Characterization of *Serratia odorifera*, *S. fonticola* and *S. ficaria* by the Electrophoretic Patterns of their Esterases

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Esterases of 15 strains of *Serratia odorifera*, 14 strains of *S. fonticola* and 13 strains of *S. ficaria* were resolved by horizontal electrophoresis in polyacrylamide-agarose gel. Esterase bands were defined by their range of activity towards several synthetic substrates and their sensitivity or resistance to di-isopropyl fluorophosphate. *Serratia odorifera* strains showed three to five major esterase bands and three minor bands. *Serratia fonticola* strains showed three or four major bands and one minor band. *Serratia ficaria* strains showed three to five major bands and one minor band. The comparative distribution of bands revealed that these three newly described species could be distinguished by the electrophoretic patterns of their esterases. Congruence was found between biotypes 1 and 2 of *S. odorifera* and electrophoretic patterns. These species-specific patterns were different from those of *S. marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* previously characterized.

INTRODUCTION

Serratia marcescens, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* have been characterized previously by the distinct electrophoretic patterns of their esterases (Goullet, 1978). The purpose of this study was to characterize three newly described species of *Serratia* – *S. odorifera*, biotypes 1 and 2 (Grimont *et al.*, 1978), *S. fonticola* (Gavini *et al.*, 1979) and *S. ficaria* (Grimont *et al.*, 1979) – by the same procedure.

METHODS

The names and sources of the test strains are given in Table 1. Growth conditions, preparation of extracts, protein estimation, heat inactivation, inhibition by di-isopropyl fluorophosphate (DFP), horizontal slab electrophoresis in polyacrylamide-agarose gel, esterase staining and estimation of electrophoretic mobility (M_F value) were all as described previously (Goullet, 1980).

RESULTS

Esterase bands migrating towards the anode and varying in sharpness and colour intensity were found for each of the 42 strains. Esterase banding patterns were sufficiently distinctive and reproducible to identify the species, and eventually the biotype, of each strain analysed. For each species, major esterase bands (E_1 to E_5) and minor bands (m_1 to m_3) were designated in order of decreasing electrophoretic mobility.

Serratia odorifera

Strains of *S. odorifera* showed two sets of esterase bands (Fig. 1*a*). One set (bands E_1 , E_2 , m_1 , m_2 and m_3) resisted DFP (10^{-3} M), whereas the other set (bands E_3 , E_4 and E_5) was sensitive to this agent. Band E_1 hydrolysed β -naphthyl acetate and, to a lesser extent,

