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THE TAXONOMIC STATUS OF SERRATIA MARCESCENS BIZIO

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SUMMARY: 1. In our work we have studied morphological, cultural and biochemical characteristics of 68 strains of Serratia marcescens. 2. We propose that the genus Serratia may have only one species. 3. We propose to acknowledge strain BS 303 (ATCC 13880) as the neotype culture of Serratia marcescens Bizio.

The oldest known species of the genus Serratia is Serratia marcescens. It was named as early as 1823 and since this time both its nomenclature and its taxonomy have undergone many changes. Recognition of this species as the type species of the genus Serratia was proposed by Buchanan (1918). It is so recognized in all seven editions of the Bergey's Manual.

The taxonomy of Serratia marcescens has been investigated by Breed et al. (1924, 1927), Topley and Wilson (1931) and Krassilnikov (1949). The two latter authors place this species in the genus Chromobacterium as Chromobacterium prodigiosum. But the name Chromobacterium prodigiosum is very little used. Much new information concerning the biochemical and antigenic structure of Serratia marcescens has been published by Davis, Ewing and Reavis (1957).

MATERIAL AND METHODS

We have studied 68 strains secured from several collections under the label Serratia marcescens. The list of strains is given in Table 1. The methods used were those described in our previous work (Martinec and Kocur, 1960). The staining of flagella was made by Zettnow's method (Kabelík 1925).

Table 1.

No. of strains	Source of strains
301, 302, 303, 304, 813 731, 545, 544	Own isolates
147, 264	Institut of Plant Production, Prague
151	Institut of Epidemiology and Microbiology, Prague
766-798 (33 strains)	Prof. E. Steinhaus, Univ. of California, Berkeley
400, 412, 414, 420, 454	
455	Culture Coll. of Entomogenous Bacteria, Prague
849	C. B. van Niel, Stanford Univ. Pacific Grove, California
772	Dept. of Agriculture, Ottawa, Canada
361	Epid. and Hygiene Station, Brno
375	Department of Microbiol. Hradec Králové
376, 377	E. Eltinge, Mont Holl. Coll. Massachusettes, U.S.A.
522	Dept. of Microbiology, Charles Univ. Prague
535	Inst. of Biology, Czech. Acad. Sci. Prague
548	Inst. of Microbiology, Univ. of Tucuman
549, 580	Dept. of Bact. Indiana Univ. Bloomington
619	B. Hampl. Dept. of Biol. Sciences, Prague
620	Dept. of Bact. Univ. of Queensland, Australia
689	Inst. of Fermentation, Sao Paulo, Brazil
696, 697	Dept. of Microbiol., Tech. School, Bratislava
761	W.C. Haynes, NRRL, Peoria, Illinois

RESULTS

Morphology. The cells of all 68 strains of Serratia marcescens studied are small Gram-negative rods occurring individually or in groups. Their size varied from 0.8-1.0 x 1.5-2μ. All the strains have motile cells.

Cultural characteristics. The colonies on nutrient agar are circular, 6-7 mm in diameter, with slight undulate margins, slightly convex, smooth. Red pigment was produced by most strains. In nutrient broth all the strains developed intense turbidity and formed slight sediment. Some strains formed red rings.

Cultural and physiological characteristics. The results of the study of biochemical characteristics of 68 strains of Serratia marcescens are given in Table 2.

DISCUSSION

Our study of 68 strains of Serratia marcescens brought many interesting notions. As it can be seen from the tabular summary No. 2, some strains showed different biochemical features. The most important variants here were: Strain 780 alone did not hydrolyze casein; strains 548 and 619 did not reduce nitrates; strains 303, 304, 772 and 787 showed positive hemolysis. It is interesting that the majority of these anomalies was observed in strains isolated from different species of insects (e.g. strains 780, 772, 787).

Relatively great variability of some strains was observed during the fermentation of carbohydrates. This problem was not met only by us but also by Davis et al. (1957). Also the differences in pigmentation are not surprising. On normal media and on media for pigmentation only 55 strains pigmented at normal cultivating temperatures. Out of these 55 strains only 15 pigmented at 37°C; 13 strains did not produce pigment at all.

The results obtained by us are essentially the same as those given in Bergey's Manual (1957), in Krassilnikov's Key (1949) and agree with the data stated by Davis et al. (1957).

