Characterization of *Serratia marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* by the Electrophoretic Patterns of their Esterases

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(Received 24 April 1978)

Esterases of 62 *Serratia marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* strains were analysed by horizontal slab electrophoresis in polyacrylamide–agarose gel. Five principal bands hydrolysed β -naphthyl acetate but differed in their range of activity towards other substrates and in their sensitivity to di-isofluoropropyl phosphate. Additional bands were detected in some strains of *S. marcescens* and *S. marinorubra*. The comparative distribution of bands showed that the four *Serratia* species were characterized by distinct electrophoretic patterns of their esterases. *Serratia marcescens*, *S. liquefaciens* and *S. plymuthica* appeared to be more closely related to one another than to *S. marinorubra*. In the case of *S. plymuthica* and *S. marinorubra*, congruence was found between biovars and esterase patterns. The numerous electrophoretotypes observed within *Serratia* species might provide useful epidemiological markers.

INTRODUCTION

Previous investigations have revealed that esterases of several taxa in the family Enterobacteriaceae (Goullet, 1973, 1975, 1977; Goullet & Richard, 1977) give distinct and reproducible species-specific zymogram banding patterns when assayed after electrophoresis in polyacrylamide–agarose gel. In the present study, the esterases produced by the four *Serratia* species recognized by Grimont *et al.* (1977*a, b*) were examined.

METHODS

The names and sources of the test strains are given in Table 1. Growth conditions, preparation of extracts, inhibition by di-isofluoropropyl phosphate (DFP), protein estimation, acrylamide–agarose gel electrophoresis, esterase staining and estimation of electrophoretic mobility (M_F value) have all been described previously (Goullet, 1977).

RESULTS

The esterase profiles resolved from multiple runs of the same and different soluble extracts from each strain were reproducible. Electrophoretic relationships were established between the strains by numerous replicate assays comparing esterase bands in adjacent positions on the same gel. Esterase bands were found in all of the test strains.

Five principle esterase bands varying in sharpness and colour intensity, designated as E_1 , E_2 , E_3 , E_4 and E_5 in order of decreasing electrophoretic mobility, were defined (Fig. 1*a*). The E_1 band hydrolysed α -naphthyl acetate and to a lesser degree α -naphthyl butyrate, indoxyl acetate and β -naphthyl acetate; the E_2 band hydrolysed α - and β -naphthyl acetates;