Table 1. *Strains of Serratia examined*

Code	Organism	Strain*	Biotype†	Source and other strain designation‡
1	<i>S. odorifera</i>	Grimont 38 (a)	1	Cultivated mushroom, Bordeaux, France
2	<i>S. odorifera</i>	Grimont 1073 (a)	1	Sputum, Bordeaux, France; ICPB 3995, holotype
3	<i>S. odorifera</i>	Richard 35.72 (b)	1	Faeces, Toulon, France
4	<i>S. odorifera</i>	Richard 2.73 (b)	1	Mussel, Saint-Etienne, France
5	<i>S. odorifera</i>	Richard 17.74 (b)	1	Blood, Lyon, France
6	<i>S. odorifera</i>	Richard 12.76 (b)	1	Pus, Paris, France
7	<i>S. odorifera</i>	Richard 11.77 (b)	1	Sputum, Paris, France
8	<i>S. odorifera</i>	Richard 12.77 (b)	1	Pleura pus, Santander, Spain
9	<i>S. odorifera</i>	CDC 4409.66 (a)	2	Lung, Connecticut, U.S.A.
10	<i>S. odorifera</i>	CDC 292.74 (a)	2	Blood, California, U.S.A.
11	<i>S. odorifera</i>	CDC 2861.74 (a)	2	Tracheal secretion, California, U.S.A.
12	<i>S. odorifera</i>	CDC 3472.74 (a)	2	Blood, Massachusetts, U.S.A.
13	<i>S. odorifera</i>	CDC 1396.75 (a)	2	Bronchial washing, Vermont, U.S.A.
14	<i>S. odorifera</i>	CDC 3567.75 (a)	2	Urine, New Jersey, U.S.A.
15	<i>S. odorifera</i>	CDC 1517.76 (a)	2	Tracheal exudate, Pennsylvania, U.S.A.
16	<i>S. fonticola</i>	CUETM 77.153 (c)		Drinking water, NF
17	<i>S. fonticola</i>	CUETM 77.168 (c)		Drinking water, NF
18	<i>S. fonticola</i>	CUETM 78.7 (c)		Surface water, NF
19	<i>S. fonticola</i>	CUETM 78.9 (c)		Drinking water, NF
20	<i>S. fonticola</i>	CUETM 78.10 (c)		Unpolluted soils, NF
21	<i>S. fonticola</i>	CUETM 78.11 (c)		Drinking water, NF
22	<i>S. fonticola</i>	CUETM 78.14 (c)		Unpolluted soils, NF
23	<i>S. fonticola</i>	CUETM 78.20 (c)		Drinking water, NF
24	<i>S. fonticola</i>	CUETM 78.22 (c)		Sewage, NF
25	<i>S. fonticola</i>	CUETM 78.26 (c)		Drinking water, NF
26	<i>S. fonticola</i>	CUETM 78.28 (c)		Drinking water, NF
27	<i>S. fonticola</i>	CUETM 78.43 (c)		Drinking water, NF
28	<i>S. fonticola</i>	CUETM 78.45 (c)		Drinking water, NF
29	<i>S. fonticola</i>	CUETM 78.49 (c)		Drinking water, NF
30	<i>S. ficaria</i>	Grimont 2602 (a)		Black ant, Bordeaux, France (July 1976)
31	<i>S. ficaria</i>	Grimont 4024 (a)		Calimyrna fig, Winters, California, U.S.A. (September 1977); ICPB 4050, CIP 79.23, ATCC 33105, holotype
32	<i>S. ficaria</i>	Grimont 4025 (a)		Caprifig, Winters, California, U.S.A. (September 1977); ICPB 4051, ATCC 33106
33	<i>S. ficaria</i>	Grimont 4026 (a)		Caprifig, Winters, California, U.S.A. (September 1977); ICPB 4052
34	<i>S. ficaria</i>	Grimont 4027 (a)		Caprifig, Winters, California, U.S.A. (September 1977); ICPB 4053
35	<i>S. ficaria</i>	Grimont 4028 (a)		Caprifig, Fresno, California, U.S.A. (October 1977); ICPB 4054
36	<i>S. ficaria</i>	Grimont 4029 (a)		Caprifig, Fresno, California, U.S.A. (October 1977); ICPB 4055
37	<i>S. ficaria</i>	Grimont 4030 (a)		Blastophaga psenes, Fresno, California, U.S.A. (October 1977); ICPB 4056
38	<i>S. ficaria</i>	Grimont 4033 (a)		Blastophaga psenes, Fresno, California, U.S.A. (October 1977); ICPB 4058
39	<i>S. ficaria</i>	Grimont 4034 (a)		Calimyrna fig, Winters, California, U.S.A. (September 1977); ICPB 4059
40	<i>S. ficaria</i>	Grimont 4037 (a)		Blastophaga psenes in a caprifig, Gammarth, Tunisia (June 1978)
41	<i>S. ficaria</i>	Grimont 4038 (a)		Caprifig, Gammarth, Tunisia (June 1978)
42	<i>S. ficaria</i>	Grimont 4039 (a)		Fig, Sfax, Tunisia (June 1978)

* (a) Strain provided by Dr P. A. D. Grimont, Service des Entérobactéries (Professeur L. Le Minor), Institut Pasteur, Paris, France; (b) strain provided by Dr C. Richard, Service des Entérobactéries, Institut Pasteur; (c) strain provided by Dr F. Gavini, Unité d'Ecotoxicologie Microbienne (Professeur H. Leclerc), Domaine du CERTIA, 59650 Villeneuve a'Ascq, France. CDC, Center for Disease Control, Atlanta, U.S.A.; CUETM, Collection de l'Unité d'Ecotoxicologie Microbienne.

† According to Grimont *et al.* (1978).

