Distinctive Electrophoretic and Isoelectric Focusing Patterns of Esterases from *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

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Esterases of 53 strains of *Yersinia enterocolitica sensu stricto*, including five previously defined biotypes, and 30 strains of *Yersinia pseudotuberculosis* were analysed by horizontal polyacrylamide-agarose gel electrophoresis and by isoelectrofocusing in thin-layer polyacrylamide gel. Esterase bands were defined by their range of activity towards several synthetic substrates, their resistance to heat and to di-isopropyl fluorophosphate. The two species were characterized by distinct electrophoretic patterns of their esterases. The apparent molecular weights of the heat-resistant esterase of *Y. enterocolitica* and of the major heat-resistant esterase of *Y. pseudotuberculosis*, as determined by polyacrylamide gradient gel electrophoresis, were estimated to be 52000 and 250000, respectively. On the basis of electrophoretic mobilities and isoelectric points of esterases produced by strains of *Y. enterocolitica*, five principal zymotypes were observed: two for strains of biotype 1, two for strains of biotypes 2 and 3, respectively, and only one for strains of both biotypes 4 and 5. The zymotypes of strains of biotypes 2, 3, 4 and 5 appeared to be more closely related to one another than to zymotypes of strains of biotype 1. Variations in number or mobility of bands observed within each biotype of *Y. enterocolitica* and within some serotypes of *Y. pseudotuberculosis* could represent an additional marker for epidemiological analysis.

INTRODUCTION

Yersinia enterocolitica and *Yersinia pseudotuberculosis*, causative agents of diverse human and animal diseases, are distinguishable from one another by both conventional biochemical tests and DNA hybridization (Brenner *et al.*, 1976). Strains of *Y. pseudotuberculosis* were found to be homogeneous in DNA relatedness and relatively uniform in biochemical tests, whereas *Y. enterocolitica* strains were found to be genetically and biochemically heterogeneous (Brenner *et al.*, 1976; Harvey & Pickett, 1980; Kaneko & Hashimoto, 1982).

Bercovier *et al.* (1980*a*) have defined the species *Y. enterocolitica sensu stricto* (SS) which contains five major biotypes based on reactions for lipase, DNAase, Indole, D-xylose, sucrose, D-trehalose and NO_3^- reduction to NO_2^- . This species which forms one DNA relatedness group (Brenner *et al.*, 1980*a*) is distinct from the newly described species *Y. intermedia* (Brenner *et al.*, 1980*b*), *Y. frederiksenii* (Ursing *et al.*, 1980) and *Y. kristensenii* (Bercovier *et al.*, 1980*b*).

The present work, which extends previous research on enterobacterial esterases (see Goullet, 1980*a, b*, 1981), proposes a new phenotypic approach for both specific and intra-specific differentiation of *Y. enterocolitica* (SS) and *Y. pseudotuberculosis* by electrophoretic and isoelectrofocusing patterns of their esterases.

Abbreviation: DFP, di-isopropyl fluorophosphate.

METHODS

Bacterial strains. The names and sources of the test strains are given in Table 1. Cultures were incubated at 28 °C.

Growth conditions, preparation of extracts, protein estimation, horizontal slab polyacrylamide-agarose gel electrophoresis, estimation of electrophoretic mobility (M_F value), polyacrylamide gradient gel electrophoresis and esterase staining. These have all been described previously (Goullet, 1980a).

Enzymic tests and substrates. The substrates used were α - and β -naphthyl acetates, α - and β -naphthyl butyrates and indoxyl acetate. Heat inactivation and inhibition by di-isopropyl fluorophosphate (DFP) were as previously described (Goullet, 1980a).

Isoelectric focusing. Thin-layer isoelectric focusing on polyacrylamide gel (Vesterberg & Svensson, 1966) was performed in Pharmacia flat bed electrophoresis apparatus FBE 3000, maintained at 4 °C by a circulating-water bath (LKB 2209 Multitemps). Ampholine LKB of pH range 3.5 to 10, Pharmalyte of pH range 4 to 6.5 and Pharmalyte of pH range 5 to 8 were used.

Before application of the cell extracts, gels were pre-electrofocussed for 30 min at a constant power of 25 W (Pharmacia electrophoresis constant power supply ECPS 2000/300). Samples at a protein concentration of 50 to 60 mg ml⁻¹ were absorbed into a strip (20 × 2 mm) of filter paper (Whatman no. 3) which was applied to the gel, 10 mm from the cathode.