Table 1. *Serratia* species examined

Code	Organism	Strain*	Biovar†	Source‡	Growth conditions§	
					30 °C	37 °C
1	<i>S. marcescens</i>	LM 24.70		Blood, Firminy, 1970	E	E, S
2	<i>S. marcescens</i>	LM 37.70		Blood, Pontivy		E
3	<i>S. marcescens</i>	LM 7.71		Nasal sample, Tangier		E, S
4	<i>S. marcescens</i>	LM 2.73		Insect, Greece		E, S
5	<i>S. marcescens</i>	LM 5.73		Pus, Toulon		E, S
6	<i>S. marcescens</i>	LM 25.73		Pus, Perpignan		E, S
7	<i>S. marcescens</i>	LM 26.73		Blood, Coulommiers		E, S
8	<i>S. marcescens</i>	LM 8.74		Urine, Paris		E, S
9	<i>S. marcescens</i>	LM 1.75		Urine, Paris		E, S
10	<i>S. marcescens</i>	LM 7.75		Urine, Paris		E, S
11	<i>S. marcescens</i>	LM 12.75		Bat, Madagascar		E, S
12	<i>S. marcescens</i>	LM 16.75		Urine, Levallois-Perret	E	E, S
13	<i>S. marcescens</i>	LM 20.75		Urine, Colmar		E, S
14	<i>S. marcescens</i>	LM 29.75		Urine, Belfort		E, S
15	<i>S. marcescens</i>	LM 38.75		Blood, Tangier		E, S
16	<i>S. marcescens</i>	LM 41.75		Faeces, Limoges		E, S
17	<i>S. liquefaciens</i>	LM 1.68		Water, Tangier		E
18	<i>S. liquefaciens</i>	LM 2.68		Water, Tangier		E
19	<i>S. liquefaciens</i>	LM 3.68		Urine, Tangier		E, S
20	<i>S. liquefaciens</i>	LM 8.68	C1b	CDC 2224-64		E
21	<i>S. liquefaciens</i>	LM 9.68	C1a	CDC 5155-57	E	E
22	<i>S. liquefaciens</i>	LM 12.68	C1b	CDC 4733-51		E
23	<i>S. liquefaciens</i>	LM 13.70	C1a	Veal, France, 1970	E	E
24	<i>S. liquefaciens</i>	LM 2.71	C1a	Blood, Fresnes, 1971		E
25	<i>S. liquefaciens</i>	LM 4.71	C1a	Urine, Tangier, 1971		E
26	<i>S. liquefaciens</i>	LM 6.71		Water, Le Pecq		E
27	<i>S. liquefaciens</i>	LM 5.72		Veal, Alfort		E
28	<i>S. liquefaciens</i>	LM 1.73		Butterfly, France		E
29	<i>S. liquefaciens</i>	LM 21.74		Throat, Villejuif		E, S
30	<i>S. liquefaciens</i>	LM 1.75		Urine, Tangier		E
31	<i>S. liquefaciens</i>	LM 2.75		Catheter, Paris		E
32	<i>S. liquefaciens</i>	LM 23.75		Blood, Garches		E, S
33	<i>S. plymuthica</i>	G 33	C2a	Brisou 3617, sea water, Oléron	E	
34	<i>S. plymuthica</i>	G 34	C2a	Brisou 3736, sea water, Oléron	E, S	
35	<i>S. plymuthica</i>	G 289	C2b	NCTC 9493	E, S	
36	<i>S. plymuthica</i>	G 299	C2b	Brisou 6912, sea water, Oléron	E	
37	<i>S. plymuthica</i>	G 325	C2b	Lab. Municipal, spring water, Creysse, 1971	E	
38	<i>S. plymuthica</i>	G 329	C2b	Lab. Municipal, well water, Izon, 1971	E, S	
39	<i>S. plymuthica</i>	G 392	C2b	Lab. Municipal, spring water, Bergerac, 1971	E, S	
40	<i>S. plymuthica</i>	G 500	C2c	ATCC 992	E	
41	<i>S. plymuthica</i>	G 501	C2c	ATCC 4261	E, S	
42	<i>S. plymuthica</i>	G 502	C2c	ATCC 7462	E	
43	<i>S. plymuthica</i>	G 510	C2c	ATCC 640	E	
44	<i>S. plymuthica</i>	G 512	C2c	NCIB 8616	E, S	
45	<i>S. plymuthica</i>	G 515	C2a	NCIB 4612	E	
46	<i>S. plymuthica</i>	G 516	C2b	NCIB 8266	E	
47	<i>S. plymuthica</i>	G 517	C2b	NCIB 8285	E	
48	<i>S. marinorubra</i>	LM 9.60		ATCC 266	E, S	
49	<i>S. marinorubra</i>	LM 36.68	B1	Animal faeces, Tangier, 1968	E, S	
50	<i>S. marinorubra</i>	LM 34.70	B2	Throat swab, Créteil, 1970	E, S	
51	<i>S. marinorubra</i>	LM 1.74		Urine, Trujillo	E, S	
52	<i>S. marinorubra</i>	G 20	B1	Brisou 90, pig	E, S	
53	<i>S. marinorubra</i>	G 22	B1	Brisou 658, sea water, La Rochelle, NCTC 10847	E, S	
54	<i>S. marinorubra</i>	G 36	B1	Santé Navale, cultivated mushroom, 1964	E, S	
55	<i>S. marinorubra</i>	G 37	B1	Santé Navale, cultivated mushroom, 1964, DSM 30124	S	
56	<i>S. marinorubra</i>	G 39	B1	Santé Navale, cultivated mushroom, 1966, NCTC 10848	E, S	
57	<i>S. marinorubra</i>	G 230	B3	IP Tunis 8376, 1971	S	
58	<i>S. marinorubra</i>	G 291	B1	Brisou 641, water, La Rochelle	E, S	
59	<i>S. marinorubra</i>	G 377	B3	Lapage T37/66, pig faeces	E, S	
60	<i>S. marinorubra</i>	G 511	B2	CCM 788	E, S	
61	<i>S. marinorubra</i>	G 513	B1	NCPPB 1126	S	
62	<i>S. marinorubra</i>	G 530	B2	Matsumoto, sewage	E, S	

