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Pseudomonas aeruginosa—its Characterization and Identification*

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SUMMARY: The species *Pseudomonas aeruginosa* is defined more precisely, and a method is presented whereby apyocyanogenic strains of this species can be correctly identified. This method depends upon the correlated characteristics: (1) ability to grow at $41^{\circ} \pm 1$; (2) ability to oxidize potassium gluconate, in shaken culture, to a reducing compound presumed to be potassium 2-ketogluconate; (3) production of slime in static culture in a medium containing potassium gluconate as the principal carbon source.

All strains of *P. aeruginosa* tested, regardless of their pigment-forming capacity or their stage of growth, could be converted to a type of growth characterized by profuse sliminess in liquid potassium gluconate medium.

Detection of pyocyanogenic capacity is shown to be equally reliable in Gessard's glycerol peptone agar and in Burton's defined medium when negative results in one or the other medium are rechecked in both, and when the observation period in Burton's medium is lengthened.

According to the International Bacteriological Code of Nomenclature (Buchanan, St John-Brooks & Breed, 1948), the type species of a genus is the species with which the genus name is permanently associated. The proper characterization of such type species is, therefore, most important. Furthermore, the systematic relationships of related organisms can hardly be made clear until type species are well enough characterized so that they can be readily differentiated. *P. aeruginosa* (Schroeter) Migula, the type species of the genus *Pseudomonas*, is a case in point.

Because of the ease of identification of pyocyanine, the blue-green water- and chloroform-soluble pigment produced by typical strains, *P. aeruginosa* is among the easiest of bacteria to identify. Atypical strains which fail to produce this pigment are common, however, and have been discussed by Lehmann, Neumann & Breed (1931), Gessard (1919), Jordan (1899) and Gaby (1946). In fact, Gaby (1946) found that non-pyocyanine-producing types predominate. He suggested that they are more typical of the species than the more striking pyocyanine-producing types. Furthermore, pyocyanine-producing strains have been reported commonly to lose the capacity to produce this pigment (Jordan, 1899; Hadley, 1927; Lehmann, Neumann & Breed, 1931; Gaby, 1946). The certain identification of such apyocyanogenic strains depends largely upon their history of having once been able to produce pyocyanine, or upon the fact of

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their isolation from pathogenic processes in higher animals. However, organisms identified as *P. aeruginosa* have been isolated from a variety of other sources including soil, water, sewage, normal skin, and contaminated media. Thus strains not producing pyocyanine, from whatever source, cannot be dismissed from consideration as *P. aeruginosa*.

As early as 1899, Jordan suggested that the chief difference between *Bacillus fluorescens liquefaciens* (*Pseudomonas fluorescens*) and his fluorescogenic, non-pyocyanine-producing variety of *P. aeruginosa* was one of temperature optima, and perhaps in their behaviour in the animal body. Although he dismissed the hypothesis for lack of convincing evidence, he mentioned the possibility that *P. fluorescens* might be a modified or degenerate form of *P. aeruginosa*. This theory has been advanced time and again over the years, most recently by Sandiford (1937), Christie (1948) and Gaby (1946). Since it could neither be proved nor disproved, it has clouded all attempts to distinguish between the two. Preoccupation with this problem perhaps accounts for the preponderance of attention given in the literature to distinguishing between *P. aeruginosa* and *P. fluorescens*.

Many bacteriologists seem unwilling to admit the existence of other distinct species. The consensus of opinion is probably expressed by Stanier (1947), who suggested the term '*Pseudomonas fluorescens* species group' for fluorescent pseudomonads unable to manufacture accessory phenazine pigments.

Although most serological studies have been unproductive in contributing to a differentiation of species in the genus, Munoz, Scherago & Weaver (1949) have recently found it possible to differentiate serologically among 10 species.

Only two characteristics other than antigenic make-up of the cells have shown much promise. They are growth temperatures and ability to haemolyse blood. The ability of *P. aeruginosa* to grow well at 37°, in contrast to the inability of *P. fluorescens* and most other fluorescent bacteria to do so, is well known and has served as the main differential characteristic for many years. The existence of pseudomonads other than *P. aeruginosa* which grow well at 37°, however, has limited the usefulness of this knowledge. In recent years it has been claimed that the ability to haemolyse blood of certain warm-blooded animals is characteristic of *P. aeruginosa* (Christie, 1948; Reid, Naghski, Farrell & Haley, 1942; Salvin & Lewis, 1946; Gaby, 1946). This knowledge is of limited usefulness, however, in the absence of information to show that other pseudomonads which grow at 37° fail to haemolyse blood.

