A Note on the Genus *Aeromonas*

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SUMMARY: Previous workers have shown that the flagellation of certain bacteria is variable, becoming lateral or polar according to age and conditions of culture. In view of these findings, and because of the morphological and biochemical similarity of the organisms, Lysenko (1958) has equated *Pseudomonas noctuarum* with *Serratia marcescens*. It is now suggested that *Pseudomonas noctuarum* would be better placed in the genus *Aeromonas*, and that it is similar to an organism provisionally named *A. margarita*. It is further suggested that all members of the genus *Aeromonas* could be regarded as non-chromogenic species of the genus *Serratia*.

Investigation of an epizootic disease which affected laboratory stocks of the desert locust *Schistocerca gregaria* Forsk., resulted in the isolation of a Gram-negative organism which was subsequently shown to be the aetiological agent of the disease (Stevenson, 1958). This organism, initially thought to be a paracolon bacillus (Stevenson, 1954), was later allocated to the group *Aeromonas*, and provisionally labelled *A. margarita*. Recent work by Lysenko (1958) raised doubts regarding both the taxonomic propriety of this classification and the validity of the genus *Aeromonas*. This paper re-examines Lysenko's classification of *Bacillus noctuarum* White in relation to *A. margarita* and the genus *Aeromonas* as a whole.

METHODS

Organisms used. Strains SL10a and SL11a of *Aeromonas margarita* isolated in this laboratory from diseased locusts. Strain 006 of *Pseudomonas noctuarum* (*Serratia marcescens*), from The Laboratory of Insect Pathology, Prague, Czechoslovakia. A strain of *Serratia marcescens* from the London School of Hygiene and Tropical Medicine.

Motility was determined by microscopic examination of hanging-drop preparations after culture in nutrient broth for 2, 4 and 24 hr. at 37°.

Sugar fermentations. Cultures were incubated at 37° in peptone water containing 1.0% (w/v) of the 'sugar', using Andrade's indicator (1.0% acid fuchsin) for the detection of acid production, and Durham tubes for the collection of gas. Examinations were made after 24 and 48 hr. and 5, 10 and 21 days.

Hydrogen sulphide production. Blackening of filter-paper strips, soaked in lead acetate and suspended above nutrient broth enriched with cysteine, was taken to indicate H₂S production. Cultures were incubated at 37° for a maximum of 10 days.

Indole formation. Cultures were incubated in peptone water for 5 days at 37° and tested with Böhm's reagents.

Urease production was tested on Christensen's (1946) urea agar slopes incubated at 37° for 5 days. A red coloration of the medium was considered to indicate a positive reaction.

Phenylalanine and malonate tests. The medium and methods of Shaw & Clarke (1955) were used.

Gelatin liquefaction was examined at 4° after incubation in nutrient gelatin for 5 days at 37°.

Citrate utilization was tested by inoculating three successive subcultures in Koser's citrate with the tip of a straight wire, and incubating at 37°.

Butanediol production. The organisms were cultured for 48 hr. at 37° in a tryptic meat digest with the addition of 1.0% (w/v) glucose. Butanediol was estimated by Neish's (1952) method and by the method recommended by Pirt (1957).

MR and VP tests. Cultures were incubated in glucose phosphate broth for 5 days at both 30° and 37°, and tested according to the details given by Vaughn, Mitchell & Levine (1939) and by Levine (1941). There was no difference in the results obtained at the different temperatures.

Flagella staining. Loeffler's (1889) and Zettnow's (1899) techniques were used.

RESULTS

All the organisms examined were similar in morphology. They were small Gram-negative rods varying in length from 0.8 to 3.0 μ , and in width from 0.8 to 1.0 μ . In 24 hr. cultures there was in all cases a preponderance of short, almost coccoid forms. Short rods with bulging sides and longer rods with parallel sides were also present. The ends were rounded and bipolar staining was common.

Motility was observed in hanging drop preparations of all strains, though in *Pseudomonas noctuarum* (*Serratia marcescens*) strain 006 it was apparent only after incubation at room temperature, and not in cultures incubated at 37°. The flagellation of *Aeromonas* and *Serratia* strains was apparently polar, though in one or two preparations observed under the light microscope, there were organisms which may have possessed peritrichate flagellation. The only strain examined for flagellation under the electron microscope was *Aeromonas margarita* strain SL10a; in this case all the flagellated organisms were monotrichate. The flagellation of *P. noctuarum* strain 006 was peritrichate.

The biochemical properties of the strains tested are given in Table 1, where they are compared with some of the results obtained by Lysenko (1958). It will be seen that, in general, all the organisms are biochemically similar.

DISCUSSION

Stevenson (1958) allocated the organism isolated from desert locusts to the genus *Aeromonas* on the grounds that it possessed polar flagella and produced butanediol from glucose. As it differed from other organisms listed in the genus, it was provisionally labelled *A. margarita*, though it was pointed out that there are other organisms which resemble it morphologically and in some

respects biochemically; these are *Bacillus sphingidis* White (1923*a*), *B. noctuarum* White (1923*b*) and *B. leptinotarsae* White (1928, 1935). These bacilli, though no doubt properly placed in the genus *Bacillus* at the time of their isolation, are now more appropriately placed in the family Pseudomonadaceae, since they are Gram-negative rods with polar flagellation.