* LM, Strain provided by Dr C. Richard from Collection du Service des Entérobactéries de l'Institut Pasteur de Paris (Professeur L. Le Minor); G, strain from Collection du Dr P. A. D. Grimont (Laboratoire de Bactériologie, Université de Bordeaux II, France) studied by Grimont *et al.* (1977b).

† According to Grimont *et al.* (1977b).

‡ ATCC, American Type Culture Collection; CCM, Czechoslovak Collection of Microorganisms; CDC, Center for Disease Control; NCIB, National Collection of Industrial Bacteria; NCPPB, National Collection of Plant Pathogenic Bacteria; NCTC, National Collection of Type Cultures. Brisou, Prof. J. Brisou, Poitiers University, France; I. P. Tunis, Institut Pasteur de Tunis, Tunisia (R. Houissa); Lab. Municipal, Laboratoire Municipal, Bordeaux, France (R. Serceau); Lapage, Dr S. Lapage, Central Public Health Laboratory, London; Matsumoto, Dr H. Matsumoto, Shinshu University, Matsumoto, Japan; Santé Navale, Ecole du Service de Santé des Armées, Bordeaux, France.

§ E, Bacteria harvested during the exponential phase; S, bacteria harvested during the stationary phase.

Fig. 1. *Serratia liquefaciens*. (a) Schematic representation of mobility and activity of esterase bands (strain 19). Horizontal slab acrylamide-agarose gel electrophoresis was performed using 7% (w/v) acrylamide and discontinuous Tris/glycine buffer, pH 8.7 (Uriel, 1966). Esterase activity was revealed by the method of Uriel (1961). Substrates used were: 1, α -naphthyl acetate; 2, α -naphthyl butyrate; 3, indoxyl acetate; 4, β -naphthyl acetate; 5, β -naphthyl butyrate. Relative intensity of staining: $\blacksquare > \boxtimes > \boxdot > \square > \square$. (b) Esterase patterns of the 16 strains (numbered as in Table 1) arranged in order of increasing mobility of E_3 esterase bands. $\blacksquare$, E_1 band; $\square$, E_2 band; $\blacksquare$, E_3 band; $\square$, E_4 band (only the slowest moving band is represented); $\square$, E_5 band. The M_F values were used only comparatively.

the E_3 band hydrolysed β -naphthyl esters and to a lesser degree the other substrates. The E_4 band, which reacted strongly with indoxyl acetate and weakly with β -naphthyl acetate, was broad and consisted of three sub-bands; the E_5 band was active towards β -naphthyl esters.

Band E_1 was inhibited by 10^{-4} M-DFP and bands E_3 and E_5 were inhibited by 10^{-3} M, whereas bands E_2 and E_4 remained active under these conditions.

Serratia liquefaciens

The five principal bands were found in 13 *S. liquefaciens* strains (Fig. 1b). Differences in mobility between bands E_1 , E_2 and E_3 were generally small and two or three of these bands were superimposed in some strains: E_1 and E_2 for strains 24, 26, 30 and 31; E_1 and E_3 for strains 25 and 27; E_2 and E_3 for strains 17 and 21; and all three bands for strains 18 and 22. Separation and characterization of bands was then achieved by varying the concentration of acrylamide and by differential DFP inhibition. In each strain, bands E_4 and E_5 showed electrophoretic uniformity with the exceptions of strain 29 (E_4 band) and strain 28 (E_5 band). Variations in esterase mobility between the strains distinguished at least 13 electrophoretotypes. For strains 21 and 23, similar esterase patterns were obtained after growth at 30 °C and 37 °C.