Differentiation of *P. aeruginosa* from other fluorescent bacteria based upon temperature relationships has been improved by Seleen & Stark (1943) who showed that good growth of *P. aeruginosa* occurs at 42°. The data obtained during the current investigation confirm the view that all strains of this organism grow well at 42°, and in addition demonstrate that this characteristic will, in company with two others, permit the identification of *P. aeruginosa* even though the ability to produce pyocyanine be lacking.

METHODS

Pyocyanine production

Four media were used in surveying the group for ability to produce pyocyanine: Gessard's glycerol peptone agar stabs, (1891; see also Seleen & Stark, 1943), Sullivan's K and M media (1905), and the complete medium of Burton, Campbell & Eagles (1948). Sullivan's K and M media were soon discarded, since only 50 % of strains which produced pyocyanine in Gessard's stabs and in Burton's complete medium produced the pigment in these substrates. Furthermore, no strain which failed to produce pyocyanine in Gessard's medium succeeded in doing so in Sullivan's media.

Burton's medium, dispensed 24 ml. to each 300 ml. flask, was incubated at 30° (air temperature). The larger quantity of medium is a departure from the method as recommended by Burton *et al.*, but was found necessary in order to make tests possible beyond the 4-day incubation period recommended. Many strains required a longer period to produce appreciable quantities of pyocyanine. Tests in Gessard's medium in 16 mm. test-tubes were conducted in a $36^{\circ} \pm 1$ water-bath because it has been shown that a more uniform temperature is thus maintained throughout the tube (Gordon & Smith, 1949).

When the intensity of blue or blue-green colour in the medium indicated that a strain had reached its limit of pyocyanine production, the pigment was extracted with chloroform. In the solid glycerol-peptone medium of Gessard the agar was macerated by means of a glass rod to obtain more intimate contact between it and the chloroform. The chloroform containing the extracted pigment was then decanted into another test-tube. In the liquid medium, the supernatant was aspirated and discarded after chloroform extraction. Approximately 1 ml. of distilled water was then added to the chloroform extract in either case to serve as a vehicle for the acid pyocyanine, which was obtained by the addition of 1 drop of N-H₂SO₄. Upon shaking, the pyocyanine became red and insoluble in the chloroform. The appearance of a red water-soluble pigment in the aqueous acid layer was accepted as confirmation of the presence of pyocyanine.

Temperature relationships

All 52 strains were streaked in quadruplicate on agar slants of the following composition (TGY slants): tryptone, 5 g.; glucose, 1 g.; yeast extract, 5 g.; K₂HPO₄, 1 g.; agar, 15 g.; distilled H₂O, 1000 ml.; pH 6.8-7.0.

Slants of each strain were then incubated at each of the following temperatures: 28-30° (air temperature), $36^{\circ} \pm 1$ (water-bath temperature), $41^{\circ} \pm 1$ (water-bath temperature) and $45^{\circ} \pm 1$ (water-bath temperature). Incubation was continued until maximum growth occurred (usually about 48 hr.), or for 1 week.

Oxidation of potassium gluconate

Each strain was inoculated from a stock slant to a 300 ml. Erlenmeyer flask containing 100 ml. of sterile medium of the following composition: tryptone,

1.5 g.; yeast extract, 1 g.; K_2HPO_4 , 1 g.; potassium gluconate, 40 g.; distilled water, 1000 ml.; pH 7.0.

The inoculated flasks were then put on a rotary shaker (Gump-type) at 28–30°. The shaker was set at 200 r.p.m. and each flask was rotated through an orbit of 2½ in. diameter. At 2, 4, 7 and 14 days, samples were tested in the following manner.