Focusing was conducted for 2 h. The sample application paper was removed after 30 min. The gel was stained for esterase activity by the same technique as for electrophoresis. For determination of the pH gradient, pieces of gel 4 mm long and 10 mm wide were cut from the gel and eluted in 0.4 ml 10 mM-KCl solution; pH was measured at 20 °C and a pH curve was obtained for each gel by plotting the pH value against the distance measured from the edge of the gel. The pI of esterase was determined by interpolation from this curve.

Table 1. *Strains examined**Yersinia enterocolitica*

Code	Strain†	Biotype‡	Serotype§	Phage typing	Source
1*	96 (a)	1	O:4	X _Z	Chinchilla, Denmark
2*	106 (a)	1	O:7	X _Z	Guinea pig, Denmark
3*	124 (a)	1	O:5	X _Z	Colt, France
4*	161 (a)	1	O:8	X _Z	Human, USA
5	846 (a)	1	O:18	X _O	Human, USA
6*	1646 (a)	1	O:6	X _Z	Human, Czechoslovakia
7*	3419 (a)	1	O:8	X _Z	Human, Canada
8*	3865 (a)	1	O:8	X _Z	Human, Canada
9*	9020 (a)	1	O:5	X _Z	Raw milk, France
10*	9023 (a)	1	O:10	X _Z	Raw milk, France
11*	10389 (a)	1	O:6	X _Z	Human, Italy
12*	10414 (a)	1	O:27	X _Z	Human, Belgium
13*	10649 (a)	1	O:4	X _Z	Human, France
14*	10660 (a)	1	O:7.8	X _O	Human, France
15	2.75 (b)	1	O:6	X _Z	Rodent, France
16*	211 (a)	2	O:9	X ₃	Human, Belgium
17*	336 (a)	2	O:9	X ₃	Human, Sweden
18*	359 (a)	2	O:9	X ₃	Human, Belgium
19	373 (a)	2	O:9	X ₃	Human, Finland
20*	825 (a)	2	O:9	X ₃	Human, Finland
21*	827 (a)	2	O:9	X ₃	Human, Finland
22*	8385 (a)	2	O:9	X ₃	Human, France
23*	10594 (a)	2	O:9	X ₃	Pig, India
24*	10658 (a)	2	O:9	X ₃	Human, France
25*	10688 (a)	2	O:9	X ₃	Human, France
26*	39 (a)	3	O:1 2a 3	II	Chinchilla, Netherlands
27*	8830 (a)	3	O:5	X _Z	Human, France
28*	8928 (a)	3	O:5	X _Z	Human, France
29*	8942 (a)	3	O:5	X _Z	Human, France
30*	8969 (a)	3	O:5	X _Z	Human, France
31*	8970 (a)	3	O:5	X _Z	Human, France
32*	10556 (a)	3	O:5	X _Z	Human, France
33*	10672 (a)	3	O:5	X _Z	Pig, Italy

Table 1. (continued)

34*	374 (a)	4	O:3	IXb	Human, Montreal, Canada
35*	577 (a)	4	O:3	VIII	Human, Czechoslovakia
36*	774 (a)	4	O:3	VIII	Human, Belgium
37*	888 (a)	4	O:3	9b	Human, Canada
38*	1615 (a)	4	O:3	9a	Pig, Johannesburg, South Africa
39*	4975 (a)	4	O:3	IXa	Human, Hungary
40*	8998 (a)	4	O:3	VIII	Human, Spain
41*	9018 (a)	4	O:4	VIII	Human, Barcelona, Spain
42*	10674 (a)	4	O:3	VIII	Human, France
43*	10707 (a)	4	O:3	VIII	Human, Poitiers, France
44	2.77 (b)	4	O:3	VIII	Human, Bordeaux, France
45*	1 (a)	5	O:2a2b3	I	Hare, France
46*	27 (a)	5	O:2a2b3	II	Hare, France
47*	37 (a)	5	O:2a2b3	XI	Hare, France
48*	178 (a)	5	O:2a2b3	II	Hare, England
49	425 (a)	5	O:2a2b3	XLc	Hare, France
50*	489 (a)	5	O:2a2b3	I	Hare, France
51*	2883 (a)	5	O:2a2b3	XI	Goat, Switzerland
52*	2893 (a)	5	O:2a2b3	XI	Goat, Switzerland
53	3662 (a)	5	O:2a2b3	XI	Hare, Hungary

