Catabolism of Nitrogenous Compounds by *Pseudomonas*

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SUMMARY

The rate of O_2 utilization by both pigmented and non-pigmented strains of *Pseudomonas aeruginosa* was greater with 2% (w/v) Casitone as substrate than with 0.1 M-glucose as substrate. *P. aeruginosa* readily catabolized Casitone, amino acids and other nitrogenous compounds with the production of an alkaline reaction even in the presence of 0.5% (w/v) glucose. Other species of *Pseudomonas* studied varied in their utilization of these compounds, suggesting a different and possibly satisfactory approach to the identification and classification of species within the genus *Pseudomonas*.

INTRODUCTION

The ability of *Pseudomonas aeruginosa* to produce acid from carbohydrates, in particular from glucose, in amounts sufficient to lower the pH value of the commonly used carbohydrate media is generally variable and to a large extent depends upon the nitrogenous compounds present in the medium. Simon (1956) stated that the production of blue-green pigment and failure to ferment any of the usual coliform test sugars, except occasionally glucose, are generally regarded as characteristic of '*P. pyocyanea*'. She found that the fermentation of sugars by *Pseudomonas* cultures was masked in the peptone medium by the production of free ammonium and a consequently high pH value. The recent studies of Warren, Ells & Campbell (1960) indicated that *P. aeruginosa* grown in a glucose ammonium phosphate medium accumulated nitrogenous materials which were oxidized during endogenous respiration. Rhodes (1959) found that after incubation for 7 days in peptone water the pH values of the media of all *Pseudomonas* strains studied were between pH 8.3 and pH 8.7. Sherris, Shoesmith, Parker & Breckon (1959) showed that nearly all of the *Pseudomonas* strains they tested were able to break down arginine more rapidly than could most other Gram-negative bacilli and suggested that this characteristic might prove to be a useful diagnostic feature. It is evident, therefore, that many of the *Pseudomonas* species produce an alkaline reaction when grown in the presence of peptides or amino acids. The present study was undertaken to determine the utilization of nitrogenous compounds by several species of *Pseudomonas* and in particular *P. aeruginosa*.

METHODS

The strains of *Pseudomonas aeruginosa* studied were obtained from our stock culture collection and from the diagnostic laboratory. All cultures were identified by the methods described by Gaby & Free (1953, 1958) and included pigmented and non-pigmented varieties. The strains, selected at random, were grown on nutrient agar or in nutrient broth at 37°. Species other than *P. aeruginosa* obtained

from the American Type Culture Collection were *P. saccharophilia* no. 9114, *P. oleovorans* no. 8062, *P. ovalis* no. 950 (grown at 25° and 37°), *P. fragi* no. 4973, *P. fluorescens* no. 11251, and *P. mucidolens* no. 4685 (grown at 25°).

Suspensions of organisms for manometric studies were obtained by growing the bacilli on agar slopes or in shaken flask broth cultures for 18 hr. at 25° or 37°. The organisms were washed thoroughly with saline or phosphate buffer, resuspended in 0.1M-phosphate buffer, and diluted to give an optical density of 1.35 at 515 m μ on a Colman Junior Spectrophotometer Model 6A. This dilution is equivalent to 6.87×10^9 viable organisms, 12.5 mg. wet weight or 2.2 mg. dry weight of organisms per ml. Oxygen consumption was measured by conventional Warburg techniques.

To determine alkalinity produced by the *Pseudomonas* species from peptides and amino acids, the various strains were inoculated into a liquid medium containing (% w/v) Casitone (Difco) 1.0, glucose 0.5, amino acid 0.5, adjusted to pH 7.2 and containing 0.02% phenol red indicator. The amino acids studied were DL-alanine, DL-serine, and DL-methionine (see Table 1). Asparagine, arginine, leucinamide and glycineamide were also included because of their different amino groupings. The cultures were incubated at either 25° or 37°, depending upon their optimum temperature requirement, and colour changes of the pH indicator were noted for periods up to 72 hr.