Fig. 2. *Serratia marcescens*. (a) Schematic representation of mobility and activity of esterase bands (strain 1). (b) Esterase patterns of the 16 strains arranged in order of increasing mobility of E₄ bands. Experimental conditions, relative intensity of staining and key as in legend to Fig. 1. ■■■, Esterase band hydrolysing α -naphthyl acetate and indoxyl acetate; ▨▨, esterase band hydrolysing α -naphthyl acetate and α -naphthyl butyrate.

Serratia marcescens

The esterase bands of *S. marcescens* strains were more widely spaced and their activity was generally less marked than in *S. liquefaciens* (Fig. 2a). Bands E₂, E₄ and E₅ were resolved in all strains but band E₁ was defined in only six of the organisms (Fig. 2b). Bands from bacteria harvested during stationary phase were more pronounced. Bands E₁, E₂ and E₃ were distinctly separated from each other. Band E₄ showed electrophoretic heterogeneity, while band E₅ exhibited two different mobilities: $M_r \approx 15$ and $M_r \approx 26$ (like strain 28 of *S. liquefaciens*). In some strains two additional moderately coloured bands were detected: one hydrolysing α -naphthyl acetate and α -naphthyl butyrate and inhibited by 10^{-4} M-DFP (strains 1, 3, 9, 11 and 16); another hydrolysing α -naphthyl acetate and indoxyl acetate and remaining active at 10^{-3} M-DFP (strains 2, 4 and 7). Some minor bands were active towards acetate esters and resistant to DFP. Fourteen electrophoretotypes were distinguished, and for strains 1 and 12 similar patterns were obtained after growth at 30 °C and 37 °C.

Serratia plymuthica

Bands E₁, E₄ and E₅ were resolved in all strains (with the exception of strain 39 for band E₄), whereas band E₃ was defined within biovar C2b only (Fig. 3). The latter was more prominent and migrated faster than the corresponding band of *S. marcescens* and *S. liquefaciens*, and was inhibited by 10^{-3} M-DFP. In biovars C2a and C2b, the E₁ band exhibited electrophoretic heterogeneity but bands E₄ and E₅ were electrophoretically homogeneous, with the single exception of strain 39 for band E₅. In biovar C2c, band E₄ migrated slower than band E₅ and all three bands exhibited electrophoretic homogeneity. Nine electrophoretotypes were found in biovar C2a and C2b, but only one was found in biovar C2c. Congruence was found between the biovars and esterase patterns.

Fig. 3. *Serratia plymuthica*. (a) Schematic representation of mobility and activity of esterase bands (strain 41). (b) Esterase patterns of the 15 strains arranged according to biovar, in order of increasing mobility of E_3 esterase bands (*, biovar C2a; †, biovar C2b; ‡, biovar C2c). Experimental conditions, relative intensity of staining and key as in legend to Fig. 1.

Fig. 4. *Serratia marinorubra*. (a) Schematic representation of mobility and activity of esterase bands (strain 58). (b) Esterase patterns of the 15 strains arranged in order of increasing mobility of E_2 esterase bands (*, biovar B1; †, biovar B2; ‡, biovar B3). Experimental conditions, relative intensity of staining and key as in legend to Fig. 1. □□□□, Esterase band hydrolysing acetate esters; ■■, cathodic band.

Table 2. M_F values of esterase bands in the four species of *Serratia*

Mean M_F values are given together with the standard deviation. Sample sizes are given in parentheses.