Yersinia pseudotuberculosis

Code	Strain†	Serotype¶	Source
1	2550 (a)	1	Blood, Paris, France
2	2552 (a)	1	Mesenteric ganglion, Grenoble, France
3*	2555 (a)	1	Blood, Saint-Etienne, France
4	2556 (a)	1	Blood, Libourne, France
5	2558 (a)	1	Bovine foetus, Limoges, France
6	2559 (a)	1	Bovine foetus, Limoges, France
7	2565 (a)	1	Mesenteric ganglion, Lyon, France
8	2568 (a)	1	Wild rodent, Bréscia, Italy
9*	2619 (a)	1	Human, France
10	2623 (a)	1	Human, New Zealand
11	A120 (c)	1	Mesenteric ganglion, Bretonneau strain (M. Piechaud) CIP
12	55.85 (c)	1	Strain 14 of E. Thal, (G. Girard) CIP
13	2.70 (b)	1	Blood, La Rochelle, France
14	2.74 (b)	1	Blood, Bordeaux, France
15	14.74 (b)	1	Ascite, Vannes, France
16*	2553 (a)	2	Blood, Tours, France
17*	2554 (a)	2	Blood, Landes, France
18	2596 (a)	2	Rabbit, St Brieux, France
19*	2613 (a)	2	Hare, Sarthe, France
20	55.86 (c)	2	Strain 16 of E. Thal (G. Girard) CIP
21	2544 (a)	3	Pig, Johannesburg, South Africa
22	2549 (a)	3	Blood, St Cloud, France
23*	2578 (a)	3	Pig, Italy
24	1830 (a)	4	Human, England
25	1833 (a)	4	Sheep, England
26	2509 (a)	4	Human, Spain
27*	2463 (a)	5	Guinea-pig, Lausanne, Switzerland
28*	2475 (a)	5	Guinea-pig, Lausanne, Switzerland
29	1553 (a)	6	Guinea-pig, Japan
30	1554 (a)	6	Guinea-pig, Japan

* Strain analysed by isoelectrofocusing.

† (a) Collection of Centre National et Centre Collaborateur O.M.S. pour les *Yersinia* (Professeur H. H. Mollaret), Institut Pasteur, Paris, France; (b) Collection of Service des Enterobacteries (Professeur Le Minor), Institut Pasteur, Paris; CIP, Collection de l'Institut Pasteur, Paris.

‡ According to the biotyping scheme of Bercovier *et al.* (1980a) which derives from the biotyping scheme of Wauters (1970) with slight modifications.

§ According to Wauters *et al.* (1971).

¶ According to Nicolle *et al.* (1967).

¶ According to Thal (1954).

RESULTS

Acrylamide-agarose gel electrophoresis

Esterase zymograms obtained by polyacrylamide-agarose gel electrophoresis gave distinctive and reproducible banding patterns for both *Y. enterocolitica* and *Y. pseudotuberculosis* strains. Electrophoretic relationships were established between the strains by multiple runs, comparing esterase bands side by side on the same gel. The M_F values were used only comparatively.

Yersinia enterocolitica. The greatest numbers of esterases were detected with β -naphthyl acetate as substrate. Five bands, designated as E_1 , E_2 , E_3 , E_4 and E_5 in order of decreasing mobility towards the anode, and one band (E_6) migrating to the cathode, were defined (Fig. 1*a*). Band E_1 reacted with β -naphthyl acetate. Band E_2 was stained by α -naphthyl and β -naphthyl acetates and included two anodic satellite components. Band E_3 hydrolysed the five substrates. Band E_4 hydrolysed α - and β -naphthyl esters and reacted slightly with indoxyl acetate. Band E_5 hydrolysed strongly α - and β -naphthyl acetates and, to a lesser extent, other substrates. Band E_4 remained stable after incubation for 10 min at 60 °C, whereas band E_5 was partly inactivated under the same conditions. Band E_6 hydrolysed β -naphthyl acetate and butyrate and reacted slightly with other substrates. Two other bands E_{aa} and E_{ab} (Fig 1*b, c*) were detected by α -naphthyl acetate and reacted slightly with indoxyl acetate. Band E_{ab} was weakly stained by β -naphthyl acetate. Bands E_2 , E_4 and E_{aa} were resistant to 10^{-3} M-DFP, whereas bands E_1 , E_3 , E_5 , E_6 and E_{ab} were inhibited at this concentration.