‡ NF, strain from the North of France; ICPB, International Collection of Phytopathogenic Bacteria, Davis, California, U.S.A.; CIP, Collection de l'Institut Pasteur, Paris, France; ATCC, American Type Culture Collection, Rockville, U.S.A.

Fig. 1. *Serratia odorifera*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 10). Horizontal slab polyacrylamide-agarose gel electrophoresis was performed using 7% (w/v) acrylamide and discontinuous Tris/glycine buffer, pH 8.7. 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: $\blacksquare$ > $\square$ > $\square$ > $\square$ > $\square$ > $\square$. (b) Esterase patterns of 15 strains (numbered as in Table 1) arranged according to biotype. $\blacksquare$, E₁ band; $\blacksquare$, E₂ band; $\blacksquare$, E₃ band; $\blacksquare$, E₄ band; $\blacksquare$, E₅ band; $\square$, m₁ band; $\square$, m₂ band; $\square$, m₃ band; $\square$, F band. The M_F values were used only comparatively.

α -naphthyl acetate. Band m₁ was faint and detected by indoxyl acetate and α -naphthyl acetate. Bands E₂, m₂ and m₃ were stained by β -naphthyl acetate. Band m₃ was resistant to heat (10 min at 60 °C). Band E₃ hydrolysed indoxyl acetate, α -naphthyl acetate and α -naphthyl butyrate, and reacted slightly with β -naphthyl acetate. Band E₄ hydrolysed α -naphthyl acetate and, to a lesser extent, α -naphthyl butyrate, indoxyl acetate and β -naphthyl acetate. Band E₅ hydrolysed β -naphthyl esters and, to a lesser extent, the other substrates. This band was faint in strain 12. Finally, one fast moving band (F) was detected by β -naphthyl acetate. Figure 1(b) shows the electrophoretic distribution of esterases in the 15 strains. All strains produced bands E₁, E₃, E₅, m₁, m₂, m₃ and F. Differences in mobilities between bands were often small and two or three bands overlapped in some strains: bands E₁ and m₁ for strains 11, 12, 14 and 15; bands E₃ and m₂ for strains 4, 6, 7 and 8; bands E₃ and m₃ for strains 1, 2, 5, 10, 11, 12, 13, 14 and 15; bands E₅ and m₂ for strains 1, 2 and 5; bands E₃, E₅ and m₂ for strains 3 and 9. Separation and characterization of bands were then achieved by using 6 or 8% (w/v) acrylamide (instead of 7%) and by noting differences in heat inactivation or in DFP inhibition. Distinct electrophoretic distributions were observed according to the biotypes. Bands E₁, E₅ and F showed differences in mobility between the two biotypes, whereas bands E₂ and E₄ were detected in biotype 2 only. Strains 10 and 13 of biotype 2 differed from other strains of this biotype in the mobility of bands m₁, E₄ and E₅.

Serratia fonticola

Strains of this species produced four or five esterase bands that hydrolysed β -naphthyl acetate but differed in activity towards other substrates (Fig. 2). Bands m₁ and E₂ hydrolysed

Fig. 2. *Serratia fonticola*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 19). (b) Esterase patterns of 14 strains. Experimental conditions and relative intensity of staining as in legend to Fig. 1. , E_1 band; , E_2 band; , E_3 band; , E_4 band; , m_1 band.

α -naphthyl acetate and to a lesser degree α -naphthyl butyrate, indoxyl acetate and β -naphthyl acetate, whereas band E_1 hydrolysed α - and β -naphthyl butyrates and, to a lesser extent, α - and β -naphthyl acetates. Band E_3 was active towards β -naphthyl esters and reacted slightly with other substrates; it showed some residual activity after incubation for 10 min at 60 °C. In some strains (16, 19, 21, 22, 23, 24, 25 and 29) this esterase showed two electrophoretically distinct components: E_{3a} and E_{3b} . Bands m_1 , E_1 , E_2 and E_3 were inhibited by 10^{-3} M-DFP. Band E_4 reacted strongly with indoxyl acetate, moderately with β -naphthyl acetate and weakly with α -naphthyl acetate; it was a prominent band consisting of several diffuse sub-bands and resisted 10^{-3} M-DFP. Only bands E_3 and E_4 were observed in all strains. Band E_1 showed the same mobility among the strains but was not recovered in strains 16 and 29. Band F was not detected.

