Further observations on *Mima polymorpha* and *Achromobacter (Bacterium) anitratum*

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SYNOPSIS Further investigations on the morphology, biochemical reactions, and serological relationships of strains of *Mima polymorpha* and *Achromobacter (Bacterium) anitratum* are reported. The results seem to indicate such a close relationship that it may yet be necessary to reconsider the nomenclature of these organisms.

In 1963, Brodie and Henderson reported investigations on two strains of *Mima polymorpha* and the results of a limited comparative study of these in relation to four strains of *Achromobacter (Bacterium) anitratum*. A further 16 isolations of *Mima polymorpha* have since been obtained in this area and have made possible a wider biochemical and serological study of all 18 *Mima* strains and seven further strains of *Achromobacter anitratum*.

MATERIALS, METHODS, AND RESULTS

STRAINS Information regarding the source of the strains of *Mima polymorpha* is given in Table I. The N.C.T.C. references for the strains of *Achromobacter (Bacterium) anitratum* are 7250, 7412, 7422, 7461, 7844, 8698, and 9427.

BIOCHEMICAL AND MORPHOLOGICAL INVESTIGATIONS The fermentative properties were read after incubation at 37°C. for 10 days in peptone water containing the relevant chemical, using bromothymol blue as indicator. Production of indole, ammonia, and catalase was determined after incubation for 10 days in peptone water, then adding Kovac's reagent, Nessler's reagent, and hydrogen peroxide respectively. Colonies, on nutrient agar plates after 24 hours' incubation, were tested for oxidase activity by adding a 1% solution of tetramethyl-p-phenylenediamine hydrochloride. Urease activity was assessed on the urea agar of Christensen (1946). Phosphatase production was determined by exposing colonies, grown on nutrient agar containing 0.01% phenolphthalein phosphate, to ammonia vapour (Barber and Kuper, 1951). The results are listed in Table II.

PREPARATION OF ANTISERA As strains were isolated continuously throughout the investigation, only the first

TABLE I
SOURCES OF AND INFORMATION CONCERNING MIMA STRAINS

Strain	Source	Patients		Clinical Summary	Other Organism(s) Isolated
		Sex	Age		
¹ V16290 ^a	Wound	M	29	Post-operative infection	<i>Staph. pyogenes</i>
¹ D8460	Nose	F	42	Routine check before chest surgery	<i>Staph. epidermidis</i>
¹ V18520	Ear	F	13	External otitis	None
AR748Q	Urethra	M	29	Suspected gonorrhoea	None
V20757	Vagina	F	50	Leucorrhoea	None
¹ V19465	Cervix uteri	F	35	Leucorrhoea	None
D9900	Throat	F	7/12	Upper respiratory tract infection	None
¹ D9682	Throat	M	24	Sore throat	None
¹ V16870	Wound	M	84	Post-operative infection	<i>Candida</i> sp.
¹ V18516	Placenta	F	27	Stillbirth	None
¹ V17750	Wound	M	84	Post-operative infection	<i>E. coli</i>
¹ V19677	Cervix uteri	F	35	Leucorrhoea	None
U28090	Urine	F	55	Cystitis following hysterectomy	None
S28366	Sputum	M	80	Respiratory infection	<i>Candida rugosa</i> <i>Cryptococcus neoformans</i> <i>Citrobacter freundii</i>
V21612	Wound	M	35	Infected compound fracture of tibia (Russian seaman)	<i>Staph. pyogenes</i> , <i>Aspergillus</i> sp.
V21784	Wound	F	66	Post-operative infection	<i>Staph. epidermidis</i>
¹ V19738	Vagina	F	28	Puerperal fever	<i>E. coli</i> , <i>Strep. faecalis</i>
¹ V16578 ^a	Wound	M	57	Osteomyelitis	<i>Staph. pyogenes</i>

¹Strains used to prepare antisera.

^aStrains previously reported (Brodie and Henderson, 1963).

