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THE BIOCHEMICAL REACTIONS GIVEN
BY MEMBERS OF THE SERRATIA GROUP

Betty R. Davis, W. H. Ewing, and R. W. Reavis

Communicable Disease Center
Public Health Service, U. S. Department of
Health, Education, and Welfare
Atlanta, Georgia

The purpose of this paper is to report the results of investigations into the biochemical reactions given by members of the Serratia group. Available descriptions of this group of microorganisms are inadequate, and often are inaccurate, hence, these studies were instituted in an attempt to characterize members of the group as fully as possible.

Fifty strains were employed in the investigations, 16 of which also were used by Davis and Woodward (1957) in their study of the O antigens of members of the Serratia group. The sources of the cultures were as follows:

Blood	9
Wounds, abscesses	7
Sputa, throats, bronchial washings	12
Urine	7
Kidney	1
Lung	1
Brain (cow)	1
Paracentesis	1
Synovial fluid	1
Spinal fluid	1
Feces	3
Aerial contaminant	1
Flies	1
Stock culture collections	2
Unknown sources	2

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The methods employed in the study of the biochemical activities of the cultures were similar to those recommended by Kauffmann (1954) and by Edwards and Ewing (1955).¹ Lysine decarboxylation was determined by the ninhydrin method, details of which were given by Carlquist (1956) and by Edwards, Fife, and Ewing (1956). Leifson's malonate broth with added yeast extract was used for determination of malonate utilization and tests for production of phenylpyruvic acid (PPA) from phenylalanine (PA) were made by the PA agar method (Ewing, Davis, and Reavis, in press). Pigment formation was determined following incubation of the cultures on infusion agar slants and in motility medium at 25 C and at 37 C. Pigment production also was assessed after the strains had been maintained at 22 to 26 C in cork stoppered stock culture tubes (infusion agar) for one week. The biochemical reactions of pigmented strains were repeated with colorless variants selected from platings in those instances where pigment interfered with interpretation of results. Gelatin liquefaction was determined after incubation at 25 C. However, these tests may be incubated at 37 C and followed by refrigeration, if desired.

The serologic methods used in the preliminary investigations reported herein were similar to those employed in Escherichia coli work. (See Ewing et al., 1956, for details and references).

The results obtained with 50 strains belonging to the Serratia group are summarized in Table 1. Attention should be directed to several important aspects of the reactions given by these cultures. Gas was produced by 32 strains (64 per cent) but the volume of gas was very small, constituting 5 per cent or less of the volume of the insert tubes. Only one strain produced acid from lactose avidly and within 24 hours incubation and this same strain also rapidly fermented raffinose and arabinose. Twenty-nine other cultures produced a slightly acid reaction in lactose tubes after 5 to 21 days. Sucrose, maltose, mannitol, and salicin were utilized within 24 hours by all strains and all except two fermented sorbitol. All cultures failed to utilize dulcitol or rhamnose and only two produced acid from raffinose. The majority of the strains failed to hydrolyze urea but 18 produced weak

¹A mimeographed outline of methods is available upon request.

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reactions on urea agar after 4 or 5 days at 37 C. None of the cultures produced hydrogen sulfide in triple sugar iron (TSI) agar in the usual incubation period but more than half of the tubes showed evidence of very slight hydrogen sulfide formation after 5 to 7 days incubation at 37 C. Only one strain failed to liquefy gelatin in 24 hours or less, two failed to show evidence of amine formation from lysine, and two failed to grow in KCN medium. All of the cultures were malonate negative and all except five failed to show evidence of production of PPA from PA. These five strains gave weak color reactions when tested with ferric chloride reagent after incubation for 20 to 22 hours on PA agar medium. Only 16, or 32 per cent, of the group of cultures under investigation produced pink or red pigment. All strains grew well at both 22 and 37 C and pigment was formed at both temperatures by those cultures in which pigment production was observed.