We do not agree, however, with Krassilnikov's ascription of S. marcescens to the genus Chromobacterium solely on the basis of its ability to produce pigment. We believe that there are insufficient grounds for shifting this species to a very different genus. As we have ascertained, Serratia

Table 2. Results of biochemical tests with 68 strains of Serratia marcescens

Test	Number positive	Number negative
Gelatin liquefaction	68	0
Casein hydrolysis	67	1
Nitrate reduction	66	2
Hydrogen sulfide I.	67	1
Hydrogen sulfide II.	0	68
Indole	0	68
Milk	68	0
Hemolysis	4	64
Glucose	68	0
Gas from glucose	43	25
Lactose	2	66
Sucrose	68	0
Maltose	63	5
Galactose	64	4
Fructose	67	1
Rhamnose	0	68
Mannose	66	2
Inulin	0	68
Xylose	6	62
Arabinose	0	68
Glycerol	66	2
Adonitol	64	4
Sorbitol	67	1
Mannitol	68	0
Dulcitol	0	68
Starch	0	68
Esculin	68	0
Koser's citrate	67	1
Simmon's citrate	66	2
Acetylmethylcarbinol	66	2
Methyl red	0	68
KCN	68	0
Phenylalanin	0	68
Catalase	68	0
Urease	0	68
Lipase	67	1
5% NaCl	68	0
7.5% NaCl	47	21
Pigment	55	13

Hydrogen sulfide I. = lead acetate papers
Hydrogen sulfide II. = modified Klinger's agar

marcescens differs from the representatives of the genus Chromobacterium not only morphologically, but especially biochemically and in the chemical structure of its pigment.

Since 1823 when the species was first described, authors have described and named other new species. These new species were classified unsatisfactorily. In 1957 Davis et al. (1957) concluded that the genus Serratia is monotypic, including only one species.

We have devoted much study to this problem and have substantiated the findings of Davis et al. (1957) (Martinec and Kocur, 1960).

Owing to the fact that there exists no culture of the original strain of Serratia marcescens, we propose the approval of our strain 303 as a neotype culture. The description of proposed neotype of Serratia marcescens:

Serratia marcescens Bizio (strain BS 303, ATCC 13880)

Small Gram negative rods, occurring individually or in groups. The size of cells was 0.8 x 1.8μ. Motile by four lateral flagella.

Agar colonies (2 days): Circular with slight undulate margin, 4-6 mm in diameter, slight convex, smooth, produce red pigment.

Gelatin colonies (5 days): Circular, smooth sinking in 2-3 days in gelatin, white.

Agar slant (1 day): Smooth, white, taking on an orange-red to carmin red colour in 4-5 days.

Broth (1 day): Intense turbid and gray sediment.

Potato (2 days): Luxuriant growth, smooth, first white, later red colour.

Peptone water with carbohydrates: Acid but no gas formed from glucose, saccharose, maltose, galactose, mannitol, sorbitol, glycerol, mannose and adonitol. Not attacked by lactose, dulcitol, arabinose, rhamnose, xylose, inulin.

Milk (2 days): Alcaline, coagulation and peptonization.

Indole: -; H_2S: -; VP: +; MR: -; nitrate +; catalase +; urease -; lipase +; KCN +; phenylalanine -; Simmons' citrate +; Koser's citrate +; hydrolysis of gelatin +; casein +; hydrolysis of starch -; esculin +; hemolysis +.

Salt tolerance: Nutrient agar with 5% NaCl +; 7.5% NaCl +; 10% NaCl -.

Aerobic.
Optimum temperature 30°C, minimum 10°C, maximum 37° C.
Optimum pH 6.8, minimum 4.4, maximum 9.2.
Habitat: Isolated from pond water.

REFERENCES

Breed, R.S. and M.E. Breed. 1924. The type species of the genus Serratia commonly known as Bacillus prodigiosus. Jour. Bact. 9:545-557.

_____, _____. 1927. The genus Serratia Bizio. Zentralbl. f. Bakt. II Abt. 71:435-440.

_____, E.G.D. Murray and N.R. Smith. 1957. Bergey's Manual of Determinative Bacteriology, 7th ed. 1094 pp. Williams and Wilkins Co., Baltimore, Maryland.

Davis, Betty R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reactions given by members of the Serratia group. Internl. Bull. Bact. Nomen. and Taxon. 7:151-160.

Kabelík, J. 1925. Bakteriologická technika drobnohledná. Praha.

Krassilnikov, N.A. 1949. Opredělitěl bakterij i aktinomycetov. Moskva.

Martinec, T. and M. Kocur. 1960. The taxonomic status of Serratia plymuthica (Lehmann and Neumann) Bergey et al. and of Serratia indica (Eisenberg) Bergey et al. Internl. Bull. Bact. Nomen. and Taxon. 10(4):247-254.

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CONTRIBUTION TO THE TAXONOMIC STUDIES OF SERRATIA KILIENSIS (LEHMANN ET NEUMANN) BERGEY

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SUMMARY: A study of the morphological, cultural and biochemical features of five strains of Serratia kiliensis (Lehmann et Neumann) Bergey from different culture collections leads to the conclusion that Serratia kiliensis is a variety of Serratia marcescens.

In 1888 a red pigmented microorganism, which was called only bacterium h, was isolated from drinking water by Breunig in Kiel. The name Bacterium kiliense was given to it later by Lehmann and Neumann (1896). This species was placed in the genus Serratia by Bergey (1923) with the name Serratia keilensis (sic). Breed (1957) corrected the spelling of the specific epithet to kiliensis. Some authors (Lehmann and Neumann 1927, Breed 1957, Davis et al., 1957) have suggested that Serratia kiliensis is a variety of or identical with S. marcescens. However, S. kiliensis is still given specific status by authors (Krasilnikov 1949, Breed 1957).