TABLE II

COMPARISON OF PROPERTIES OF STRAINS OF *MIMA* AND *B. ANITRATUM*

Characteristic	<i>Mima</i> Strains															<i>B. anitratum</i> (N.C.T.C. Nos.)									
	V16290	D8460	V18520	AR748Q	V20757	V19465	D9900	D9682	V16870	V18516	V17750	V19677	U28090	S28366	V21612	V21784	V19738	V16578	9427	7461	7412	7844	7250	8698	7242
Morphology																									
Pleomorphic—mainly coccoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on																									
Nutrient agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Horse blood agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MacConkey's agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility																									
Aerobic (strict)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	—	(+)	—	—	—	—	—	—	(+)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Phosphatase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Urease	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Indole	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl red test	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Voges-Proskauer test	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Citrate utilization	—	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrite production	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ammonia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen sulphide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carbohydrates																									
Pentoses																									
Arabinose	±	±	±	—	—	±	—	±	—	—	—	—	±	—	—	—	—	—	±	±	—	±	±	±	±
Xylose	±	±	±	±	±	±	±	—	—	—	—	—	±	±	—	—	—	±	±	±	±	±	±	±	±
Hexoses																									
Rhamnose	—	—	—	±	±	—	—	—	—	(±)	—	—	±	±	±	±	—	—	—	—	—	—	—	—	—
Glucose	—	—	±	—	—	—	±	—	—	—	—	—	±	±	±	—	—	—	—	±	—	—	—	—	—
Fructose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Galactose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mannose	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Disaccharides																									
Sucrose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Maltose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Trehalose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Trisaccharide																									
Raffinose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Polysaccharides																									
Inulin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dextrin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Alcohols																									
Glycerol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adonitol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glycosides																									
Salicin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aesculin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Inositol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

+ = reaction obtained

(+) = reaction weak after seven days

± = acid produced without gas

(±) = initially acid produced then reversion to neutrality

(1) = beta haemolysis

TABLE III

TUBE AGGLUTINATION TITRES OF 11 MIMA ANTISERA TESTED AGAINST *MIMA POLYMORPHA* AND STRAINS OF *ACHROMOBACTER* (*BACTERIUM*) *ANITRATUM*

Suspension of Strain		Antiserum to Strain											Pattern of Agglutination
		V16290	D8460	V18520	V18516	V19465	D9682	V16870	V17750	V16578	V19677	V19738	
A ^a <i>Mima</i>	V16290	4096 ¹	1024	256	256	4	8	0	0	0	0	0	A
	D8460	2048	4096	2048	128	8	8	0	0	0	0	0	
	V18520	2048	4096	1024	128	16	32	0	0	0	0	0	
	AR748Q	128	2048	512	128	8	16	0	0	0	0	0	
	V20757	1024	1024	1024	512	4	0	0	0	0	0	0	
	V19465	1024	1024	1024	256	2048	2048	0	0	0	0	0	B
	D9900	512	256	1024	128	2048	2048	0	0	0	0	0	
	D9682	1024	512	32	32	2048	2048	8	0	0	0	0	
	V16870	0	0	0	0	2048	2048	2048	0	0	0	0	C
	V18516	0	0	0	512	0	0	0	256	1024	0	0	
	V17750	0	0	0	512	0	0	0	128	1024	0	0	D
	V19677	0	0	0	0	0	0	0	0	0	64	0	
	U28090	0	0	0	0	0	0	0	0	0	64	0	E
	S28366	0	0	0	0	0	0	0	0	0	0	0	
	V21612	0	0	0	0	0	0	0	0	0	0	0	F (?) G (?)
	V21784	0	0	0	0	0	0	0	0	0	0	0	
	V19738												
	V16578												
		Autoagglutinable											
		Autoagglutinable											
B ^a <i>Achromobacter</i>	N.C.T.C. 9427	2048	4096	2048	128	16	32	8	0	0	4	8	A
	N.C.T.C. 7461	2048	2048	128	1024	64	64	32	128	1024	32	2048	
	N.C.T.C. 7412	4096	512	64	512	32	32	8	64	1024	32	512	
	N.C.T.C. 7844	2048	512	64	512	32	32	16	128	1024	32	1024	
	N.C.T.C. 7250	128	4	0	1024	64	32	16	64	2048	32	2048	
	N.C.T.C. 8698	0	4	0	0	0	8	0	0	0	32	0	E
	N.C.T.C. 7422	0	0	0	0	0	0	0	0	0	64	8	
		Autoagglutinable											

¹Titre is expressed as the reciprocal of the final dilution giving complete agglutination of the bacterial suspensions.^aA, 18 strains of *Mima polymorpha* and B, seven strains of *Achromobacter* (*Bacterium*) *anitratum*.

11 *Mima* strains isolated were used for the preparation of antisera. These are appropriately marked in Table I. Each strain was grown on nutrient agar for 24 hours, washed off and suspended in 0.2% formal saline, then diluted with saline to the opacity of Brown's tube no. 4 (Brown, 1919). Adult rabbits were inoculated intravenously on the first three days of each of three consecutive weeks, using the following doses:—0.2 ml., 0.4 ml., 0.8 ml., 0.8 ml., 1.0 ml., 1.0 ml., 1.0 ml., 1.0 ml., and 1.0 ml. After a further five days' rest, the rabbits were bled out under pentobarbitone anaesthesia. The serum was separated, inactivated at 56°C. for 20 minutes, then merthiolate added to a final concentration of 1/10,000. The antisera were stored at -20°C.