Significant heterogeneity in the number and mobility of esterase bands was observed within this species. However, species identification was possible by combination of bands E_1 , E_3 and E_4 for most strains and by combination of bands m_1 , E_2 , E_3 and E_4 for strains 16 and 29.

Serratia ficaria

Between five and seven esterase bands were recovered from *S. ficaria* strains (Fig. 3). Band E_1 was stained by α - and β -naphthyl acetates. In most of the strains this band showed two electrophoretically distinct components: E_{1a} and E_{1b} . Band m_1 was faint and detected by indoxyl acetate and α -naphthyl acetate as was the corresponding band of *S. odorifera*. Band E_2 hydrolysed α -naphthyl acetate. Band E_3 hydrolysed β -naphthyl acetate and was resistant to heat. Band E_4 reacted strongly with indoxyl acetate, moderately with β -naphthyl acetate and weakly with α -naphthyl acetate; it was a prominent band composed of several sub-bands distinctly separated from each other. Band E_5 hydrolysed β -naphthyl esters. Band F, detected

Fig. 3. *Serratia ficaria*. (a) Diagrammatic representation of mobility and activity of esterase bands (strain 34). (b) Esterase patterns of 13 strains. Experimental conditions and relative intensity of staining as in legend to Fig. 1. , E₁ band; , E₂ band; , E₃ band; , E₄ band; , E₅ band; , m₁ band; , F band.

by β -naphthyl acetate, was electrophoretically distinct ($M_F \approx 97$) from the corresponding band of *S. odorifera* biotype 1 ($M_F \approx 95$) and biotype 2 ($M_F \approx 92$). Bands E₂, E₅ and F were completely inhibited by treatment with 10^{-3} M-DFP, whereas bands E₁, E₃, E₄ and m₁ remained active. Bands E₁, m₁, E₄ and E₅ were observed in all strains (Fig. 3b). The electrophoretic mobility of bands F ($M_F \approx 97$), m₁ ($M_F \approx 76$) and E₅ ($M_F \approx 26$) showed no 'between-strains' variation.

Heterogeneity in esterase patterns was also observed within this species. On the basis of variation in the number and mobility of esterase bands, three subclusters of strains could be distinguished. Subcluster 1 (strains 31, 32, 34, 36 and 39) contained band E₃, whereas subclusters 2 and 3 lacked this band. Subcluster 2 (strains 30, 33, 35, 37 and 38) differed from subcluster 3 (strains 40, 41 and 42) in the mobilities of bands E₁ and E₄.

DISCUSSION

Electrophoretic analysis has demonstrated that *S. odorifera*, *S. fonticola* and *S. ficaria* may be characterized by the distinct banding patterns of their esterases and that biotypes 1 and 2 of *S. odorifera* can be identified by differences in distribution of their esterases (Fig. 1, 2, 3; Table 2). The specific patterns of these species were clearly distinguishable from those of *S. marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* (Goulet, 1978). These findings correlate well with DNA homology studies which showed that the new *Serratia* species constitute three hybridization groups clearly distinct from the other *Serratia* species (Grimont *et al.*, 1978, 1979; Gavini *et al.*, 1979). Recognition of the two biotypes of *S. odorifera* by differences in esterase patterns supports the subdivision based on biochemical characteristics and reflects the genetic divergence observed within this species (Grimont *et al.*, 1978).

Table 2. M_F values of esterase bands of *S. odorifera* (biotypes 1 and 2), *S. fonticola* and *S. ficaria*Mean M_F values are given together with the standard deviation.