Differentiation of members of the Serratia group from other Enterobacteriaceae was possible if the above-mentioned characteristics were taken into consideration. The IMViC reactions (--++) given by members of the Serratia group distinguished them from strains belonging to the Salmonella, Arizona, Citrobacter (Escherichia freundii), Shigella, and Escherichia groups and indicated that the Serratia group should be placed near the Klebsiella, Aerobacter (Cloaca), and Hafnia groups in the Enterobacteriaceae schema. Members of the Serratia group may be separated from Klebsiella on the basis of their motility, failure to utilize malonate, slowness and reluctance to ferment lactose, slight delayed, or negative urease reactions, gelatin liquefaction, and by the small volumes of gas produced by aerogenic cultures (Table 2). They may be distinguished from members of the Aerobacter (Cloaca) group by their rapid liquefaction of gelatin, reluctance to produce acid from lactose, failure to utilize malonate, and by the small volumes of gas produced from fermentable substrates (Table 2). Serratia strains may be differentiated from those belonging to the Hafnia group by negative methyl red tests at 37 C, by rapid liquefaction of gelatin, and by the small volumes of gas produced by aerogenic cultures (Table 2). Sorbitol fermentation also served to differentiate between members of these two groups, since the majority of Serratia strains produced acid from this substance, whereas known members of the Hafnia group did not utilize it. Proteus mirabilis cultures, if urease negative and Voges-Proskauer

positive, may be differentiated from members of the Serratia group by means of PPA production, positive methyl red tests, hydrogen sulfide production within 24 to 48 hours, and failure to produce acid from mannitol. When present, pink or red pigment served to distinguish between members of the Serratia group and members of all the other groups mentioned above.

Members of the Serratia group and Erwinia strains also must be differentiated, since several species of the latter appeared to be similar to Enterobacteriaceae in many of their characteristics.¹ Serratia strains, as well as other Enterobacteriaceae, and certain Erwinia species may be differentiated on the basis of nitrate reduction, since some species of the latter failed to reduce nitrate. Many of the nitrate negative species also differed from Enterobacteriaceae in their general growth characteristics. The nitrate positive Erwinia species and Serratia group strains may be differentiated by means of various combinations of the following reactions given by these Erwiniae: the majority of strains fermented lactose rapidly and avidly, gelatin was liquefied slowly by some cultures while others were gelatin negative, some strains were methyl red positive and Voges-Proskauer negative, gas volumes were larger than those produced by Serratia strains, and nonmotile forms were common.

Antigen suspensions prepared from the above-mentioned 50 Serratia group cultures were tested in O antisera prepared with representatives of each of the six O antigen groups delineated by Davis and Woodward (1957). The results of these studies (Table 3) indicated that it was possible to determine the O antigen group of 47 of the 50 strains with these six antisera. Living suspensions prepared from many of the strains were agglutinable in O antisera but some were inagglutinable, indicating the probable presence of K antigen.

Further studies on the O and H antigens of the Serratia group are in progress with a view to the eventual establish-

¹Representative cultures of the various Erwinia species were received for comparative studies through the courtesy of Drs. D. D. Sutton and M. P. Starr, of the University of California, Davis.

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ment of an antigenic schema and to facilitate the study of intra and intergroup relationships.

COMMENT

Cultures that belong to the Serratia group often constitute a diagnostic problem and it is probable that nonpigmented strains often are mistakenly identified as Aerobacter cloacae (Cloaca cloacae), as members of the Hafnia group, as intermediate coliform bacteria, or possibly as urease negative P. mirabilis. The results of the biochemical studies reported herein indicated that the strains investigated composed a homogeneous group. Parenthetically, it may be added that the results of preliminary serological studies also attested to the homogeneity of the group, since it was possible to determine the O antigen group of 47 of the 50 strains with only six antisera. Although the number of cultures studied was small, they were considered as fairly representative of the group, since they originated in a variety of sources and were collected from widely separated areas. Hence, it was felt that the following characterization or definition of the Serratia group based upon the reactions given by the majority of cultures, was justified.

Gram negative bacteria that conform to the definition of the family Enterobacteriaceae. Some strains produce pink or red pigment. Biochemical characters:

Glucose	+
Gas	d (when gas is formed, volumes are small, 5 per cent or less)
Lactose	- (or + weak and delayed)
Sucrose	+
Maltose	+
Mannitol	+
Dulcitol	-
Rhamnose	-
Arabinose	-
Inositol	d
Xylose	d
Raffinose	-
Sorbitol	+

Salicin	+
Adonitol	d
Trehalose	+
Motility	+
Simmons' citrate	+
Christensen's citrate	+
Urease	- (occasionally + weak and delayed)
Indol	-
MR	-
VP	+
H ₂ S (TSI)	- (or + weak and delayed)
Nitrate	+
Ammonia glucose	+
Gelatin	+
PA deaminase	-
Malonate	-
Lysine decarboxyla- tion	+ weak
KCN	+
Pigment	d

