AND METHODS

Table 1 gives some of the corresponding strain numbers of the 8 P. aeruginosa strains studied. The histories of these strains are given in Table 2. Kavanagh and Woodruff

Table 1. Corresponding numbers of the 8 Pseudomonas aeruginosa strains.

ATCC	97	256	260	262	10145	14149	14209	14216
RH	145	146	147	148	815	1091	1448	1455
NCTC					10332	2000		
NRRL	B-247	B-249	B-249	B-250	B-771			
van den Ende								D
NCIB					8295			
CCEB					481			
IBCAS					277			
Drake							44	

Key:

- ATCC American Type Culture Collection, Washington, D.C.
- NCTC National Collection of Type Cultures, London
- NRRL Northern Regional Research Laboratory, Peoria
- NCIB National Collection of Industrial Bacteria, Aberdeen
- CCEB Culture Collection of Entomogenous Bacteria, Prague
- IBCAS Institute of Biology, Czechoslovak Academy of Sciences, Prague

Table 2. The histories of 8 Pseudomonas aeruginosa strains.

ATCC strain number	Received at ATCC as strain	Date deposited at ATCC	Deposited by	Remarks
97		1925	Walter Reed Army Medical Center	Jordan strain.
256	151	1925	E.O. Jordan	Isolated (1898) by LeCount from gangrenous tissue of a patient with leukemia.
260	LeCount IV	1925	E.O. Jordan	Isolated by LeCount from an amputation stump of a patient with leukemia.
262		1925	E.O. Jordan	Gessard strain
10145		1946	F. Kavanagh	Used in antibiotic sensitivity studies. 1947 J. Bacteriol. <u>54</u> :761-766.
14149	Eyre I	1961	S.T. Cowan	NCTC 2000, 1958 J. Gen. Microbiol. <u>19</u> :551-65
14209	44	1961	C.H. Drake	Drake strain 44. 1960 J. Bacteriol. <u>80</u> :420-21
14216	D	1961	R.M. Wood	van den Ende strain D of human origin. 1952 J. Hyg. <u>50</u> :405-414

were unable to provide information concerning the date and source of isolation of P. aeruginosa ATCC strain 10145. The procedures used to characterize the 8 strains of P. aeruginosa have been described by Hugh and Ryschenkow (1961). Blood agar base (Difco 0045) which contains beef heart infusion was used to detect pyocyanine production.

RESULTS

Each of the 8 Pseudomonas aeruginosa strains studied have the same 26 positive characteristics and the same 23 negative characteristics enumerated in Table 3. Some of these 8 strains are positive, while others are negative in respect to the 8 characteristics enumerated in Table 4.

ATCC strain 10145 is described below:

Morphology: Gram-negative, asporogenous, rod-shaped; cells straight with soma size $0.5 \times 1.5-2.5 \mu$. Motile by means of predominantly one polar flagellum having a mean wavelength of 1.6μ (Figs. 1, 2, 3 in the plate). Two flagella may occasionally be found at one pole of some cells. Formalin treatment does not significantly change the flagellar shape. Capsules are not evident on the media employed in the study.

Physiology: This strict aerobe produces a dense turbidity in neutral peptone broth in 18-24 hours at temperatures of 22, 37 and 41°C , but fails to grow at 25 to 37°C in brain heart infusion broth at pH 4.5. It is neither halophilic nor halophobic. There is good growth in broth containing potassium cyanide.

An anaerogenic acid reaction is produced in unsealed tubes of Oxidative Fermentative (OF) Basal Medium (Difco 0668, Hugh and Leifson 1953) containing the following carbon compounds: arabinose, dextrose, ethanol 3%, fructose galactose, glycerol, mannitol, mannose, melibiose, d-ribose, trehalose, and xylose. The acid reaction is apparent in 1-3 days except with trehalose which may require 10 days of incubation before acid appears. An acid reaction is produced in unsealed tubes of OF dextrose medium containing 0.001 molar iodoacetic acid sodium salt. An acid reaction is not produced in OF dextrose medium sealed with petrolatum. Citrate (Simmons and Christensen), catalase, indophenol oxidase, gelatin, 2-ketogluconate, malonate, nitrate

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Table 3. Similar Characteristics of 8 *Pseudomonas aeruginosa* strains.