To 1 ml. of the culture in a 16 mm. test-tube, 10 ml. of a copper sulphate sugar reagent (Shaffer & Hartmann, 1921—'Carbonate-citrate reagent for cupric titration') were added. The contents of the tubes were thoroughly mixed, heated for 10 min. in boiling water, rapidly cooled in cold water and then set aside until the next morning to settle the precipitate of reduced copper. The percentage conversion of potassium gluconate to potassium 2-ketogluconate was then estimated by comparison with previously prepared standards made with weighed quantities of calcium 2-ketogluconate. The conversion was recorded as 75, 50 or 25 %, or none. Exact quantitative relationships were deemed unessential to establish the capability of these organisms to oxidize potassium gluconate to potassium 2-ketogluconate.

Slime production

The same medium used for determining the ability of these organisms to oxidize potassium gluconate was used for slime production. (Slime production as a significant diagnostic characteristic of *P. aeruginosa* was first suspected when an associate, Dr H. J. Koepsell, detected its presence in a flask which had been allowed to stand for several hours.)

On the completion of observations for oxidation of potassium gluconate, the flasks were allowed to stand at room temperature until maximum slime formation occurred. This was usually 4 days after the flasks were removed from the shaker. Maximum slime formation was recognized when all of the following phenomena, listed in the order of appearance, were observable.

Slimy pellicle. The pellicle which forms in still culture appears slimy when the flask is gently swirled. This pellicle usually appears after overnight incubation in still culture.

Reverse swirl. When a flask containing slime is vigorously swirled and then set on a table, the swirling liquid rapidly slows to a stop and then abruptly reverses direction. This phenomenon is usually weak after overnight incubation, but rapidly becomes stronger until about the fourth day. Thereafter, in some strains this phenomenon increases in intensity, although more slowly, until the liquid becomes quite viscous. With such strains no diminution has been observed, even after several weeks. In other strains, however, a maximum is reached, after which the sliminess subsides until it can scarcely be detected.

Transitory film formation. After the pellicle has been submerged by vigorous swirling, and while it is still being gently swirled, examination of the flask by transmitted light reveals a slimy, transparent film on the walls of the flask where the liquid has splashed. This film slowly flows down into the body of the liquid again.

'Oyster' formation. After film formation and the reverse swirl phenomenon have become strong there usually is a differentiation of a part of the slime into an 'oyster-like mass' which becomes visible when the centrifugal action of swirling throws the remainder of the fluid towards the walls of the flask, leaving the 'oyster' at the apex of the vortex.

When slime formation has progressed to the stage where there is a good reverse swirl, a wire hook or loop inserted into the liquid and withdrawn draws the slime out into a thread which is quite thick and viscid, and in some cases may be stretched a foot or more before it breaks.

Strains of bacteria observed during this study included all strains received as *P. aeruginosa*, strains re-identified by the writer as *P. aeruginosa*, and finally a few strains isolated at this Laboratory and identified as *P. aeruginosa*. The sources of these strains are shown in Table 1.

RESULTS

Pyocyanine production

Thirty-six strains produced pyocyanine in Gessard's glycerol peptone agar stabs and in Burton's medium. It must be pointed out, however, that strain NRRL-B-219, although it usually produces pyocyanine in either medium, has failed to do so on at least one occasion in each. Another strain, NRRL-B-255, has always produced pyocyanine in Burton's medium, but failed to do so on one occasion in a Gessard stab.

Nine strains have been consistently negative in regard to pyocyanine production in both media. On the other hand, three strains which have never given a positive test for pyocyanine in Gessard's medium have done so in Burton's medium, two of these producing but a trace. Likewise, four strains consistently positive in Gessard stabs have produced but a trace of pyocyanine (three strains) or none (one strain) in the medium of Burton *et al.* Thus these media are equally effective in detecting pyocyanogenic strains. However, neither can be depended upon to reveal all of them, nor can a single negative result be accepted as reliable.

It is suggested that Burton's medium be used for routine surveys and that strains which fail at the initial test to produce pyocyanine be re-checked in the same medium and also in Gessard stabs. Burton's medium is selected since its liquid form makes it more easily prepared and dispensed and it is possible to test samples at intervals during an observation period of 10 days to 2 weeks. A summary of results obtained with each medium for each strain is shown in Table 1.