We have therefore undertaken to ascertain whether S. kiliensis and S. marcescens are synonyms.

MATERIAL AND METHODS

We have studied 5 strains supplied as Serratia kiliensis by various culture collections. Strain 274 was from the Department of Agriculture, Ottawa, Canada; strain 300 from Department of Biological Sciences, Purdue University, Indiana; strain 526 from J. Simpson, Prairie Regional Laboratory, Saskatoon, Canada, strains 7462 and 922 from the American Type Culture Collection.

The methods used were the same as in our previous work (Martinec and Kocur 1960).

RESULTS

Morphology. All five strains formed small Gram negative rods, singly or in groups. 0.8 x 1.5-2.0μ. All strains were motile.

Cultural characteristics. Colonies were round with undulate margin, slightly convex, smooth. On nutrient agar two strains only were pigmented. In nutrient broth all strains produced turbidity and sediment, two strains formed a fragile pellicle. On potato, gelatin and on medium Dewey and Poe (1943) only 2 strains were pigmented.

Biochemical characteristics. The ascertained biochemical characteristics are shown in Table 1.

DISCUSSION

No essential differences in morphological and cultural characters were found among the five strains studied. Some minor differences appeared in the biochemical characteristics. Strain 526 hemolysed blood and did not form gas from glucose, strains 582, 274 and 581 fermented lactose. The variability in fermenting lactose and producing gas agrees with the description given by Davis et al. (1957).

In other respects our results agree with the original description (1888) as well as with the data given by Krasilnikov (1949) and Breed (1957). Breed (as well as some other authors) suggests that S. kiliensis is probably identical with Serratia marcescens but does not prove this identity experimentally. He stated that it is uncertain whether the microorganism isolated by Breunig (1888) was a strain of Serratia marcescens not heavily pigmented. Our results confirm this supposition. Breed also states that one of the features differentiating S. kiliensis from S. marcescens is the fact that S. kiliensis does not produce acethylmethylcarbinol. Out of five strains studied only the strain 526 produced acethylmethylcarbinol. Besides this difference, however, we have ascertained no other difference between S. kiliensis and S. marcescens. Comparison of our results with those of Breed (1957), Davis et al. (1957) and with our earlier work (Martinec and Kocur 1959) leads us to the conclusion that S. kiliensis is only a variety of S. marcescens. This conclusion is reached because no production of acethylmethylcarbinol is the only single one of the substantial biochemical

Table 1. Results of biochemical tests with 5 strains of Serratia kiliensis.

Test	Number Positive	Number Negative
Gelatin liquefaction	5	0
Casein hydrolysis	5	0
Nitrate reduction	5	0
Hydrogen sulfide I.	5	0
Hydrogen sulfide II.	0	5
Indole	0	5
Milk	5	0
Hemolysis	1	4
Glucose	5	0
Gas from glucose	4	1
Lactose	3 d	2
Sucrose	5	0
Maltose	4	1
Galactose	5	0
Fructose	5	0
Rhamnose	0	5
Mannose	4	1
Inulin	4	1
Xylose	4	1
Arabinose	0	5
Glycerol	4	1
Adonitol	0	5
Sorbitol	5	0
Mannitol	5	0
Dulcitol	0	5
Starch	0	5
Esculin	5	0
Koser's citrate	5	0
Simmons' citrate	5	0
Acethylmethylcarbinol	1	4
Methyl red	0	5
KCN	5	0
Phenylalanin	0	5
Catalase	5	0
Urease	0	5
Lipase	5	0
5% NaCl	5	0
7.5% NaCl	4	1
Pigment	2	3

Hydrogen sulfide I. = lead acetate papers
Hydrogen sulfide II. = modified Klinger's agar
d = delayed

features which is suitable in taxonomic differentiation of the Serratia genus. According to our opinion the mentioned biochemical test is a more substantial feature than the strain differences in fermenting of carbohydrates or in pigmentation, etc.

From our previous work (Martinec and Kocur, 1961) and from present study it follows that the genus Serratia includes only one species - S. marcescens with the variety Serratia marcescens var. kiliensis.

REFERENCES

Breed, R.S. and M.E. Breed. 1927. The genus Serratia Bizio. Zentralbl. f. Bakt. II. Abt. 71:435-440.

_____, E.G. Murray and R.N. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

Breunig, J. 1888. Bacteriologische untersuchung des Trinkwasserss des Stadt Kiel. Diss. Kiel.

Davis, B.R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reactions given by members of Serratia group. Internatl. Bull. Bact. Nomen. and Taxon. 7: 151-160.

Krasilnikov, N.A. 1949. Opredelitel bakterij i aktinomycetov. Moskva.

