TAXONOMIC PROBLEMS RELATING TO THE
IDENTIFICATION OF SPECIES WITHIN
THE GENUS *PSEUDOMONAS*

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The 6th ed. of Bergey's Manual (1948) lists 261 names of species in the genus *Pseudomonas*, of which 148 are fully accepted and described in detail, 15 are believed better placed in the genus *Xanthomonas*, and 98 are listed in the genus as names of uncertain position. The genus is the largest in the 1948 edition of Bergey's Manual.

New species names are constantly being added to the genus *Pseudomonas*, frequently on the basis of some variable minor characteristics or solely on the basis of flagellation regardless of physiological characteristics. In many instances the species characteristics now listed are so vague and inadequate that laboratory reidentification of a culture that had lost its label would be impossible. This point is emphasized by the fact that, with few exceptions, investigators studying the genus (primarily the species *P. aeruginosa* and *P. fluorescens*) do not agree on the major characteristics of the group. These controversies include such basic and fundamental differences as typical colonial morphology, pigmentation, serological relationships, biochemical or physiological characteristics, odor, optimum temperature of growth, and even the ease, or difficulty, of overall identification of the genus. (1, 2, 5, 6, 7, 9, 10, 11, 13, 15, 16, 17)*

The type species of the genus, *P. aeruginosa*, can be isolated not only from feces, urine, skin burns, abscesses, chronic lung diseases, etc., but also from soil, water, and even plants. Because of the ubiquitous distribution of this and other species in the genus, when studying their characteristics, one must recognize and consider carefully their adaptability and dissociation. It is well known, for example, that cultures of *P. aeruginosa* frequently lose their

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*For a complete list of references, see Gaby, 1946 (6); Munoz et al., 1949 (13); and Haynes, 1951 (9).

ability to produce pigment and that freshly isolated strains may occasionally be nonpigmented. Under these conditions P. aeruginosa may be indistinguishable from numerous other Pseudomonas species. Studies carried out in this laboratory show that similarities exist among many of the species of the genus and that differences in their biochemical characteristics can be due to variations which commonly occur within this group of organisms.

Methods and Materials

Special attention has been given to all cultures isolated from clinical specimens and identified as P. aeruginosa in this diagnostic laboratory. Over 300 such cultures have been examined to date. Identification was based on the characteristics previously described by Gaby and Free (7). In addition, the physiological and morphological characteristics of 12 species of Pseudomonas, other than P. aeruginosa, obtained from the American Type Culture Collection, were compared with the clinical cultures and stock cultures of P. aeruginosa. All cultures were grown on heart infusion agar or broth. Biochemical characteristics were determined by standard techniques. All carbohydrate media were prepared in a peptone base broth. Cultures were incubated at 37°C, and room temperature (approx. 24°-25°C) for appropriate periods of time ranging up to three weeks before the final readings in litmus milk, gelatin, and carbohydrate media were recorded.

Results and Discussion

The biochemical characteristics of these species and strains are given in the table. Only the results obtained with five of the clinical cultures are shown to represent the degree of variation obtained in biochemical characteristics. Although the reactions observed are relatively constant, variations do occur. The two most variable reactions noted were those in litmus milk and carbohydrate media. It should be noted that the reactions listed here do not necessarily agree with those found in Bergey's Manual (1948). All of the species fell within the limits of biochemical variations found for strains of P. aeruginosa, with the possible exception of the last four species listed in the table. A sharp distinction

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

between several of the species, however, is evident in their optimum growth temperature range. Those species that grew well at 37°C, also grew well at room temperature (24°-25°C). However, 7 of the species did not grow as well at 37° as they did at room temperature.

It is evident from the data presented that the problem of species differentiation has two aspects. First, and perhaps the more important, is the similarity of P. aeruginosa to several other species of Pseudomonas; e.g., P. chlororaphis and P. putida were indistinguishable from P. aeruginosa on the basis of biochemical and growth characteristics, whereas, P. mucidolens, P. fluorescens, P. putrefaciens, P. graveolens, P. ovalis, P. mildenbergii, and P. fragi differed primarily from P. aeruginosa in their optimum growth temperature. Second, there is the problem of distinguishing among species of Pseudomonas; e.g., P. mildenbergii was found to be indistinguishable from P. ovalis and P. fluorescens was indistinguishable from P. putrefaciens.