Temperature relationships

Of 138 pseudomonads tested for growth on TGY slants in the $41^{\circ} \pm 1$ water-bath, 57 grew well. In addition to these, 43 others grew at $36^{\circ} \pm 1$, while all 138 grew well at 30° . Among the 57 which grew well at $41^{\circ} \pm 1$ were the 43 pyocyanogenic strains of *P. aeruginosa* and the 9 apyocyanogenic strains identified as members of the same species on the basis of the three other characteristics shown herein to be diagnostic for it. These data are given in

Table 1. *Strains studied and pyocyanogenic capacity of each in two media*

NRRL no.	Received as	Pyocyanine production in		Donor
		Gessard stabs	Burton's medium	
B-7	<i>Pseudomonas fluorescens</i>	—	—	N. R. Smith, as no. 112
B-12	<i>P. fluorescens</i>	—	—	ATCC, as no. 142
B-23	<i>P. vendrelli</i>	+++	+++	ATCC, as no. 7700
B-26	<i>P. aeruginosa</i>	++	++	N. R. Smith, as no. 110
B-27	<i>P. aeruginosa</i>	+++	+++	N. R. Smith, as no. 111
B-79	<i>P. synxantha</i>	++	+++	H. F. Long
B-189	<i>P. fluorescens</i>	++	+++	E. McCoy
B-211	<i>P. aeruginosa</i>	++	++	NRRL isolate
B-217	<i>Pseudomonas</i> sp.	++	+++	L. D. Bushnell, as Bear
B-218	<i>Pseudomonas</i> sp.	++	+++	L. D. Bushnell, as no. 82
B-219	<i>Pseudomonas</i> sp.	++	++	L. D. Bushnell, as K-4
B-220	<i>Pseudomonas</i> sp.	++	++	L. D. Bushnell, as PS L
B-221	<i>Pseudomonas</i> sp.	—	—	L. D. Bushnell, as Ps
B-222	<i>Pseudomonas</i> sp.	+	++	L. D. Bushnell, as YS-6
B-241	<i>P. aeruginosa</i>	—	—	ATCC, as no. 257
B-247	<i>P. aeruginosa</i>	—	—	ATCC, as no. 97
B-248	<i>P. aeruginosa</i>	—	—	ATCC, as no. 256
B-249	<i>P. aeruginosa</i>	—	—	ATCC, as no. 260
B-250	<i>P. aeruginosa</i>	—	—	ATCC, as no. 262
B-255	<i>P. aeruginosa</i>	++	++	K. H. Lewis, as A-4
B-256	<i>P. aeruginosa</i>	++	+++	K. H. Lewis, as D-1
B-257	<i>P. aeruginosa</i>	+++	++	K. H. Lewis, as A-3
B-264	<i>Pseudomonas</i> sp.	+++	+++	C. N. Stark, as no. 1
B-265	<i>Pseudomonas</i> sp.	++	+++	C. N. Stark, as no. 2
B-275	<i>Pseudomonas</i> sp.	—	—	C. N. Stark, as no. 150
B-323	<i>P. fluorescens</i>	+	+	M. Solowey, as no. 4089
B-325	<i>P. aeruginosa</i>	++	+++	NRRL isolate
B-428	<i>P. fluorescens</i>	++	++	M. Solowey, as no. 6918-1
B-431	<i>P. fluorescens</i>	+++	+++	M. Solowey, as no. 4215
B-450	<i>P. aeruginosa</i>	+	++	NRRL isolate
B-451	<i>P. aeruginosa</i>	+	+++	NRRL isolate
B-452	<i>P. aeruginosa</i>	+++	+++	NRRL isolate
B-534	<i>P. aeruginosa</i>	++	+++	NRRL isolate
B-716	<i>P. aeruginosa</i>	+	++	NRRL isolate
B-743	<i>P. chlororaphis</i>	+++	+++	R. Y. Stanier, as A.3.30
B-771	<i>P. aeruginosa</i>	++	+++	ATCC, as no. 10145
B-786	<i>P. synxantha</i>	++	++	P. H. H. Gray
B-800	<i>P. aeruginosa</i>	+++	+++	ATCC, as no. 9027
B-853*	<i>P. polycolor</i>	+++	++	W. H. Burkholder, as PP2
B-903†	<i>P. aeruginosa</i>	++	++	E. W. Schultz, as U-21 <i>d</i>
B-904†	<i>P. aeruginosa</i>	++	±	E. W. Schultz, as U-21 <i>e</i>
B-937	<i>P. chlororaphis</i>	+++	+++	M. P. Starr, as MD 44.1
B-982*	<i>P. marginalis</i>	+++	++	NCTC, as no. 1995
B-996	<i>P. pyocyanea</i> var. <i>erythrogenes</i>	—	±	NCTC, as no. 5083
B-997	<i>P. pyocyanea</i> var. <i>erythrogenes</i>	++	—	NCTC, as no. 6749
B-1000‡	<i>P. aeruginosa</i>	—	++	E. J. Hehre, as 'Henriksen'
B-1019‡	<i>P. aeruginosa</i>	++	±	L. H. Schwarz, as LS-1
B-1069	<i>Malleomyces haemorrhagicogenes</i>	—	±	NCTC, as no. 8028
B-1080	<i>Pseudomonas pyocyanea</i> var. <i>erythrogenes</i>	++	++	NCTC, as no. 4844
B-1083	<i>P. aeruginosa</i>	++	+++	NRRL isolate
B-1087	<i>P. aeruginosa</i>	+	++	NRRL isolate
B-1088‡	<i>P. aeruginosa</i>	++	±	ATCC, no. 10197