Studies are being continued, as additional cultures are received, and it is possible that some changes in the above-mentioned characterization will be necessary in the future. In the meantime, it may be used as an aid in the differentiation of members of the Serratia group from other Enterobacteriaceae. This characterization or definition of the Serratia group also may be used in determining the position of the group, in relation to other groups, within the family Enterobacteriaceae. Members of this group differed from members of the Aerobacter (Cloaca) and Hafnia groups in several ways but they appeared to be more closely related to these groups than to any others. Hence, the writers would locate the Serratia group near the Aerobacter (Cloaca) and Hafnia groups.

Several species of Serratia have been described (see e.g. Bergey's Manual, 6th Ed., 1948) but the descriptions were inadequate, particularly as regards fermentative characteristics, and the differences between the species were slight. Thus, it seems that in the future it probably would be better to recognize only one species (S. marcescens) within the genus.

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SUMMARY

The results of studies on the biochemical reactions of fifty Serratia group cultures indicated that the strains composed a homogeneous group. The characteristics of the group were enumerated and means by which members of the Serratia group may be differentiated from other Enterobacteriaceae were given. Forty-seven of the fifty cultures studied belonged to one or another of six previously described O antigen groups.

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TABLE 1

Summary of Results of Biochemical Tests with
Fifty Serratia Group Cultures

Test	No. +	No. + delayed	No. -
Glucose	50	0	0
Gas	32	0	18
Lactose	1	29 w	20
Sucrose	50	0	0
Maltose	50	0	0
Mannitol	50	0	0
Dulcitol	0	0	50
Rhamnose	0	0	50
Arabinose	1	0	49
Inositol	31	16	3
Xylose	3	13	34
Raffinose	1	1	48
Sorbitol	48	0	2
Salicin	50	0	0
Adonitol	38	8 w	4
Trehalose	50	0	0
Motility	50	0	0
Simmons' citrate	50	0	0
Christensen's citrate	50	0	0
Urease	0	18 w	32
Indol	0	0	50
Methyl red	0	0	50
Voges-Proskauer	50	0	0
Hydrogen sulfide (TSI)	0	26 w	24
Nitrate reduction	50	0	0
Ammonia glucose agar	50	0	0
Gelatin (22 C.)	49	0	1
Phenylalanine deaminase	5 w	0	45
Malonate	0	0	50
Lysine decarboxylation	49 w	0	1
KCN	48	0	2
Pigment	16	0	34

w, weak; indicates slight, and often delayed, reactions.

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TABLE 2

Group Differentiation by Biochemical Tests
(Reactions of Typical Cultures)

Biochemical Tests	Klebsiella-Aerobacter-Serratia Division				
	Klebsiella	Aerobacter	Hafnia	Serratia	
	Group	(or Cloaca) Group	Group 37 C 22 C	Group	
Lactose	+	+	-	-	(+) or -
Methyl red	-	-	+	-	-
Voges- Proskauer	+	+	d	+	+
Hydrogen sulfide (TSI)	-	-	-	(+)	- or (+), 37 C
Urease	++	d*	-	-	- or (+)*
Gelatin	-	(+)	-	-	+
Malonate	+	+	d	d	-
Motility	-	+	d	+	+
Gas	+	+	+	+	d**
Pigment	-	-	-	-	d

*, Urease reactions given by Klebsiella strains are not as rapid as those given by Proteus cultures and the majority of Aerobacter (Cloaca) strains are urease negative. Positive reactions given by members of either of these groups are more extensive and more rapid than those given by Serratia strains.

**, When gas is formed, volumes are small, 5 per cent or less.

+, Positive 1 or 2 days.

-, No reaction.

(+), Lactose fermented slowly and slightly, hydrogen sulfide produced slowly and in small amounts, or gelatin liquefied slowly.

d, Different biotypes.

TABLE 3

Distribution of Aerogenic, Anaerogenic,
and Pigmented Serratia Strains
among the O Antigen Groups

O Antigen Group	Pigment Only	Gas Only	Both Pigment and Gas	Neither Pigment nor Gas	Total
1		1			1
2		7		5	12
3		1	1	3	5
4	1	3		2	6
5		7	8	2	17
6	1		3	2	6
Undetermined			2	1	3
Total	2	19	14	15	50