	ATCC Strain Number							
	97	256	260	262	10145	14149	14209	14216
Gram-negative rod-shaped	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
Flagellar wavelength 1.5-2 μ	+	+	+	+	+	+	+	+
Polar monotrichous	+	+	+	+	+	+	+	+
Growth at 22, 37 and 41 °C	+	+	+	+	+	+	+	+
Adonitol	-	-	-	-	-	-	-	-
Arabinose	+ ₂	+ ₂	+ ₂	+ ₂	+	+	+	+
Cellobiose	-	-	-	-	-	-	-	-
Dextrose open	+	+	+	+	+	+	+	+
Dextrose sealed	-	-	-	-	-	-	-	-
Dextrose gas	-	-	-	-	-	-	-	-
Dextrose with iodoacetic acid	+	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-
Ethanol, 3%	+ ₂	+	+	+ ₆	+	+ ₂	+	+
Galactose	+	+	+	+ ₂	+	+	+	+
Glycerol	+ ₃	+	+	+ ₂	+	+	+	+
Inositol	-	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-
Mannose	+	+	+	+ ₂	+	+	+	+
Melezitose	-	-	-	-	-	-	-	-
Melibiose (Difco)	+ ₂	+ ₂	+ ₂	+ ₃	+ ₃	+ ₂	+	+
Raffinose	-	-	-	-	-	-	-	-
d-Ribose	+ ₂	+ ₂	+	+ ₂	+	+	+	+
Salicin	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-
Xylose	+	+	+	+ ₂	+	+	+	+
Indole	-	-	-	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-	-
Acetylmethyl carbinol	-	-	-	-	-	-	-	-
Citrate-Simmons	+	+	+	+	+	+	+	+
Citrate-Christensen	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Indophenol oxidase	+	+	+	+	+	+	+	+
Gelatin-charcoal	+ ₂	+ ₃	+ ₂	+ ₃	+	+ ₂	+ ₂	+
Hydrogen sulfide-Kligler	-	-	-	-	-	-	-	-
2-ketogluconate	+	+	+	+	+	+	+	+
Lysine decarboxylase-Carlquist	-	-	-	-	-	-	-	-
Malonate	+	+	+	+	+	+	+	+
Nitrate to gas	+	+	+	+	+	+	+	+
Nitrate to nitrite	+	+	+	+	+	+	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-
Urea-Christensen	+	+	+	+	+	+	+	+
Lysine-Møller	-	-	-	-	-	-	-	-
Arginine-Møller	+	+	+	+	+	+	+	+
Ornithine-Møller	-	-	-	-	-	-	-	-
Growth at pH 4.5	-	-	-	-	-	-	-	-
Spores	-	-	-	-	-	-	-	-

EXPLANATION OF THE PLATE

Figure 1. Pseudomonas aeruginosa ATCC 10145 showing the polar monotrichous flagellation of the organism from broth culture without formalin fixation. The soma of this strain generally stains very faintly. Leifson flagella stain, X2,000.

Figures 2 and 3. Pseudomonas aeruginosa ATCC 10145 showing a rod from broth culture with a polar monotrichous flagellum. Electron micrograph, palladium shadowed, X22,000.

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Table 4. Dissimilar characteristics of 8 *Pseudomonas aeruginosa* strains.

	ATCC Strain Number							
	97	256	260	262	10145	14149	14209	14216
Fructose	wk+ ₂	wk+ ₂	-	-	+	+	+	+
Lactose	-	-	-	-	-	+ ₁₁	+ ₂₀	+ ₁₈
Mannitol	+	wk+	+	-	+	+	+	+
Rhamnose	-	-	-	-	-	+ ₇	-	+ ₇
Trehalose	-	-	-	-	+ ₁₀	+	+ ₈	-
Growth in KCN broth	+ ₇	-	+ ₄	-	+	+	+	+
Water soluble pigment	-	-	-	-	++++	-	-	-
Chloroform soluble pigment	-	-	-	-	++++	-	-	wk+

Key:

- + positive test on the first day; subscripts indicate the day test became positive
- negative test
- wk weak
- ++++ strongly positive

reduction to nitrite and nitrogen gas, urea (Christensen), and arginine (Møller) tests are positive. Pyocyanine is visibly produced in infusion agar (Difco 0045) and is readily extracted with chloroform.