* Presumably phytopathogens. The strain of *P. polycolor* and one other, previously has been shown by Elrod & Braun (1942) to be the same as *P. aeruginosa*. Mehta & Berridge (1924) and Elrod & Braun (1942) believe *P. marginalis* is the same as *P. aeruginosa*. St John-Brooks, Nain & Rhodes (1925) confirm this relationship so far as this particular strain is concerned, but not with another strain received from Erwin F. Smith.

† Strains B-903 and B-904 are two strains of E. W. Schultz's gelatinous variant (1947).

‡ Strains B-1019 and B-1088 are strains of the mucoid *P. aeruginosa* described by Schwarz & Lazarus (1947). B-1000 is a strain of the mucoid *P. aeruginosa* described by Henriksen (1948).

ATCC=American Type Culture Collection. NCTC=National Collection of Type Cultures (Great Britain). NRRL=Northern Regional Research Laboratory (U.S.A.).

Table 2. The remaining five strains which grew at $41^{\circ} \pm 1$ failed to meet the other requirements for inclusion in the species.

Table 2. Comparison of selected growth temperatures with potassium gluconate oxidizing capacity of some pseudomonads

Grow well at	No. of strains	No. of strains converting gluconate to 2-ketogluconate to extent of		
		75 %	50 %	25 % or less
$41^{\circ} \pm 1$	57	52	0	5
$36^{\circ} \pm 1$	43	30	4	9
30°	38	26	0	12
Total	138	108	4	26

Thus, this investigation confirms reports that *P. aeruginosa*, whether or not pyocyanogenic, grows well at 42° . Like other investigators, I have encountered strains able to grow at this temperature which are not *P. aeruginosa*. Growth of *P. aeruginosa* at 45° is poor, when it occurs.

Oxidation of potassium gluconate

Lockwood, Tabenkin & Ward (1941) reported the production from glucose of 2-ketogluconic acid by 22 strains of *Pseudomonas* representing 12 species. Five strains of *P. aeruginosa* were among the 22 strains investigated, including *P. vendrelli*, which subsequently was shown to be *P. aeruginosa* (Tobie, 1946); and two strains listed by Lockwood *et al.* as *P. fluorescens* (ATCC-142 and the N.R. Smith strain), which have been currently re-examined and are now identified as apyocyanogenic strains of *P. aeruginosa*. Their observation points to the possibility that the entire genus may be characterized on the basis of an oxidative attack on glucose and other carbon sources. With a view to elucidating the value of this property in the systematic relationships of these and related organisms, a survey of several hundred strains representing many species was undertaken. Results of the survey will be published later. During the survey it was found that all strains of *P. aeruginosa* oxidized glucose to a compound which was shown to be 2-ketogluconic acid. The five strains of *P. aeruginosa* included in the report by Lockwood *et al.* (1941) are among the strains herein reported.