On the basis of the fermentative metabolism of carbohydrates by P. hydrophila and other similar species, their inclusion in the genus Pseudomonas may be questioned. Hugh and Leifson (1953) pointed out the basic differences between the fermentative and oxidative metabolism of carbohydrates by Gram-negative bacilli and discussed the taxonomic significance of these reactions. On the basis of the results obtained in this laboratory with the various species and strains of Pseudomonas as well as the routine examinations of other Gram-negative bacilli isolated in the clinical laboratory, such as the paracolon, Proteus, Salmonella, and Shigella, it was concluded that the following four biochemical characteristics should tend to exclude an organism from the genus: 1. A fermentative carbohydrate metabolism; 2, acid production from sucrose; 3, acid production from lactose; and 4, production of acid curd in litmus milk.

The question naturally arises as to what are the characters of the type species P. aeruginosa, and what procedures should a laboratory follow in making a positive identification. Should such metabolic activities as the nonphosphorylated oxidation of glucose by way of gluconic and 2-ketogluconic acids by P. aeruginosa as described by Campbell and Norris (3) be considered a basic characteristic? Haynes (9) considers this characteristic along with maximum growth temperature and slime formation as sufficient evidence for the

identification of P. aeruginosa. However, it has been demonstrated in this laboratory that several strains of Proteus and paracolons possess one or more of these characteristics.

It should perhaps be reemphasized at this time that the pigmented strains are easily identified. The nonpigmented strains offer a real problem of correct or satisfactory identification if one adheres strictly to the keys and descriptions in Bergey's Manual. For example, where should one place a Gram-negative, nonpigmented, motile bacillus, producing only acid in glucose and sucrose, lactose negative, urea and gelatin negative, isolated from urine, or water? The taxonomist immediately points out that in this so-called hypothetical question the presence or absence of polar flagella is not mentioned. However, flagella staining is not routine in most laboratories and would not usually be regarded as necessary in this case as most clinical laboratories would regard this organism as a paracolon rather than as a Pseudomonas on the basis of acid production from sucrose. However, a lack of pigment alone does not rule out allocation to the genus Pseudomonas. Numerous examples could be cited to illustrate possible conflicting views as to the identity of many such borderline microorganisms. Studies are currently under way in this laboratory to determine the extent of variation and dissociation of P. aeruginosa. Until this has been satisfactorily determined one cannot expect to get agreement as to the circumscription of the type species. There is no doubt, however, as the data presented indicate, that these boundaries will incorporate many of the species now recognized. These findings are adequately substantiated in the literature, as Mehta and Berridge (12) have reported that not only did a close relationship exist between certain species of the genus Pseudomonas, but also that P. aeruginosa and P. marginale were identical in morphological and cultural characteristics and in pathogenicity on lettuce. Paine and Branfoot (14) found that P. aptata is identical with, and probably a strain of, P. aeruginosa. Elrod and Braun (4) concluded P. polycolor to be indistinguishable from cultures of P. aeruginosa. These authors also confirmed the results of Mehta and Berridge as to the identity of P. aeruginosa and P. marginale. There are many such examples in the literature, but for some reason they have been, with few exceptions, ignored in Bergey's Manual.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