Weiser & Lysenko (1956) placed *Bacillus noctuarum* in the genus *Pseudomonas*. However, since it ferments glucose and other sugars, and since *Pseudomonas* by definition excludes fermentative bacteria, it would be better placed in the genus *Aeromonas* were it not VP-negative and therefore not a producer of butanediol (Weiser & Lysenko, 1956). The present tests have confirmed that the organism is VP-positive (Lysenko, 1958). In all probability,

Table 1. *Biochemical reactions of certain strains of Serratia marcescens, Pseudomonas noctuarum and Aeromonas margarita*

Strain ...	<i>S. marcescens</i>		<i>P. noctuarum</i>		<i>A. margarita</i>	
	Lko.	Stn.	Lko.	Stn.	Stn.	Stn.
			006	006	SL 10 a	SL 11 a
Motility	+	+	+	+	+	+
Glucose	d	AG	(G)	AG	AG	AG
Mannitol	AO	AG	AO	AG	AG	Ag
Adonitol	d	nt.	—	nt.	nt.	nt.
Dulcitol	—	—	—	—	—	—
Lactose	x	—	(A)O	—	—	—
Sucrose	AO	AG	AO	AG	A	A
Maltose	AO	AG	(A)O	Ag	A	A
Salicin	AO	AG	AO	Ag	A	A
Inositol	dO	nt.	(A)O	nt.	nt.	nt.
Glycerol	nt.	A	AG	AG	A	A
Gelatin	+	+	+	+	+	+
H ₂ S	x	+	—	+	+	+
Urease	x	nt.	(+)	nt.	—	—
Indole	—	—	—	—	—	—
MR	—	—	—	—	—	—
VP	+	+	+	+	+	+
Citrates	+	+	(+)	+	+	+
Malonate	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—

+ = positive; — = negative; A = acid produced; G = gas produced; g = small quantity of gas produced; O = gas production not tested; x = indeterminate and delayed reaction; d = mutative character (+ or —); () = delayed action; nt. = not tested. Lko. = results obtained by Lysenko (1958); Stn. = results in present paper.

therefore, it will produce butanediol and could be an aeromonad. Lysenko (1958), however, allocated it to the genus *Serratia*, and the present work confirms his opinion that strains of *Serratia* tested are, apart from chromogenesis, very similar to Lysenko's strain of *P. noctuarum* (= *Bacillus noctuarum*). In addition, strains of *Aeromonas margarita* are morphologically and biochemically very similar to *P. noctuarum* and to *S. marcescens*.

These findings bring into question the validity of the genus *Aeromonas*. First proposed by Kluyver & van Niel (1936) for the bacterium previously known as *Aerobacter liquefaciens* (Beijerinck, 1900), the genus was further

studied by Miles & Miles (1951) who came to the conclusion that if the production of butanediol from the fermentation of glucose was a stable character in an otherwise homogeneous group, the genus *Aeromonas* should be accepted. Stanier (1953) confirmed that *A. hydrophila* carried out a butanediol fermentation similar to that of *A. aerogenes* and concurred in its inclusion in the genus *Aeromonas*. The editors of *Bergey's Manual*, 7th edition (1957), list only four members of the genus, but since the preparation of that book, Pivnick & Sabina (1957) have re-examined *Pseudomonas formicans*, isolated by Crawford (1954) from soluble oil emulsions, and have concluded that this organism too, should be included in the genus *Aeromonas*.

An examination of the flagellation of *Aeromonas margarita* leaves no doubt that most of the individuals are mono- or lophotrichate. However, as pointed out by Lysenko (1958) and Stevenson (1958), the work of Leifson (1951), Leifson & Hugh (1953), Sneath (1956) and others leads to the view that flagellation in the genera *Aeromonas* and *Serratia* may vary according to conditions of culture. Breed (1957) expressed the opinion that such variations have little taxonomic significance, the flagellation of some species having undergone a retrogressive specialization, the organisms becoming primarily dependent on one flagellum as their chief methods of locomotion. Breed said: 'This apparently intermediate type of flagellation seems to be a comparatively recent development...', but produced no evidence for this hypothesis. It may not be so much a recent development, as one that has been recently investigated.

In view of the similarities between the species of *Aeromonas* and *Serratia*, together with the changeable character of the flagellation, it is here proposed that the organism provisionally labelled *A. margarita* is in reality a non-chromogenic strain of *S. marcescens*. Further, it is suggested that all members of the genus *Aeromonas* are unpigmented members of the genus *Serratia*. Obviously, it would be necessary to carry out serological or other studies before such a conclusion could be substantiated; but if it were, it would assist, at least in some degree, to simplify the complicated taxonomy of the Gram-negative bacteria.

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