RESULTS

The results shown in Fig. 1 illustrate the stimulation in the rate of O₂ consumption of a representative pigmented strain of *Pseudomonas aeruginosa* by increasing concentrations of Casitone from 0.1 to 2.0% (w/v). Three pigmented and three non-pigmented strains tested under these conditions gave essentially the same results. On the other hand, the rate of O₂ utilization of four brown (brownish-purple) pigmented strains was only one-half to three-quarters of that shown in Fig. 1. The results in Fig. 2 show that with both pigmented and non-pigmented strains the rate of O₂ utilization was greater with 2% (w/v) Casitone as substrate than with 0.1M glucose. All the strains produced a slight amount of acid in a glucose+peptone basal medium on primary isolation. It is evident from these results that the six *P. aeruginosa* strains tested not only oxidized Casitone at a faster but also more uniform rate than glucose. The results at least partially explain the frequently reported observations that *P. aeruginosa* strains may or may not produce acid in a glucose medium, particularly when the medium contains peptone.

Since Casitone contains amino acids and peptides, experiments were carried out to determine the rate of O₂ utilization of *Pseudomonas aeruginosa* with various single amino acids as substrate. The results in Table 1 show that the rate of O₂ utilization by *P. aeruginosa* varied considerably among the 18 different amino acids tested, ranging from 18.5 μ l. O₂ uptake/90 min./mg./dry wt. bacteria with DL-methionine to 74.1 μ l. O₂ uptake with DL-serine. These results are in agreement with those of Silberman & Gaby (1961) which showed a correlation between the rate of O₂ consumption by *P. aeruginosa* of various amino acids as substrates and the uptake of the corresponding ¹⁴C labelled amino acid by the lipid complex of *P. aeruginosa*.

The results in Table 2 list the reactions of the cultures in the media containing the various nitrogenous compounds. All strains of *Pseudomonas aeruginosa* gave an alkaline reaction within 24 hr. in the media containing asparagine, arginine, leucinamide and glycineamide with the exception of four strains which required

48 hr. to produce an alkaline reaction in the media containing glycineamide and leucinamide. Four of the *P. aeruginosa* strains produced either an acid reaction or no indicator colour change in the media containing the four amino acids. The reactions of the other *Pseudomonas* species in the media differed considerably, not only from *P. aeruginosa*, but also indicated distinct differences between individual species. When peptone was substituted for Casitone in the test medium the results were similar with the exception that alkaline production was less rapid. The other Gram-negative bacilli which normally ferment glucose with the production of much stronger acids continued to do so in the presence of the nitrogenous compounds tested.

Fig. 1

Fig. 1. The effect of increasing concentrations of Casitone on rate of O_2 utilization by *P. aeruginosa*. See legend Table 1, p. 382 for conditions.

Fig. 2

Fig. 2. Comparison of the rate of O_2 utilization by 6 strains of *P. aeruginosa* with 2% Casitone and 0.1 M glucose as substrates. Vertical lines represent the extent of individual strain variation. See legend Table 1, p. 382 for conditions.

DISCUSSION

The results emphasize the ability of *Pseudomonas aeruginosa* to catabolize nitrogenous compounds. It is of interest to note that there was an excellent correlation found between pigmentation, ability of these strains to utilize nitrogenous compounds, and mouse pathogenicity. The green pigmented and non-pigmented strains of *P. aeruginosa* were found to be weakly pathogenic for mice, whereas 0.5 ml. of an 18–24 hr. undiluted broth culture of the brown pigmented strains failed to kill when injected intraperitoneally into mice (Gaby & Logan, 1961).

Table 1. *Effect of amino acids on O₂ uptake by washed suspension of Pseudomonas aeruginosa*

The Warburg vessels contained, 2.0 ml. suspension in phosphate buffer (pH 7.0); 1.15 mg. amino acid in 0.3 ml. phosphate buffer in the side arm; 0.2 ml. 20 % KOH in the centre well; atmosphere, air; bath temperature 37°.