Fig. 1. Esterase bands of *Y. enterocolitica* revealed by horizontal acrylamide-agarose gel electrophoresis using 7% (w/v) acrylamide. (a) Esterases of strain 1 (biotype 1) visualized by β -naphthyl acetate. (b) Esterases of strain 48 (biotype 5) visualized by β -naphthyl acetate. (c) Esterases of strain 48 visualized by α -naphthyl acetate.

Fig. 2. Biotype 1 of *Y. enterocolitica*. Diagrammatic representation of electrophoretic esterase patterns of 15 strains (numbered as in Table 1). , E₁ band; , E₂ band; , E₃ band; , E₄ band; , E₅ band; , E₆ band; , E_{aa} band.

Figures 2, 3 and 4 show the electrophoretic distribution of esterases in the five biotypes. Bands E₁, E₂ and E₄ were identified in all biotypes whereas bands E₃ and E₅ were identified in biotype 1 only. Band E₇ of strains of biotypes 2 and 3 showed hydrolysing activity towards synthetic substrates similar to that observed for band E₆ of the other biotypes, but migrated to the anode. All strains of biotype 1 (Fig. 2) produced bands E₁, E₂, E₃, E₄, E₅ and E₆ with the exception of strain 5 for bands E₃ and E₅ and of strain 7 for band E₆. Bands E₂ and E₄ showed electrophoretic uniformity among the strains, whereas bands E₁, E₃ and E₅ displayed two types of mobility. Variations in mobilities of bands E₃ and E₅ defined two principal groups of strains: group A for strains 1, 4, 5, 7, 11, 12 and 14 and group B for strains 2, 3, 6, 8, 9, 10, 13 and 15. Band E_{aa} was detected in strains 3, 4, 5, 7 and 10 only. Strains of biotypes 2 and 3 (Fig. 3) showed identical mobilities for bands E₁, E₄ and E₇, respectively. In these two biotypes band E₁ was faint. Band E₂ of strains 28 to 33 (biotype 3) migrated slower than the corresponding band of other strains. Band E_{aa} was produced by all strains of biotype 3. This band overlapped with band E_{ab}, produced by strains 28 to 33. Separation and characterization of bands were then achieved by using 6 or 8% (w/v) acrylamide (instead of 7%) and by noting differences in DFP inhibition. Some additional bands were observed in biotype 2; band E_a revealed by α -naphthyl acetate in strains 17 to 19; band E_b revealed by β -naphthyl acetate in strains 16, 19 to 21, 24 and 25; and band E_c detected by α -naphthyl butyrate in some strains of biotype 3 (not shown). Strains of biotypes 4 and 5 (Fig. 4) showed very similar patterns in mobilities of bands. However for strains of biotype 4, (except strain 42) the band E₁ was faint or not detected whereas for strains of biotype 5 (except strain 49) the band E₁ was well stained. In these two biotypes, band E₆ migrated towards the cathode faster than the corresponding band of biotype 1, with the exception of strain 53 (biotype 5); in some strains, band E₂ was not detected.

Yersinia pseudotuberculosis. Five esterase bands, detected with β -naphthyl acetate and designated as P₁, P_{2a}, P_{2b}, P₃ and P₄ in order of decreasing mobilities, were defined (Fig. 5a).

Fig. 3

Fig. 4

Fig. 3. Biotypes 2 and 3 of *Y. enterocolitica*. Diagrammatic representation of electrophoretic esterase patterns of 18 strains. Key as in legend to Fig. 2, with the additions: —, E_1 faint band; ▨, E_7 band; □, E_{ab} band; —, E_a band; ▤, E_b band.

Fig. 4. Biotypes 4 and 5 of *Y. enterocolitica*. Diagrammatic representation of esterase patterns of 20 strains. Key as in legend to Figs 2 and 3.

