Comparative Electrophoretic Polymorphism of Esterases and Other Enzymes in *Escherichia coli*

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The electrophoretic polymorphism of esterases was compared with that of other enzymes in *Escherichia coli* populations by investigating allozyme distribution of four esterases (A, B, C and I) within both the subspecific groups I, II and III and the new groups A, B1, B2, C, D and E, which have been distinguished by electrophoretic analysis of 11 and 35 enzymes respectively in the 72 reference strains of the ECOR collection. Electrophoretic distribution of esterases was distinct for each of the three subspecific groups as indicated by distributions of allozymes and electrophoretic types (distinctive combination of allozyme for the four esterases). Esterase polymorphisms of the three subspecific groups appeared to have similar features to those of three previously studied natural populations of strains obtained from human and animal gastrointestinal tracts and extra-intestinal infections in humans. Multiple correspondence analyses using data obtained from the four esterases and the 11 other enzymes also distinguished the groups A, B1, B2, C, D and E. All strains of group B2 showed the B₂ electrophoretic pattern of esterase B, which appeared to be a marker of a distinct cluster of strains frequently implicated in extra-intestinal infections.

INTRODUCTION

Four kinds of electrophoretically variable esterases, designated A, B, C and I, have been characterized in *Escherichia coli* by their distinct spectra of activity on synthetic substrates and by their sensitivity or resistance to di-isopropyl fluorophosphate (Goullet, 1973, 1980). A survey of the electrophoretic polymorphism of these esterases revealed three associations of allozymes in natural *E. coli* populations obtained from three distinct sources [animal intestinal strains (AIS), human intestinal strains (HIS) and human extra-intestinal infection strains (HES)]. The first association, characterized by slow electrophoretic mobilities (M_F values) of esterase B (from $M_F \simeq 57$ to $M_F \simeq 63$), was frequently observed in HES. The second, characterized by the fast mobilities of esterases A and B ($M_F \simeq 81$ and $M_F \simeq 72$ respectively), was frequently found in AIS. The third, characterized by prominence of the most common mobilities of esterases A and B ($M_F \simeq 75$ and $M_F \simeq 70$ respectively), was found in all three populations (Goullet & Picard, 1986*a*).

Ochman & Selander (1984) have assembled a set of 72 *E. coli* reference (ECOR) strains isolated from a variety of hosts and geographical locations. These strains are representative of the genotypic diversity in the species and are available to investigators concerned with the analysis of variation and genetic structure in natural populations. These strains, which were initially characterized by electrophoresis of 11 enzymes other than esterases and distributed according to the subspecific groups I, II, III (Ochman *et al.*, 1983; Whittam *et al.*, 1983) were

Abbreviations: AIS, animal intestinal strains; E_i , electrophoretic type; GDC, genetic diversity coefficient; HES, human extra-intestinal infection strains; HIS, human intestinal strains; IEF, isoelectrofocusing; MCA, multiple correspondence analysis; M_F , electrophoretic mobility; MRHA, mannose resistant haemagglutinin.

recently characterized by electrophoresis of 35 enzymes and redistributed into six new groups designated A, B1, B2, C, D and E (Selander *et al.*, 1987).

In the present work, we analysed electrophoretic variations of esterases A, B, C and I in the ECOR collection to investigate their allozyme distribution within both the three subspecific groups and the six new groups of strains. Esterase polymorphism of the ECOR strains was also compared with that of the three previously surveyed populations (AIS, HIS and HES).

METHODS

Bacterial strains. The 72 strains of the ECOR collection were provided by R. K. Selander. Details of the sources of the strains were indicated by Ochman & Selander (1984). All isolates were cultured in Fernbach flasks containing 500 ml L broth without glucose and, concomitantly for some strains, in minimal salt medium M-63 (Pardee *et al.*, 1959) supplemented with 2 g glycerol l⁻¹. The flasks were shaken vigorously for 18 h at 37 °C in a reciprocating water-bath shaker set at about 70 oscillations min⁻¹.

Electrophoretic analysis. The preparation of extracts, protein estimation, horizontal slab polyacrylamide agarose gel electrophoresis (7%, w/v, acrylamide; Tris/glycine buffer, pH 8.6), estimation of M_F , isoelectrofocusing (IEF) and esterase staining have been described previously (Goullet & Picard, 1985). Each M_F variant was designated as an allozyme (electromorph). When an esterase was not detected in a strain, it was recorded as 'null allozyme' (and scored 0). Each distinctive combination of allozymes for the four varieties of esterases was designated as an electrophoretic type (E_i).

α -Haemolysin and mannose resistant haemagglutinin (MRHA) assays. These were done as previously described (Goullet & Picard, 1986b).

Statistical analyses. Multiple correspondence analyses (MCA) (Greenacre, 1984; Lebart *et al.*, 1984; Tenenhaus & Young, 1985) were done using the SPAD.N program from Centre International de Statistique et d'Informatique Appliquées, Sèvres, France.

Genetic diversity. Genetic diversity coefficient (GDC) values were calculated according to Nei (1978). $GDC(H) = 1 - \sum x_i^2 [n/(n-1)]$ where x_i is the frequency of each allozyme and n is the number of strains in the samples.