Lehmann, K.B. and R.O Neumann 1886. Bakteriologische diagnostik. München.

Martinec, T. and M. Kocur. 1960. The taxonomic status of S. plymuthica (Lehmann and Neumann) Bergey et al. and of S. indica (Eisenberg) Bergey et al. Internatl. Bull. Bact. Nomen. and Taxon. 10(4): 247-254.

_____, _____. 1961. The taxonomic status of Serratia marcescens Bizio. Internatl. Bull. Bact. Nomen. and Taxon. 11(1): 7-12.

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A TAXONOMIC STUDY OF THE MEMBERS OF THE GENUS SERRATIA

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SUMMARY: A study of the morphological, cultural, and biochemical characteristics of 12 strains of different species of the genus Serratia indicates that most strains of species thus far recognized are identical with Serratia marcescens.

The names S. piscatorum (Lehmann and Neumann) Breed, S. pyoseptica (Fortineau) Bergey, S. fuschsina (Boeckhout et de Vries) Bergey, S. anolium (Duran-Reynals et Clausen) are to be regarded as synonyms of S. marcesceens Bizio and Serratia marinorubra as a synonym of Serratia marcescens var. kiliensis.

The strains S. saponaria I and II Markov should be removed from the genus Serratia and placed provisionally in the genus Alcaligenes.

For our studies of the species of the genus Serratia, we have obtained from various collections cultures of strains without designation of species or with obsolete names. Among these cultures were strains names, e.g. Serratia pyoseptica (Fortineau) Bergey, Serratia anolium Duran-Reynals and Clausen, Serratia fuchsina (Boeckhout and de Vries) Bergey and others. Breed (1948) regards most of these names as synonyms of Serratia marcescens, while Krasilnikov (1949) includes them in his Manual as names of accepted species.

In the 1957 edition of Bergey's Manual neither of the two new strains of Serratia, isolated and described by Markov (1956) as Serratia saponaria I and II, is included.

In our present work we have studied whether the several aforesaid "species" have taxonomic significance and whether some of them belong to the genus Serratia at all.

MATERIAL AND METHODS

In our work we have thoroughly studied 12 strains of Serratia acquired from various collections. The list of strains is given in Table 1.

The methods used were the same as in our previous work (Martinec and Kocur, 1960).

Table 1.

No. of strain	Source of strain
Serratia anolium	ATCC 6065
S. anolium B 1700	W.C. Haynes, NRRL, Peoria, Illinois
S. pyoseptica S 4	Dept. of Appl. Biol. NRC, Ottawa, Canada
S. pyoseptica 523	J. Simpson, Prairie Reg. Lab., Saskatoon, Canada
S. saponaria I. II.	W. Markov, Sofia, Bulgaria
S. fuchsina 150	B. Hampl, Dept. of Biol. Sci. Prague
S. marinorubra 318	C.E. Zobell, La Jolla, Calif.
S. piscatorum 415	Inst. of Plant Product., Prague
Serratia sp. S 13, S 26	J. Simpson, Prairie Reg. Lab. Saskatoon, Canada
Serratia sp. 846	C.B. van Niel, Stanford Univ., California
Alcaligenes faecalis 09	Inst. of Epidemiology and Microbiol., Prague

RESULTS

Morphology. All studied strains were short, gram-negative rods occurring singly and in clumps. Size of individual cells 0.8-1 x 1.5-2.5 μ. All strains were motile except Serratia saponaria II.

Cultural characteristics. Colonies of most strains were round, with undulate margin, slightly convex, smooth. Eight strains were at first creamy or slightly rose colored, later red, very slightly pigmented was S. saponaria I. and there were three non-pigmented strains S. saponaria II., no. 6065 and B 1700. In nutrient broth all strains produced turbidity and sediment except S. saponaria I. and II. which grew very poorly. S. saponaria I. and II., however, grew well in nutrient broth at pH 9.6. The mentioned strains did not grow on potato. On the medium recommended by Dewey and Poe (1943) 6 strains only were pigmented.

Biochemical characteristics. The ascertained biochemical characteristics are shown in Table 2.

DISCUSSION

The results indicated that all the strains with the exception of Serratia saponaria I and II agreed with the characteristics of Serratia marcescens Bizio. The differences as shown in Table 2 (lactose fermented by strain 318, gas not formed from glucose by strains 523, 318, S 26, maltose not fermented by strain S 4 and adonitol not fermented by strain 527) are characteristic for Serratia marcescens. Serratia marinorubra BS 318 (acethylmethylcarbinol not formed) is identical with Serratia marcescens var. kiliensis.

We conclude that the aforesaid species S. anolium Duran-Reynals and Clausen, S. pyoseptica (Fortineau) Bergey, Serratia fuchsina (Boeckhout and de Vries) Bergey are to be regarded as synonyms of Serratia marcescens Bizio and Serratia marinorubra as a synonym of Serratia marcescens var. kiliensis.