BIOCHEMICAL REACTIONS

Species	Source	Optimum temperature	Nitrate reduction	Gelatin liquefaction	Litmus milk	Indole production	Glucose	Sucrose	Xylose	Lactose
<i>P. aeruginosa</i> R*	No. 51 stock	24°-37°	+	+	P	-	A	-	A	-
<i>P. aeruginosa</i> R*	No. 52 stock	24°-37°	+	+	P	-	A	-	A	-
<i>P. aeruginosa</i>	Clinical lab.	24°-37°	+	+	P	-	A	-	A	-
<i>P. chlororaphis</i>	ATCC	24°-37°	+	+	P	-	A	-	A	-
<i>P. aeruginosa</i>	Clinical lab.	24°-37°	+	+	P	-	A	-	-	-
<i>P. aeruginosa</i>	Clinical lab.	24°-37°	+	+	ALK.	-	A	-	A	-
<i>P. mucidolens</i>	ATCC	24°**	+	+	ALK.	-	A	-	A	-
<i>P. aeruginosa</i> S*	No. 52 stock	24°-37°	+	+	P	-	-	-	-	-
<i>P. fluorescens</i>	ATCC	24°**	+	+	P	-	-	-	-	-
<i>P. putrefaciens</i>	ATCC	24°**	+	+	P	-	-	-	-	-
<i>P. aeruginosa</i> S*	No. 53 stock	24°-37°	-	+	P	-	A	-	A	-
<i>P. aeruginosa</i> S*	No. 51 stock	24°-37°	-	-	ALK.	-	A	-	A	-
<i>P. putida</i>	ATCC	24°-37°	-	-	ALK.	-	A	-	A	-
<i>P. aeruginosa</i>	Clinical lab.	24°-37°	-	-	ALK.	-	A	-	A	-
<i>P. ovalis</i> R*	ATCC	24°**	-	-	ALK.	-	A	-	A	-
<i>P. mildenbergii</i>	ATCC	24°**	-	-	ALK.	-	A	-	A	-
<i>P. graveolens</i>	ATCC	24°**	-	-	-	-	A	-	A	-
<i>P. aeruginosa</i>	Clinical lab.	24°-37°	-	-	-	-	A	-	A	-
<i>P. fragi</i>	ATCC	24°**	-	-	-	-	A	-	-	-
<i>P. pavonacea</i>	ATCC	24°-37°	-	-	ALK.	-	-	-	-	-
<i>P. oleovorans</i>	ATCC	24°-37°	-	-	ALK.	-	-	-	-	-
<i>P. ovalis</i> S*	ATCC	24°**	-	-	ALK.	-	-	-	-	-
<i>P. hydrophila</i>	ATCC	24°-37°	+	+	P	+	AG	AG	AG	-

A = Acid. AG = Acid and gas. P = Peptonization. Alk = Alkaline. + = Positive reaction. - = No reaction.

* R = Rough, S = Smooth. From those species containing easily recognized R and S colonies.
 ** All cultures grew at 37°C. On repeated transfers at 37°, growth improved slightly but did not approach that observed at the optimum temperature.

Although no 'type culture' of the species P. aeruginosa has thus far been designated, it is possible to delimit the type species more accurately than is done in Bergey's Manual. The genotype P. aeruginosa is comprised of a highly dissociable group of Gram-negative, non-encapsulated rods. Cells are monotrichous, lophotrichous, or rarely, nonmotile. Pigments may or may not be produced. If pigments are formed they may be green (pyocyanin), brown (pyorubin), or fluorescent. The ability to produce pigments, especially pyocyanin, is frequently lost on repeated subculturing. Indeed, many freshly isolated cultures identified as P. aeruginosa on the basis of other characteristics do not produce pigment. Glucose and occasionally xylose are usually the only carbohydrates fermented with the production of acid in a peptone base medium, whereas in a synthetic medium acid may be produced from glucose, xylose, arabinose, galactose, glycerol, and mannose. Nitrates are usually reduced to nitrites. Gelatin is usually liquefied, especially in freshly isolated cultures. Indole formation is variable. Litmus milk usually becomes alkaline and peptonized. Fats and hydrocarbons may or may not be attacked. A pellicle is usually produced in liquid culture media. The colonial morphology is variable but can be classified into three basic types. 1. Large, spreading, with generally smooth, convex, translucent centers; effuse, flat, wavy transparent periphery; and irregular, lobulated edges. 2. Round, convex, translucent, finely granular or mucoid with entire edges. 3. Round or slightly irregular, slightly raised, umbilicate or umbonate, finely or coarsely granular, simulating a rough colony. Representatives of this species are commonly found in water, soil, vegetable matter, and are opportunists, frequently causing secondary infections in man.

Summary

A study of clinical and stock cultures of P. aeruginosa and 12 other species of Pseudomonas obtained from the American Type Culture Collection indicated that many of these cultures are indistinguishable from one another and from P. aeruginosa when their morphological and biochemical characteristics are determined by standard procedures. While there are, no doubt, several distinct species of Pseudomonas, it is evident that there have been placed in the

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

genus many ill-defined and illegitimate species. No type culture of Pseudomonas aeruginosa has thus far been designated. It is therefore as yet impossible accurately to describe it. An amended description of the type species, however, is given which is more accurate than that given in Bergey's Manual.

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