Working exclusively with *P. aeruginosa*, ATCC-9027 (NRRL-B-800) Norris & Campbell (1949) identified 2-ketogluconic acid as an intermediate in the oxidation of glucose, and also demonstrated that the resting cells were able to oxidize gluconate. The strain with which they worked is included among the strains studied in this investigation. Stubbs, Lockwood, Roe, Tabenkin & Ward (1940) demonstrated the ability of an organism later identified as a pseudomonad to produce 2-ketogluconic acid from calcium gluconate.

Inasmuch as gluconic acid does not reduce Benedict's reagent and yet may be oxidized to 2-ketogluconic acid which does reduce it, the advantage of gluconic acid as the carbon source in studying the oxidative metabolism of these organisms is obvious. The potassium salt of gluconic acid was selected

for use in our medium because it gives clear solutions and is readily available. It was furnished at a concentration of 4 % (w/v), at which level potassium 2-ketogluconate accumulated and could be estimated at convenient intervals.

All pyocyanogenic strains of *P. aeruginosa* rapidly oxidized potassium gluconate to a reducing compound, presumably potassium 2-ketogluconate, and at the end of 2 days had effected a conversion of at least 50 % of the gluconate to 2-ketogluconate. By the fourth day the conversion usually was 75 % or more. Shaking was continued until a conversion equivalent to at least 75 % was obtained at two successive observations. This had usually been accomplished by the seventh day. The nine apyocyanogenic strains, tested in like manner, produced similar results.

The fact that many strains, other than those identifiable as *P. aeruginosa*, oxidized gluconate to 2-ketogluconate does not invalidate use of this characteristic for identification of the species; as shown in Table 2, only 52 strains of 112 able to oxidize gluconate were also able to grow well at $41^{\circ} \pm 1$. Those 52 strains are *P. aeruginosa* on the basis of this and two other characters found to be characteristic of the species.

Slime formation in potassium gluconate broth

From time to time reports have appeared in the literature of the isolation of mucoid variants (Schwarz & Lazarus, 1947; Henriksen, 1948; Sonnenschein, 1928; Dahr & Kolb, 1936) and of gelatinous variants (Schultz, 1947) of *P. aeruginosa*. Neither type of growth is common, such forms being regarded as more or less unstable variants of the usual butyrous type. However, it was possible to provoke, in all strains of *P. aeruginosa* tested, a style of growth which is best described as 'slimy'. This type of growth develops in a tryptone yeast-extract K_2HPO_4 basal medium containing 4 % potassium gluconate. When glucose is substituted for the potassium gluconate, slime formation does not occur. (Calcium gluconate was not able to replace potassium gluconate as the carbon source.) The optimum temperature for slime production is 36° , although slime forms also at 30° and 25° , but more slowly. At 41° there is rapid production of slime, but this never becomes as well developed as at 36° . Phosphate ion appears to be essential for maximum development of the slimy material; yeast extract is not essential and may be excluded when the tryptone is increased to at least 0.25 % (w/v).

In shake flasks slime is seldom observed. It is easily recognized, however, when flasks that have been shaken for 3 or 4 days are allowed to stand for an additional 18-24 hr.

In static culture at 36° (water-bath temperature), which represents optimum conditions, sliminess may be observed after 18-24 hr. As in shake flasks which are allowed to stand, the degree of sliminess increases until about the fourth day, but with no appreciable change thereafter.

Slime formation in this medium may also be observed in test-tubes incubated at 36° . The various manifestations of slime are not so readily seen under these circumstances, but the reverse swirl effect can be demonstrated.

On agar slants, prepared by adding 1.5 % of agar to the medium used in

liquid culture, sliminess developed only in the tubes inoculated with a gelatinous variant received from E. W. Schultz (NRRL-B-904), and with a mucoid strain (NRRL-B-1019) sent by L. H. Schwarz. The other gelatinous strain and the other mucoid strains were not tested. All other strains tested produced a growth which may be described as 'elastic'.