Serratia marcescens differs significantly from Serratia saponaria I and II. The differences in the biochemical activity of these two strains in comparison with the strain Serratia marcescens are so substantial, that we conclude that these two strains should not be placed in the genus Serratia. We have compared biochemical characteristics of Serratia saponaria I and II with one strain of Alcaligenes faecalis 09 and with the characteristics of this species in Bergey's Manual (1957). We have ascertained that biochemical features of Serratia saponaria I and II and Alcaligenes faecalis are very similar (liquefaction of gelatin, no fermentation of carbohydrates, growth at pH 9.6). On the

Diagnostic Tests Used	S. anolium 6065	S. anolium B 1700	S. pyoseptica S 4	S. pyoseptica 523	S. saponaria I.	S. saponaria II.	S. fuchsina 150	S. marinorubra 318	S. piscatorum 415	Serratia sp. S 13	Serratia sp. S 26	Serratia sp. 846
Gelatin liquefaction	+	+	+	+	+	-	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	-	-	+	-	+	+	+	+
Nitrate reduction	+	+	+	+	-	+	+	+	+	+	+	+
Hydrogen sulfide I.	+	+	+	+	-	-	+	+	+	+	+	+
Hydrogen sulfide II.	+w	+w	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Milk	+	+	+	+	-	-	+	+	+	+	+	+
Hemolysis	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	-	-	+	-	+	-	-	+
Gas from glucose	+	+	+	-	-	-	+	-	+	-	-	+
Lactose	-	-	-	-	-	-	-	+	-	-	-	-
Sucrose	+	+	+	+	-	-	+	+	+	+	+	+
Maltose	+	+	-	+	-	-	+	+	+	+	+	+
Galactose	+	+	+	+	-	-	+	+	+	+	+	+
Fructose	+	+	+	+	-	-	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	-	-	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	+	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	+	+	+	-	-	-	+	+	+	+	+	+
Adonitol	+	+	+	-	-	-	-	+	+	-	-	+
Sorbitol	+	+	+	+	-	-	+	-	+	+	+	+
Mannitol	+	+	+	+	-	-	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	+	+	+	+	-	-	+	+	+	+	+	+
Koser's citrate	+	+	+	+	-	-	+	+	+	+	+	+
Simmons' citrate	+	+	+	+	-	-	+	+	+	+	+	+
Acethylmethylcarbinol	+	+	+	+	-	-	+	-	+	+	+	+
Methyl red	-	-	-	-	-	-	-	-	-	-	-	-
KCN	+	+	+	+	+	-	+	+	+	+	+	+
Phenylalanine	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	+	-	-	-	-	-	-
Lipase	+	+	+	+	-	-	+	+	+	+	+	+
5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+
7.5% NaCl	+	+	-	+	+	+	+	+	+	+	+	+
Pigment	-	-	+	+	+	-	+	+	+	-	-	-

w = weak
Hydrogen sulfide I. = lead acetate papers
Hydrogen sulfide II. = modified Kliger medium

basis of this observation we recommend placing Serratia saponaria I and II provisionally in the genus Alcaligenes. The definitive placing of this species will be possible only after having compared it with other species of the genus Alcaligenes.

Special attention should be given to the species S. piscatorum (Lehmann and Neumann) Breed. This species has been recognized both by Krasilnikov (1949) and Breed (1957). But this species is not deposited in any collection and therefore it cannot be compared with Serratia marcescens. The only strain assigned to S. piscatorum, which we could compare, was isolated several years ago from soil (see Table 1). We have ascertained that this strain is identical with Serratia marcescens. On comparison of the original characteristics (1884) as well as the description in Bergey's Manual (1957) with the features of Serratia marcescens, we find only small differences. The fact that S. piscatorum can produce pigment at 37° and that this pigment is soluble in water, cannot be taken for such an essential feature, on the basis of which it would be possible to acknowledge S. piscatorum a valid species, because this feature can be found also in some strains of Serratia marcescens. We have ascertained that out of 68 strains studied at 37°C only 15 strains produced pigment (unpublished data). . Also the fact pigment of some heavily pigmenting strains of Serratia marcescens is soluble in water, was stated already in 1948 by Breed and recently it has been experimentally proven by Williams et al. (1958). For these reasons we regard Serratia piscatorum (Lehmann and Neumann) Breed as a synonym of S. marcescens.

REFERENCES

Breed, R.S. and M. E. Breed. 1927. The genus Serratia Bizio. Zentralbl. f. Bakt. II. Abt. 71: 435-440.

_____, E.G.D. Murray and A.P. Hitchens. 1948. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

_____, _____ and R.N. Smith. 1957. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore, Maryland.

Boeckhout, F.W.J. and J.J. Ott de Vries. 1898. Über einem neuen chromogenen Bacillus. Zentralbl. f. Bakt. 4: 487-501.

Davis, B.R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reaction given by members of the Serratia group. Internatl. Bull. Bact. Nomen. and Taxon. 7: 151-160.