Band	<i>S. marcescens</i>	<i>S. liquefaciens</i>	<i>S. plymuthica</i>	<i>S. marinorubra</i>
E ₁	64.50 ± 6.35 (6)	68.27 ± 3.01 (15)	68.27 ± 4.98 (15)	67.38 ± 2.20 (8)
E ₂	60.88 ± 3.50 (16)	66.06 ± 1.57 (16)		76.00 ± 3.24 (13)
E ₃	50.23 ± 7.34 (13)	65.00 ± 3.80 (15)	80.83 ± 2.86 (6)	55.50 ± 4.95 (2)
E ₄	37.75 ± 4.40 (16)	44.93 ± 0.26 (15)	40.86 ± 2.98 (14)	
E ₅	23.25 ± 4.92 (16)	34.44 ± 2.25 (16)	37.40 ± 5.58 (15)	
E _{M1}				52.00 ± 0 (7)
E _{M2}				-14.58 ± 6.16 (12)

Serratia marinorubra

Only faint esterase bands were observed with bacteria harvested in exponential phase but in those from stationary phase the bands were defined more clearly (Fig. 4). Band E₁ migrated more slowly than E₂ and was inhibited by 5×10^{-5} M-DFP. In some strains, especially in biovar B3, these bands were very faint. Band E₃ was present in two strains only and bands E₄ and E₅ were not detected. Two additional bands, both sensitive to DFP, were visualized: the one in biovar B1, designated E_{M1}, hydrolysed acetate esters and was inhibited by 10^{-5} M-DFP; the other, which was present in all three biovars and designated E_{M2}, migrated cathodically, hydrolysed β -naphthyl acetate and was inhibited by 10^{-4} M-DFP. Eleven electrophoretotypes were distinguished, and for the 13 strains of known biovar, good correlation was obtained between the biovars and esterase patterns.

DISCUSSION

Nine types of esterase bands differing in their hydrolysing activity towards carboxylic esters, sensitivity to DFP and electrophoretic mobility were found in extracts of *Serratia* strains. The distribution of the esterases varied among species (Figs 1 to 4; Table 2). Band E₁ was observed in all four *Serratia* species but was absent in numerous strains. The mobility of band E₂ increased from *S. marcescens* through to *S. marinorubra* (Table 2) but was not resolved in *S. plymuthica* strains. The mobility of band E₃ increased from *S. marcescens* through to *S. plymuthica*, its electrophoretic distribution ranging from $M_F \approx 36$ (Fig. 2) to $M_F \approx 83$ (Fig. 3). Bands E₄ and E₅ were present in all strains of *S. liquefaciens*, *S. marcescens* and *S. plymuthica*, with two exceptions for band E₄ in strains 29 and 39, but were not detected in *S. marinorubra*. The distance between bands E₄ and E₅ decreased from *S. marcescens* through to *S. plymuthica*. For each of the principal bands *S. marcescens* exhibited the slowest mobility. Additional bands were observed in some *S. marcescens* and *S. marinorubra* strains.

The finding that *Serratia* species may be characterized by the distinct electrophoretic patterns of their esterases agrees with earlier reports for *Proteus* and *Providencia* (Goullet, 1975), *Salmonella* (Goullet, 1977), *Levinea malonatica*, *Levinea amalonicata* and *Citrobacter* (Goullet & Richard, 1977). The differentiation of *Serratia* by esterase profiles is consistent with the results obtained by numerical taxonomy (Bascomb *et al.*, 1971; Grimont *et al.*, 1977b), DNA hybridization (Steigerwalt *et al.*, 1976) and electrophoresis of proteases (Grimont *et al.*, 1977a). On the basis of the esterase profiles in the present study, *S. marcescens*, *S. liquefaciens* and *S. plymuthica* appear to be more closely related to one another than to *S. marinorubra*. Differentiation of a biovar within *S. plymuthica* and *S. marinorubra* by esterase bands was possible. The numerous electrophoretotypes observed within *Serratia* species could represent a potential method for epidemiological analyses.

The author thanks Drs C. Richard and P. A. D. Grimont for *Serratia* strains, and Madame Colette Gaillard and Mademoiselle Catherine Lamesch for technical assistance. This work was supported by grants from the Université de Paris VII and from the Institut National de la Santé et de la Recherche Médicale (no. 75.1.114.1 and no. 78.1.136.1).

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