Several hundred strains belonging to many genera in the family Pseudomonadaceae, primarily in the genus *Pseudomonas*, have been inspected for slime formation. Although the degree of sliminess observed has never approached that attained by the *P. aeruginosa* cultures, a few have developed a relatively low degree of sliminess. This fact does not invalidate the criteria herein offered for the identification of *P. aeruginosa*, since the identification is not dependent upon slime formation alone but upon two additional characteristics which correlate with it, as shown in Table 3. None of the slime-forming organisms other than *P. aeruginosa* were able also to grow at 41° and to oxidize potassium gluconate to a reducing compound.

Table 3. Comparison of selected growth temperatures with ability to produce slime in potassium gluconate broth

Grow well at	No. of strains	No. of strains showing intensity of slime formation as		
		Strong	Moderate	Weak
41° ± 1	53	52	0	1
36° ± 1	16	0	0	16
30°	17	2	2	13
Total	86	54	2	30

The 52 strains which grew at 41° ± 1 and produced profuse sliminess were the 43 pyocyanogenic and 9 apyocyanogenic strains of *P. aeruginosa*. The remaining strain which grew at 41° ± 1 did not approach *P. aeruginosa* in slime-forming ability and, more important, did not oxidize gluconate to 2-ketogluconate.

DISCUSSION

It is believed that the three correlating characteristics presented here will make it possible to remove from the '*Pseudomonas fluorescens* species group' those organisms which are truly *P. aeruginosa* and perhaps thereby diminish confusion to the point where further progress can be made in establishing the true relationships of the remaining members of the group.

The ability of *P. aeruginosa* to oxidize potassium gluconate to 2-ketogluconate was implied by the work of Norris & Campbell (1949) and Stubbs *et al.* (1940). It has been confirmed in the present investigation and shown to be of taxonomic value when used as a diagnostic characteristic along with ability to grow at 42° and to produce slime from potassium gluconate.

Slimy or mucoid strains of *P. aeruginosa* are seldom encountered, and have usually been associated with pathogenic conditions in man. Upon the potassium gluconate medium used in this investigation we have found that all strains of

P. aeruginosa grow in the mucoid form. These included pyocyanogenic and apyocyanogenic strains, gelatinous variants, mucoid strains, and the usual types which produce butyrous growth on solid media. Slime production is a constant characteristic, showing correlation with two other constant characteristics, and is believed to be, in company with them, a reliable characteristic for use in identification of *P. aeruginosa*.

In 1896, Pottien described *Bacillus fluorescens capsulatus*, a mucoid organism isolated from intestinal contents in several fatal cases of cholera nostras. It has been suspected that this was another instance where the investigator was dealing with a mucoid strain of *Pseudomonas aeruginosa*. However, according to the original description, such is definitely not the case, since *Bacillus fluorescens capsulatus* fermented glucose and glycerol with the evolution of gas and produced an unidentified crystalline compound in several media. Gas production in glycerol media relegates Pottien's species to the acid- and gas-producing group (*Aeromonas*).

The value of more precise means of identifying *Pseudomonas aeruginosa* is illustrated by the following occurrence. Dr Aldo Castellani isolated a micro-organism from a pathogenic condition of human beings and assigned it the name *Malleomyces haemorrhulcogenes*. He sent cultures to S. T. Cowan (National Collection of Type Cultures, Colindale, London) who reidentified it as *Pseudomonas pyocyanea* (*P. aeruginosa*). A culture was forwarded to me and I have confirmed Dr Cowan's opinion by the methods presented in this report. In the meantime, Castellani (1950) has reassigned this organism to the genus *Pseudomonas*, but has retained the specific epithet *haemorrhulcogenes*.

The value of means for the identification of *P. aeruginosa* supplementary to pyocyanine formation is demonstrated further by the fact that 11 strains received as members of five other species of the genus were identified as *P. aeruginosa*. Nine of them produced pyocyanine in Gessard's medium and in Burton's medium, while two failed to do so in any medium tried. It is apparent that the certain identification of *P. aeruginosa* requires the use of media and conditions specifically designed and proved most suitable to elicit characteristic responses. Failure to do so is bound to result in misnaming of many isolates.

Strain NRRL-B-853 received as *P. polycolor* is one of two strains shown by Elrod & Braun (1942) to be pyocyanogenic and identical with *P. aeruginosa*. We have confirmed this relationship by means of three additional characters found to be characteristic of the latter.

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