Du Bois, Saint Sévrin. 1894. Panaris des pêcheurs et microbe rouge dela sardine. Ann. Inst. Pasteur. 8: 152-160.

Duran-Reynals and H.J. Clausen. 1934. A contagious tumor-like condition in the lizard (Anolis equestris) as induced by new bacterial species, Serratia anolium (sp. n.). Jour. Bact. 33: 369-376.

Fortineau, L. 1905. L'Erythrobacillus pyosepticus et les bactéries rouges. Bull. Inst. Pasteur. 3: 13-14.

Lehmann, K.B. and R.O. Neumann. 1886. Bacteriologische Diagnostik. München.

Markov, W. 1956. Serratia saponaria I. und II. Schädliche Bakterien in der Seife. Zentralbl. f. Bakt. II. Abt. 110: 26-32.

Martinec, T. and M. Kocur. 1960. The taxonomic status of Serratia plymuthica (Lehmann and Neumann) Bergey et al. and of Serratia indica (Eisenberg) Bergey et al. Internatl. Bull. Bact. Nomen. and Taxon. 10(4): 247-254.

_____, _____. 1961. The taxonomic status of Serratia marcescens Bizio. Internatl. Bull. Bact. Nomen. and Taxon. 11(1): 7-12.

Williams, R.P., W.W. Taylor, D. Hawkins and I.L. Roth. 1958. A water soluble diffusible pigment produced by a strain of Serratia marcescens (Chromobacterium prodigiosum) Nature. 182: 1028-1029.

ZoBell, C.E. and H.C. Upham. 1944. A list of Marine Bacteria including descriptions of sixty new species. Bull. Scripps. Inst. Oceanography, La Jolla. 5: 255.

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THE TAXONOMIC STATUS OF SERRATIA PLYMUTHICA (LEHMANN AND NEUMANN) BERGEY ET AL. AND OF SERRATIA INDICA (EISENBERG) BERGEY ET AL.

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Serratia plymuthica is one of the commonly recognized species of the genus Serratia. This microorganism, first isolated by Fischer (1887), was named Bacterium plymuthicum by Lehmann and Neumann (1896) who concerned themselves with its classification. These authors showed that the characteristics of this species allied it closely with Serratia marcescens, perhaps it is quite identical. It has been given species status rather uncritically even in such recent manuals on taxonomy as that of Krassilnikov 1949 and in Bergey's Manual, ed. 7, 1957.

Inasmuch as neither the original description of Serratia plymuthica or the characterizations given by Krassilnikov (1949) and Breed (1957) are complete on the basis of present-day needs, we have studied critically the justification for the recognition of this as a distinct species.

Serratia indica (Eisenberg) Bergey et al. was first isolated in 1884 by Koch from the alimentary tract of a Java ape. The organism was not named by Koch, the binomial—Bacillus indicus Eisenberg—was given by Eisenberg (1886). The history of the discovery of this species, the nomenclature and the justification of its validity was worked out by Breed (1926).

Recently there have appeared opinions that this species is identical with Serratia marcescens, e.g., Breed (1957) thinks that it represents the R-form of S. marcescens. Davis et al. (1957), as the result of a study of 50 strains of the genus Serratia, concluded that this genus has only one species, i.e. S. marcescens.

In this contribution we are able experimentally to verify the hypothesis of Davis et al.

MATERIAL AND METHODS

We have studied 6 strains under the name of Serratia plymuthica, secured from several collections: strain 192 from Prof. J. De Ley, Brussels, strains 139 and 162 from Department of Bacteriology, Indiana University, Bloomington, Indiana, strain 183 from the American Type Culture Collection, strain 847 from Prof. van Niel, Pacific Grove, California and strain 497 from Dept. of Agric. Ottawa.

We have also studied in detail 10 strains acquired from various collections under the name Serratia indica. The origin of these strains is given in Table 1.

Table 1.

Serratia indica Strain No.	Source of Strain
305	Own isolates
T72	Research Institute of Plant Production, Prague
33 and 135	Dept. of Bacteriology, Purdue University, Indiana
Bu 209	Biol. Inst. Czech. Acad. Sci., Prague
341	W.C. Haynes, Northern Regional Res. Lab., Illinois
IZ 358	Institute of Fermentation, Sao Paulo, Brazil
447	Dept. of Agriculture, Ottawa, Canada
4002 and 4003	American Type Culture Collection

We have studied the morphological, cultural, and biochemical characteristics of strains of both S. plymuthica and S. indica, using the following methods: Gram's stain as modified by Hucker, motility was detected by the Hajna method, gelatin liquefaction was tested by Frazier's method and by stab inoculation on 15% gelatin, nitrate reduction by Gries-Illosway's agent, hydrogen sulfide by means of lead acetate strips and in modified Klinger agar (Davis et al. 1957), indole by Kovács' reagent.