<i>S. odorifera</i>			<i>S. fonticola</i>		<i>S. ficaria</i>	
Band	M_F value		Band	M_F value	Band	M_F value
	Biotype 1	Biotype 2				
F	95 $\pm$ 0	92.50 $\pm$ 0	m ₁	74.22 $\pm$ 0.67	F	97.50 $\pm$ 0
E ₁	81 $\pm$ 0	77 $\pm$ 0	E ₁	71 $\pm$ 0	E ₁	81.50 $\pm$ 1.58
m ₁	75 $\pm$ 0	76.33 $\pm$ 1.03	E ₂	65.11 $\pm$ 1.69	m ₁	76 $\pm$ 0
E ₂		71.50 $\pm$ 0	E ₃	61.71 $\pm$ 1.82	E ₂	61.58 $\pm$ 2.46
m ₂	60.44 $\pm$ 0.52	65 $\pm$ 0	E ₄	32.86 $\pm$ 4.20	E ₃	52.50 $\pm$ 0
m ₃	64 $\pm$ 0	63 $\pm$ 0			E ₄ *	29.62 $\pm$ 3.07
E ₃	61.89 $\pm$ 3.10	64 $\pm$ 0			E ₅	26.50 $\pm$ 0
E ₄		55.67 $\pm$ 2.58				
E ₅	59.67 $\pm$ 1.49	38.33 $\pm$ 2.58				

* M_F for slowest moving band.

However, similarities appeared in the range of hydrolysing activities of several esterase bands produced by *Serratia* species. Band E₄ of *S. odorifera* (biotype 2) and band E₂ of *S. fonticola* showed the same range of hydrolysing activity as bands E₁ of *S. marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra*. Bands E₃ of *S. odorifera* and E₃ of *S. fonticola* resembled band E₃ of these four last species in their preferential hydrolysis of β -naphthyl esters. Bands E₄ of *S. fonticola* and of *S. ficaria* were practically identical to the corresponding bands of *S. marcescens*, *S. liquefaciens* and *S. plymuthica* with respect to their strong activity towards indoxyl acetate and their resistance to inhibition by DFP. Band E₅ of *S. ficaria* reacted with β -naphthyl acetate and β -naphthyl butyrate as did the corresponding bands of *S. marcescens*, *S. liquefaciens* and *S. plymuthica*. These similarities between some esterase bands are in agreement with the 25 to 55% DNA-DNA relatedness observed between *Serratia* species (Grimont *et al.*, 1978, 1979; Gavini *et al.*, 1979; Steigerwalt *et al.*, 1976). As observed for the *Serratia* species analysed previously, *S. odorifera*, *S. fonticola* and *S. ficaria* showed intraspecies variations in the number and mobilities of esterase bands. This heterogeneity appeared even for *S. ficaria* strains which constitute a homologous taxon with 90% DNA-DNA relatedness (Grimont *et al.*, 1979).

The present work supports the phenotypic and genotypic individuality of the newly described species of *Serratia* and shows that esterases give discriminative and reliable information for both specific and subspecific differentiation.

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REFERENCES

- GAVINI, F., FERRAGUT, C., IZARD, D., TRINEL, P. A., LECLERC, H., LEFEBVRE, H. & MOSSEL, D. A. A. (1979). *Serratia fonticola*, a new species from water. *International Journal of Systematic Bacteriology* **29**, 92-101.
- GOULLET, PH. (1978). Characterization of *Serratia marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* by the electrophoretic patterns of their esterases. *Journal of General Microbiology* **108**, 275-281.
- GOULLET, PH. (1980). Distinctive electrophoretic patterns of esterases from *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes* and *E. gergoviae*. *Journal of General Microbiology* **117**, 483-491.
- GRIMONT, P. A. D., GRIMONT, F., RICHARD, C., DAVIS, B. R., STEIGERWALT, A. G. & BRENNER, D. J. (1978). Deoxyribonucleic acid relatedness between *Serratia plymuthica* and other *Serratia* species, with a description of *Serratia odorifera* sp. nov. (type strain: ICPB 3995). *International Journal of Systematic Bacteriology* **28**, 453-463.

- GRIMONT, P. A. D., GRIMONT, F. & STARR, M. P. (1979). *Serratia ficaria* sp. nov., a bacterial species associated with smyrna figs and the fig wasp *blastophaga psenes*. *Current Microbiology* **2**, 277–282.
- STEIGERWALT, A. G., FANNING RICHARD, G., FIFE-ASBURY, M. A. & BRENNER, D. J. (1976). DNA relatedness among species of *Enterobacter* and *Serratia*. *Canadian Journal of Microbiology* **22**, 121–137.