Band P_1 was faint and reacted only with β -naphthyl acetate. Bands P_{2a} and P_{2b} hydrolysed β -naphthyl acetate and, slightly, α -naphthyl acetate and β -naphthyl butyrate. Band P_3 was active towards β -naphthyl esters. Band P_4 was prominent, hydrolysing strongly α - and β -naphthyl acetates and to a lesser extent other substrates. The bands P_3 and P_4 remained active after 10 min at 60 °C. In addition, one fast-migrating band, P_{3a} (Fig. 5b), was detected with α -naphthyl acetate and indoxyl acetate. The esterases P_1 , P_3 and P_4 were inhibited by 10^{-4} M-DFP, whereas esterases P_{2a} and P_{2b} were resistant to 10^{-3} M-DFP. Figure 6 shows the electrophoretic distribution of esterases in strains of different serotypes. Individual mobilities were found to be identical among the strains for bands P_{3a} , P_3 and P_4 , respectively. Band P_{3a} was not recovered in strains 22 and 24. Band P_4 was not recovered in strain 3 (serotype 1) and in strains 27 and 28 (serotype 5). Variations in mobility appeared only for band P_1 of strains 29 and 30 (serotype 6) and for bands P_{2a} and P_{2b} of strain 16 (serotype 3).

Isoelectric focusing

Strains indicated in Table 1 by asterisks were also analysed by isoelectrofocusing. A broad pH gradient (3.5 to 10) was initially employed but the two narrow pH gradients (4 to 6.5 and 5 to 8) were subsequently preferred because they gave higher resolution of esterase bands. The better results were obtained with α -naphthyl acetate as substrate. Esterase bands of *Y. enterocolitica* strains resolved by isoelectric focusing had pI values ranging from 4.9 to 6.7 (Fig. 7). Esterase E_2 was visualized at pH 4.9 for strains of biotype 1 but was not recovered for strains of other biotypes. Esterases E_3 and E_5 (biotype 1) showed two distinct isoelectric focusing patterns: one

Fig. 5. Esterase bands of *Y. pseudotuberculosis* revealed by horizontal acrylamide-agarose gel electrophoresis using 7% (w/v) acrylamide (strain 20). (a) Esterases visualized by β -naphthyl acetate. (b) Esterases visualized by α -naphthyl acetate.

for strains of group A with the higher pI values, another for strains of group B with the lower pI values. Esterase E_4 focused at pH 6.4 for strains of biotype 1 and at pH 6.6 for strains of other biotypes. Esterase E_7 showed the same pI at 6.7 among the strains of biotypes 2 and 3. Esterase E_{aa} focused uniformly at pH 5 for strains of biotypes 3, 4 and 5, whereas esterase E_{ab} focused at pH 5.25 for strains of biotype 3 and at pH 5.4 for strains of biotypes 4 and 5. Anodic band E_1 and cathodic band E_6 were not resolved by isoelectric focusing. However, using this technique two esterase bands, designated as E_{F1} and E_{F2} , produced by strains of biotype 2, were seen at pH 4.9 and 5.2, respectively whereas these two bands were undetected by conventional polyacrylamide-agarose gel electrophoresis.

For strains of *Y. pseudotuberculosis*, one major band and one minor band were visualized at pH 7 and pH 5, respectively. The major band was identified as esterase P_4 and the minor band as esterase P_{aa} . This latter band was diffuse, while esterase E_{aa} of *Y. enterocolitica* focused as a sharp band. No detectable variation of esterase pI among the strains of *Y. pseudotuberculosis* was observed.

Polyacrylamide gradient gel electrophoresis

Esterase E_4 produced by all strains of the five biotypes of *Y. enterocolitica* and esterase P_4 produced by *Y. pseudotuberculosis* strains, two kinds of esterase resistant to heat, were investigated by polyacrylamide gradient gel electrophoresis for 500, 1000, 1500, 2000 and 2500 Vh. Figure 8 shows that asymptotic migration necessary to determine molecular weight (Rodbard & Chrambach, 1971) was obtained with both *Y. pseudotuberculosis* and *Y. enterocolitica*. Using human serum albumin and *Escherichia coli* alkaline phosphatase as standard protein, the apparent molecular weights of esterases E_4 and P_4 were estimated to be approximately 52000 and 250000, respectively.

Fig. 6

Fig. 6. Diagrammatic representation of electrophoretic esterase patterns of 30 strains of *Y. pseudotuberculosis* (numbered as in Table 1). $\square$, P_1 band; $\boxtimes$, P_2 band; $\blacksquare$, P_3 band; ▬ , P_4 band; ▨ , P_{aa} band.