The fermentation of sugars was studied in peptone water with 1% sugar, starch hydrolysis was tested by Lugol solu-

tion, and the utilization of citrate determined in Koser's and Simmons' medium. Production of acetylmethylcarbinol we ascertained by Voges-Proskauer's medium by Leifson's reagent (Methods for Pure Culture Study of Bacteria (1946)).

Production of phenylpyruvic acid from phenylalanine and growth in presence of KCN were studied by the methods recommended by the Enterobacteriaceae Subcommittee (1958). Urease we ascertained by Christensen's method (1946) and by Cowan's microtest (1952), lipase by Bulder's method (1955). Pigment production was noted on potato, nutrient agar, gelatin and on the medium recommended by Dewey and Poe (1943).

RESULTS

Serratia plymuthica

Morphology: All six strains of S. plymuthica formed small Gram-negative rods occurring individually or in groups. The size varied from 0.8-1.0 x 1.5-2.0μ. All the strains were motile.

Cultural characteristics: The colonies on nutrient agar, circular with undulate margins, slightly convex, smooth. Red pigment was produced only by the strains 183 and 847, the others were cream-colored. In nutrient broth all the strains developed intense turbidity and formed a slight sediment.

Biochemical characteristics: The results of the study of biochemical characteristics of both S. plymuthica and S. indica are given in Table 2.

Discussion. We wished to determine the taxonomic status of the species S. plymuthica (Lehmann et Neumann) Bergey et al., by testing Lehmann and Neumann's hypothesis that it might well be identical with the species S. marcescens Bizio. In a study of the morphological and cultural characteristics of S. plymuthica we found no significant differences among the six strains. Differences occurred only in pigment production (3 strains formed no pigment). The differences in biochemical characteristics were as follows: One strain did not liquefy gelatin or hydrolyze casein, 5 strains showed delayed fermentation of lactose, 2 strains fermented inulin, 1 strain fermented adonitol. We attach no importance to these differences. Other biochemical characteristics

Table 2. Results of biochemical tests with 6 strains of Serratia plymuthica and 10 strains of Serratia indica.

Test	S. plymuthica No. +	S. plymuthica No. -	S. indica No. +	S. indica No. -
Gelatin liquefaction	5	1	10	0
Casein hydrolysis	5	1	10	0
Nitrate reduction	6	0	10	0
Hydrogen sulfide I	6	0	10	0
Hydrogen sulfide II	0	6	2w	8
Indole	0	6	0	10
Milk	6	0	10	0
Hemolysis	0	6	0	10
Glucose	6	0	10	0
Gas from glucose	6	0	5w	5
Lactose	5d	1	0	10
Sucrose	6	0	10	0
Maltose	6	0	10	0
Galactose	6	0	10	0
Fructose	6	0	10	0
Rhamnose	0	6	0	10
Mannose	6	0	10	0
Inulin	2	4	0	10
Xylose	0	6	0	10
Arabinose	0	6	0	10
Glycerol	6	0	10	0
Adonitol	1	5	10	0
Sorbitol	6	0	10	0
Mannitol	6	0	10	0
Dulcitol	0	6	0	10
Starch	0	6	0	10
Esculin	6	0	10	0
Koser's citrate	6	0	10	0
Simmons' citrate	6	0	10	0
Acetylmethylcarbinol	6	0	10	0
Methyl red	0	6	0	10
KCN	6	0	10	0
Phenylalanine	0	6	0	10
Catalase	6	0	10	0
Urease	0	6	0	10
Lipase	6	0	10	0
5% NaCl	6	0	10	0
7.5% NaCl	6	0	10	0
Pigment	3	3	10	0

Hydrogen sulfide I = lead acetate papers. w = weak.
Hydrogen sulfide II = modified Klinger's agar. d = delayed.

agree with the work of Davis et al. (1957).

Our results agree to some extent with Fischer's description (1887) in which the difference between S. plymuthica and S. marcescens is said to lie in the fact that the cells of S. plymuthica are thicker than those of S. marcescens, and that a crimson pigment is produced only by S. plymuthica. Neither these differences or those recognized by Breed seem adequate today. Breed regarded as the main difference between these species the production of gas from some sugars by S. plymuthica, especially from glucose. This is also true of S. marcescens (Bergey's Manual 1957), as was found also by Davis (1957) and by us. In our opinion it is not advisable to use the ability of the genus Serratia to produce gas from sugars as a taxonomic criterion for distinguishing species; we regard it as a variable characteristic.

Having compared the characteristics of the species Serratia plymuthica with the results of Davis et al. (1957) and with the characteristics of Serratia marcescens (in Bergey's Manual (1957) as well as with the characteristics of 68 strains studied by us, we came to the opinion that Serratia plymuthica is a junior synonym of Serratia marcescens.

Serratia indica

Morphology: All strains of Serratia indica were morphologically identical, small rods, occurring singly and in chains; size of individual cells 0.8-1.0 x 1.8μ. Cells were motile and Gram-negative.