An acid reaction is not produced in tubes of unsealed OF basal medium with the following carbon compounds: adonitol, cellobiose, dulcitol, inositol, inulin, lactose, maltose, melezitose, raffinose, Rhamnose, salicin, sorbitol, and sucrose. The indole, methyl red, acetylmethyl carbinol, hydrogen sulfide (Kligler, after 2 days' incubation), lysine decarboxylase (ninhydrin), phenylalanine deaminase, lysine and ornithine (Møller) tests are negative.

DISCUSSION

It is apparent that the 8 candidate strains of *Pseudomonas aeruginosa* are very homogeneous in respect to the 49 characteristics enumerated and compared in Table 3. They are alike in 26 positive and 23 negative characteristics. The strain differences recorded in Table 4 are not interpreted to be of profound significance in taxonomy except for pigment production, which may require some discussion as it relates to the selection of the neotype strain.

Some strains of *P. aeruginosa* readily produce large quantities of pyocyanine in almost every medium capable of supporting growth. Other strains are apyocyanogenic even when grown in many special media devised to induce pigment

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production (Gessard, Burton, Paton, Sellers, King, etc.). Between these two extremes are strains which appear to be apyocyanogenic except when grown on one of the special media devised to induce pigment production. Each pseudomonadologist appears to prefer his own favorite pyocyanine-inducing medium since there is little evidence that one medium is universally superior in inducing pyocyanine production in all strains. It should be observed that P. aeruginosa strains can be identified even though pyocyanine is not produced.

P. aeruginosa strains 97, 256, 260, 262, 14149, 14209, and 14216 did not produce visible water-soluble pigment on the infusion agar medium employed in this study (see Table 4). However, a very small quantity of pyocyanine was extractable with chloroform from the infusion agar medium on which strain 14216 had grown for 7 days. Pyocyanine could not be extracted from the infusion agar medium on which strains 97, 256, 260, 262, 14149, and 14209 had grown for 7 days. Haynes (1951) reported that strains 97, 257, 260 and 262 were apyocyanogenic in Gessard's and Burton's media, however these strains produce pyocyanine in Sellers medium. Each of the 8 P. aeruginosa strains produce pyocyanine in at least one of the special pigment-inducing media. None of the 8 strains are dramatically prolific pigment producers. However, of these 8 strains ATCC 10145 is unusual in that it readily produces visible pigment in infusion agar medium, the usual routine medium employed in the clinical diagnostic laboratory. This blue pigment is readily extracted with chloroform.

The quantitative equivalence of all characteristics to establish phenons in numerical taxonomy has created the problem of evaluating the significance of the various phenon levels in terms of the classically established species. Liston et al. (1963) published observations on the over-all similarity, expressed in S values, of 33 strains of P. aeruginosa as compared to a calculated hypothetical median organism, and Lockhart et al. (1963) emphasized the concept of monothetic core properties as the characteristic feature of a species, these properties being normally distributed among strains. The 17 minimal-core properties described by Liston et al. (1963) represent the minimum calculated hypothetical organism for P. aeruginosa. P. aeruginosa strains 14209 and 14216 were reported by Liston et al. (1963)

to show 97% similarity to their concept of the calculated median organism whereas strain 10145 has 82% similarity. The specific differences in the characteristics of strain 14216 and Lysenko's proposed neotype strain 10145 are not readily apparent from the data presented in the report by Liston *et al.* (1963).

Undoubtedly many strains of P. aeruginosa can meet the requirements of a neotype strain. Each of the 8 strains studied possesses the monothetic-core properties of P. aeruginosa; however, each would not be equally useful as a neotype strain of P. aeruginosa.

As a result of this comparative study of the 8 candidate strains and particularly because of pigment production, ATCC strain 10145 is judged to be the most suitable to recommend for the neotype strain of Pseudomonas aeruginosa. Lysenko (1961) proposed Pseudomonas aeruginosa ATCC 10145 as the neotype. The authors support this proposal and hereby request an Opinion on this proposal by the Judicial Commission of the International Committee on Bacteriological Nomenclature.

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