Fig. 7

Fig. 7. Diagrammatic representation of isoelectric focusing in acrylamide gel (see Methods) of strains of *Y. enterocolitica* and *Y. pseudotuberculosis*. 1A, biotype 1, strains of group A; 1B, biotype 1, strains of group B; 2, strains of biotype 2; 3, strains of biotype 3; 4, strains of biotype 4; 5, strains of biotype 5; P, strains of *Y. pseudotuberculosis*. Key as in legends to Figs 2, 3 and 6, with the addition: ▨ , E_{F1} and E_{F2} bands.

Fig. 8. Plots of mobilities of esterase E_4 from *Y. enterocolitica* (strain 1) and esterase P_4 from *Y. pseudotuberculosis* (strain 20) against time of electrophoresis in a polyacrylamide gradient gel. $\bullet$, Esterase E_4 ; $\blacksquare$, esterase P_4 .

DISCUSSION

Polyacrylamide-agarose gel electrophoresis and thin-layer isoelectrofocusing demonstrated that strains of *Y. enterocolitica* and *Y. pseudotuberculosis* were characterized by distinct esterase banding patterns and that esterase data may be sufficient to identify the species of the 83 *Yersinia* strains analysed. This differentiation is supported by the molecular sieving effect in gradient gel electrophoresis which revealed that esterase E₄ from *Y. enterocolitica* and esterase P₄ from *Y. pseudotuberculosis* were very distinct in molecular weight. The distinctive esterase patterns of these organisms reflect their taxonomic distance as observed in DNA relationships (Brenner *et al.*, 1976) and correlate with the specific differences in protein electrophoretograms (Hudson & Quan, 1975), whereas only small interspecies differences were detected in cellular fatty acid composition (Jantzen & Lassen, 1980).

On the basis of comparative distribution of electrophoretic bands and isoelectric points of esterases produced by strains of *Y. enterocolitica*, five principal zymotypes may be distinguished: zymotypes A and B for strains of biotype 1, zymotypes C and D for strains of biotypes 2 and 3, respectively, and zymotype E for strains of both biotypes 4 and 5 (Table 2). Zymotypes A and B were distinguishable from the other zymotypes by the presence of bands E₃ and E₅ and by differences in mobility of band E₂. Zymotypes C, D and E appeared similar in terms of the E₁ and E₄ band mobilities and in terms of E₄ band pI. Strains of biotype 2 differed from strains of biotype 3 by the absence of bands E_{aa} and E_{ab} and by the presence of two esterase bands detected by isoelectric focusing and not recovered by electrophoresis. Strains of biotypes 2 and 3 differed from strains of biotypes 4 and 5 by the presence of band E₇ and absence of band E₆. According to electrophoretic and isoelectric focusing patterns of esterases, strains of biotypes 2, 3, 4 and 5 appear to be more closely related to one another than to strains of biotype 1, as was observed with β -lactamase electrofocusing patterns (Wauters, 1981).

Variations in number or in mobility of bands observed respectively within each biotype of *Y. enterocolitica* and within some serotypes of *Y. pseudotuberculosis* could represent an additional marker for epidemiological analysis.

Table 2. *Distribution of esterase bands in the five biotypes of Y. enterocolitica*

<i>M_F</i> and pI values of esterase band	Biotype				
	1		2	3	4-5
	Zymotype A	Zymotype B	Zymotype C	Zymotype D	Zymotype E
E ₁ <i>M_F</i>	79/82	79/82	79	79	79
E ₂ <i>M_F</i>	65	65	63	60*	63
pI	4.9	4.9	—	—	—
E ₃ <i>M_F</i>	55	59	—	—	—
pI	5.35	5.15	—	—	—
E ₄ <i>M_F</i>	48	48	46	46	46
pI	6.4	6.4	6.6	6.6	6.6
E ₅ <i>M_F</i>	20	22	—	—	—
pI	6	5.4	—	—	—
E ₆ <i>M_F</i>	—7	—7	—	—	—21†
E ₇ <i>M_F</i>	—	—	16	16	—
pI	—	—	6.7	6.7	—
E _{aa} <i>M_F</i>	d‡	d‡	—	69	69
pI	—	—	—	5	5
E _{ab} <i>M_F</i>	—	—	—	69	65
pI	—	—	—	5.25	5.4
E _{F1} pI	—	—	4.9	—	—
E _{F2} pI	—	—	5.2	—	—

* *M_F* approx. 63 for strains 26 and 27

† *M_F* approx. —5 for strain 53

‡ Band showing variations in mobility among the strains.

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