Cultural characteristics: Colonies were round with slight undulate margins, smooth, some strains were rough. Most strains at first were creamy or slightly rose-colored, later red. In nutrient broth all strains produced turbidity and white sediment. On potato and in medium recommended by Dewey and Poe (1943) all strains were pigmented.

Biochemical characteristics: The ascertained biochemical characteristics are shown in Table 2.

Discussion. The task of our present work was to ascertain experimentally whether these strains of Serratia indica were identical with Serratia marcescens. We conclude that there are no substantial differences between the individual strains of Serratia indica studied. Some differences in results appeared in the production of hydrogen sulfide as determined by two methods. By use of the first method (lead

acetate paper strips) we found sulfide produced by all ten strains, with the second method (modified Klinger's agar) we ascertained weak production in 2 strains only. These results agree with those of other authors (Clarke 1953, Davis et al. 1957).

Certain differences between individual strains were noted in the production of gas from glucose. Five strains out of the total of 10 produced a small amount of gas. We do not attach great importance to this difference. Similar results were obtained by Davis et al. (1957).

Only a small part of our results can be compared with those recorded in the original description by Eisenberg (1886) which included morphological and cultural characteristics only. Our results agree with the characteristics recorded by Eisenberg.

Breed (1957) described Serratia indica more fully but emphasized the morphological and cultural characteristics without adequate consideration of the biochemical characteristics. He does not list the kinds of sugar that this species utilizes. On the basis of Reed's work (1937) Breed suggests that Serratia indica is probably an R-form of Serratia marcescens. Our results in part verify this hypothesis.

Krassilnikov (1949) in his Guide gives no new data about this species, he places it in the genus Chromobacterium.

We attach the greatest importance to the comparison of our work with the results of Davis et al. (1957). On the basis of the study of 50 strains of genus Serratia, these authors formulated the hypothesis that this genus has only one species. We have proved experimentally that this hypothesis is correct. We have ascertained that the characteristics of 10 strains of Serratia indica agree not only with the results of the authors mentioned above but also with the characteristics of 68 strains of Serratia marcescens, which we have also studied in detail.

SUMMARY

From our study of the morphological, cultural and biochemical characters of 68 strains received as Serratia marcescens Bizio, 6 strains as S. plymuthica (Lehmann and Neumann) Bergey et al. and 10 strains as S. indica (Eisenberg) Bergey et al., we conclude that the species

names S. plymuthica and S. indica should be regarded as junior synonyms of S. marcescens.

REFERENCES

Breed, R.S. and M.E. Breed. 1924. The type species of the genus Serratia commonly known as Bacillus prodigiosus. Jour. Bact. 9: 545-557.

_____, _____. 1926. Serratia indica Bergey, the red chromogenic bacterium originally isolated by Koch in India. Jour. Bact. 11: 76-77.

_____, _____. 1927. The genus Serratia Bizio. Zentral. f. Bakt. II. abt. 71: 435-440.

_____, E.G.D. Murray and N.R. Smith. 1957. Bergey's Manual of Determinative Bacteriology, 7th ed. 1094 pp. Williams and Wilkins Co., Baltimore, Maryland.

Bulder, C.J.E.A. 1955. Some observations on the lipolytic activity of microorganisms and a new method for its detection. Antonie van Leeuwenhoek 21: 433-445.

Christensen, W.B. 1946. Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from Salmonella and Shigella types. Jour. Bact. 52: 461-466.

Clarke, P.K. 1953. Hydrogen sulphide production by bacteria. Jour. Gen. Microbiol. 8: 397-497.

_____ and S.T. Cowan. 1952. Fermentations: Biochemical micromethods for bacteriology. Jour. Gen. Microbiol. 6: 784-794.

Davis, Betty R., W.H. Ewing and R.W. Reavis. 1957. The biochemical reactions given by members of the Serratia group. Intern. Bull. Bact. Nomen. and Taxon. 7: 151-160

Dewey, B.T. and Ch. F. Poe. 1943. A simple artificial medium for pigment production by members of the genus Serratia. Jour. Bact. 45: 485-498.

Eisenberg, J. 1886. Bakteriologische Diagnostik. Hamburg and Leipzig.

Enterobacteriaceae Subcommittee. 1958. Report of the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological Societies. Intern. Bull. Bact. Nomen. and Taxon. 8: 25-70.

Kocur, M. and T. Martinec. 1957. O výskytu pigmentujících bakterií ve vodách Knínické přehrady. Publ. Fac. Sci. Univ. Masaryk. Bruno, No. 388, 1-12.

Krassilnikov, N.A. 1949. Opredělitěl bakterij i aktinomycetov. Moskva.

Lehmann, K.B. and R.O. Neumann. 1896. Bakteriologische Diagnostik. München.

Manual of Methods for Pure Culture Study of Bacteria. 1946. Geneva, New York.

Reed, G.B. 1937. Independent variation of several characteristics in Serratia marcescens. Jour. Bact. 34: 255-266.