TUBE AGGLUTINATION TESTS Suspensions of each of the 25 strains of organisms, in the live state and diluted to the opacity of Brown's tube no. 4, were tested against each of the 11 *Mima polymorpha* antisera. Agglutination was allowed to take place in tubes in a water bath at 56°C. for four hours, then the tubes allowed to stand overnight at room temperature. The results are set out in Table III.

DISCUSSION

The first account of an unusual Gram-negative coccus causing meningitis was given by Sen in 1936, but a systematic search for these organisms was not made until 1939, when de Bord described a group of organisms in normal and diseased conjunctivae and

vaginas which he named *Mima*. Numerous instances of the isolation of this group of bacteria from pathological materials have now been recorded and an account of these was given in a previous publication by Brodie and Henderson (1963).

A further group of pleomorphic organisms, sometimes cocci, sometimes coccobacilli and usually Gram-negative, was described by Schaub and Hauber (1948) and named *Bacterium* (*Achromobacter*) *anitratum*. In 1949, Ewing suggested that *Mima polymorpha* and *Achromobacter anitratum* might be related.

The previous findings of Brodie and Henderson (1963) on the two strains of *Mima polymorpha*, V16290 and V16578, and four strains of *Achromobacter* (*Bacterium*) *anitratum*, N.C.T.C. nos. 7362, 7363, 7364, and 8102, suggested that on morphological grounds *Mima* is mainly coccoid and the four *B. anitratum* strains mainly bacillary. Now, however, with the number of *Mima* strains increased to 18 and these compared biochemically and morphologically with a further seven *B. anitratum* strains (Table II) little, if any, evidence is forthcoming suggesting their entitlement to separate names.

The serology of *Mima polymorpha* has been investigated by Cary, Lindberg, and Faber (1956). Using a precipitin technique, after extracting the bacteria by the method of Maxted (1948), 29 strains

were examined. These investigators found evidence of the existence of 19 serotypes, distinguished by type-specific antigens, but found no evidence of group-reacting antigens.

In the present study of agglutinogens, 18 strains of *Mima* have been agglutinated against 11 *Mima* antisera (Table III, A). Certain 'patterns of agglutination'—A, B, C, D, and E—have appeared. Five strains fell within pattern A, three in B, one in C, two in D, and two in E. Two strains, V19738 and V16578, unfortunately were autoagglutinable. Of V16578 it is known from past work that it had agglutinated with antiserum to strain V16290 to a dilution of 1 in 8,192 (Brodie and Henderson, 1963), and this evidence, together with the reasonable assumption that, in the saline-stable state, it would agglutinate with its homologous serum, suggests that its reactions would constitute yet another pattern. Again, since the antiserum to V19738 fails to agglutinate any of the 16 stable suspensions and would be expected to agglutinate its homologue when saline-stable, the resultant agglutination pattern would be different from the others. Thus, patterns A, B, C, D, and E are demonstrable with the antisera and strains available and the further patterns F and G seem feasible. The three remaining strains of the 18 tested failed to react with any of the 11 antisera, providing almost certain evidence that further serotypes exist, as the work of Cary *et al.* (1956) would indicate.

As previously reported by Brodie and Henderson (1963), absorption of antiserum to *Mima* strain V16290 with all four of the previously examined strains of *B. anitratum*, N.C.T.C. nos. 7462, 7463, 7464, and 8102, resulted in no drop in titre, suggesting then that these strains of *B. anitratum* were unrelated serologically to *Mima*. Now, with seven further strains of *B. anitratum* tested against 11 *Mima* antisera (Table III, B), it is evident that a serological relationship does exist. Indeed, two of the 'patterns of agglutination' obtained with *Mima*

antisera against *Mima* strains, namely patterns A and E, have been reproduced with *B. anitratum* strains, N.C.T.C. no. 9427 giving pattern A and N.C.T.C. no. 8698 with N.C.T.C. no. 7422 yielding pattern E. The remaining four strains give agglutination with almost all the *Mima* antisera. If the previously examined strains of *B. anitratum* had possessed antigen(s) as in patterns A or B or had shown the wide range of reactions of the present four strains mentioned above, absorption of pattern A *Mima* antiserum V16290 would have been demonstrated. It seems, therefore, that the original four strains, examined by Brodie and Henderson in 1963, might have been in pattern E group, or some other group as yet not defined, and on this account have failed to indicate a serological relationship.

The present results indicate a far closer relationship in morphology, biochemical, and serological reactions than was at first apparent, so lending support to the suggestion of Ewing (1949) that *Mima polymorpha* and *Achromobacter* (*Bacterium*) *anitratum* might be related.

If these groups of organisms are regarded finally as related, there will arise the problem of a name. By the principle of prior names, one would expect the name of the group to continue to be *Mima*. Many investigators, however, have described pathogenic organisms under the name of *Achromobacter* (*Bacterium*) *anitratum* and, if the principle of the *nomen conservandum* is employed, it is possible that the latter name will be applied.

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