DL-Amino acid substrate	O ₂ uptake (μl./90 min./mg. bacterial dry wt.)		Ratio: substrate/ endogenous
	Endogenous	Substrate	
Methionine	15.7	18.5	1.18
Hydroxyproline	20.0	24.2	1.21
Histidine	20.0	27.4	1.37
Cystine*	20.8	28.9	1.39
Tyrosine	15.7	22.6	1.44
Leucine	21.8	32.5	1.49
Tryptophane	18.3	29.3	1.60
Lysine	21.0	37.6	1.79
Glycine	16.4	30.8	1.84
Isoleucine	20.8	40.5	1.94
Phenylalanine	21.2	45.0	2.03
Valine	17.8	36.5	2.05
Glutamic acid	19.1	39.8	2.08
Cysteine	18.3	37.6	2.11
Threonine	15.7	33.5	2.13
Asparagine	21.2	45.6	2.15
Alanine	21.2	70.3	3.31
Serine	26.4	74.1	3.63

* Added in a slurry.

The limited number of *Pseudomonas* species studied, other than *Pseudomonas aeruginosa*, indicated differences in their ability to catabolize nitrogenous compounds. These reactions were consistent on repeated testing and indicate the possibility of using this type of procedure as an aid in differentiating the numerous species with the *Pseudomonas* genus. There is general agreement among those working in this field that the species comprising the genus are ill-defined, due in part to the emphasis which has been placed on the ability of these bacilli to attack glucose and other sugars either oxidatively or fermentatively. The inadequacy of these commonly used procedures emphasizes the obvious need for the development of new techniques and methods.

Recent publications by Colwell & Liston (1961) and Rhodes (1961) using the electron computer technique to study the taxonomic relationship among the *Pseudomonas*, stress the difficulty of species identification within the genus. Most of the cultures examined by Colwell & Liston (1961) fell into four groups. They were unable, however, to identify most of their freshly isolated strains with named strains listed in *Bergey's Manual* (Breed, Murray & Smith, 1957). Rhodes (1961) also found that the strains which she examined fell into groups, but concluded that none of the groups merit species rank but should perhaps be regarded merely as variants of *Pseudomonas fluorescens*.

All of the *Pseudomonas* strains tested in this laboratory have given a positive cytochrome oxidase test (Gaby & Hadley, 1957) while Colwell & Liston (1961) found that all of the strains within their four groups were positive by both the

Table 2. Change of reaction produced by *Pseudomonas* and other species on incubation for 72 hr. in media containing Casitone + glucose + added nitrogenous compounds

Organisms	Asparagine alk	Arginine alk	Leucinamide alk	Glycinamide alk	Alanine 1-n 2-a 18-alk
<i>P. aeruginosa</i> 21 strains					
<i>P. saccharophilia</i>	alk	alk	alk	alk	n
<i>P. oleovorans</i>	n	n	n	n	alk
<i>P. ovalis</i>	alk	alk	a	a	a
<i>P. fragi</i>	alk	alk	a	a	a
<i>P. fluorescens</i>	alk	alk	alk	a	a
<i>P. mucidolens</i>	a	a	n	a	a
<i>E. coli</i>	a	a	a	a	a
<i>Salmonella</i> *	a	a	a	a	a
<i>Shigella</i> *	a	a	a	a	a
<i>Proteus</i> *	a	a	a	a	a

Organisms	Serine 2-n 2-a 17-alk	Lysine 1-n 1-a 19-alk	Methionine 4-n 1-a 16-alk	Glucose (peptone- base) 7-a 14-n	Xylose (peptone- base) 2-a 19-n
<i>P. aeruginosa</i> 21 strains					
<i>P. saccharophilia</i>	n	n	n	a	a
<i>P. oleovorans</i>	n	n	n	n	n
<i>P. ovalis</i>	alk	a	n	a	a
<i>P. fragi</i>	a	a	a	a	a
<i>P. fluorescens</i>	a	a	a	a	n
<i>P. mucidolens</i>	a	a	a	a	n
<i>E. coli</i>	a	a	a	a	a
<i>Salmonella</i> *	a	a	a	a	a
<i>Shigella</i> *	a	a	a	a	a
<i>Proteus</i> *	a	a	a	a	a

alk = alkaline; a = acid; n = neutral; no change in indicator.

* Several strains and species tested.

oxidase test (Kovacs, 1956) and cytochrome oxidase test (Gaby & Hadley, 1957). These results would seem to indicate that it should not be too difficult to identify a Gram-negative bacillus as a *Pseudomonas*. Proper emphasis, however, has not been placed on the catabolism of nitrogenous compounds by this group of micro-organisms.

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