RESULTS

Electrophoretic types and pI of esterase B

Among the 72 strains, 49 electrophoretic types (distinct combination of allozymes for esterases B, A, C and I: E_{iBACI}) were characterized (Table 1). They were 16, 20 and 14 E_{iBACI} for subspecific groups I, II and III (Ochman *et al.*, 1983) respectively. Strain 38 of subspecific group II and strain 70 of subspecific group III showed the same E_{iBACI} but were distinguished by the pI value of esterase B. In five other cases, strains having an identical E_{iBACI} could be distinguished by the pI of esterase B (strains 30, 39 and 40; strains 58 and 72; strains 56 and 57; strains 51, 52 and 54; and strains 63, 64 and 67). Differentiation between (i) strains 32 and 33, (ii) strains 38, 40 and 41, (iii) strains 51 and 54–56 and (iv) strains 53 and 60, which was not possible

Table 1. Esterase electrophoretic data on the 72 ECOR strains

ECOR strain no.	Subspecific group characterized by electrophoresis of 11 enzymes (Ochman & Selander, 1984)	New group characterized by electrophoresis of 35 enzymes (Selander <i>et al.</i> , 1987)	Esterase electrophoretic type (E_{iBACI})				IEF
			B* (M_F)	A (M_F)	C (M_F)	I (M_F)	B (pI)
1	I	A	70	75	60	0	4.6
2	I	A	70	75	44	70	4.6
3	I	A	70	75	60	70	4.6
4	I	A	68	75	0	60	4.75
5	I	A	70	75	60	70	4.6
6	I	A	68	75	55	68	4.75
7	I	A	70	75	44	72	4.6
8	I	A	70	75	60	70	4.6
9	I	A	70	75	60	70	4.6

Table 1. (continued)

ECOR strain no.	Subspecific group characterized by electrophoresis of 11 enzymes (Ochman & Selander, 1984)	New group characterized by electrophoresis of 35 enzymes (Selander <i>et al.</i> , 1987)	Esterase electrophoretic type (E_{tBACI})				IEF B (pI)
			B* (M_F)	A (M_F)	C (M_F)	I (M_F)	
10	I	A	70	75	60	70	4.6
11	I	A	70	75	60	70	4.6
12	I	A	70	75	60	70	4.6
13	I	A	70	75	63	0	4.6
14	I	A	70	75	44	70	4.6
15	I	A	68	75	44	0	4.75
16	I	A	68	75	60	68	4.75
17	I	A	68	75	60	0	4.75
18	I	A	68	75	48	70	4.75
19	I	A	68	75	40	68	4.75
20	I	A	68	75	40	73	4.75
21	I	A	68	75	40	73	4.75
22	I	A	68	75	57	62	4.75
23	I	A	70	75	59	0	4.6
24	I	A	68	75	59	65	4.75
25	I	A	70	75	60	70	4.6
26	II	C	70	78	57	75	4.6
27	II	C	70	78	57	75	4.6
28	II	B1	72	78	57	72	4.75
29	II	B1	70	81	57	70	4.75
30	II	B1	70	75	57	70	4.75
31	II	B1	73	78	57	72	4.5
32	II	B1	72	75	57	72	4.75
33	II	B1	72	75	59	72	4.75
34	II	B1	72	81	57	70	4.75
35	II	D	70	75	62	68	4.6
36	II	D	70	75	62	68	4.6
37	II	E	70	75	0	61	4.6
38	II	D	72	75	57	70	4.75
39	II	D	70	75	57	70	4.6
40	II	D	70	75	57	70	4.6
41	II	D	73	75	57	70	4.5
42	II	B1	70	0	60	72	4.6
43	II	B1	70	75	0	70	4.5
44	II	D	70	75	55	65	4.6
45	II	B1	72	78	55	72	4.75
46	II	D	70	0	0	65	4.6
47	II	D	72	75	48	0	4.9
48	II	C	72	75	55	74	4.5
49	II	D	72	75	0	65	4.5
50	II	D	72	75	0	65	4.5
51	III	B2	57	78	60	72	5
52	III	B2	57	78	60	72	5
53	III	B2	60	75	55	70	4.85
54	III	B2	57	78	60	72	4.9
55	III	B2	57	75	60	75	4.9
56	III	B2	57	78	61	75	4.9
57	III	B2	57	78	61	75	5
58	III	C	70	78	57	70	4.75
59	III	B2	62	75	59	61	4.85
60	III	B2	60	75	61	61	4.85
61	III	B2	62	78	60	70	4.85
62	III	B2	62	78	60	70	4.85
63	III	B2	57	78	60	70	4.9
64	III	B2	57	78	60	70	5
65	III	B2	57	75	60	72	5
66	III	B1	57	78	60	0	5

Table 1. (*Continued*)

ECOR strain no.	Subspecific group characterized by electrophoresis of 11 enzymes (Ochman & Selander, 1984)	New group characterized by electrophoresis of 35 enzymes (Selander <i>et al.</i> , 1987)	Esterase electrophoretic type (E_{tBACI})				IEF B (pI)
			B* (M_F)	A (M_F)	C (M_F)	I (M_F)	
67	III	B1	57	78	60	70	5
68	III	B1	72	0	57	70	4.75
69	III	C	70	78	57	72	4.6
70	III	C	72	75	57	70	4.8
71	III	B1	72	0	57	70	4.75
72	III	C	70	78	57	70	4.6

* Electrophoretic mobilities corresponding to pattern B₂ are shown in **bold** type.

Table 2. *Electrophoretic distribution of esterases A, B, C and I in groups I, II and III*

Values are the number of strains with each allozyme; particularly significant values are in **bold** type. GI, II, III, groups I, II and III; Na, null allozyme.

	Esterase A			Esterase B			Esterase C			Esterase I		
	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII
Na		2	2				1	5		5	1	1
40							3					
44							4					
48							1	1				
55							1	3	1			
57						11	1	12	6			
59							2	1	1			
60						2	11	1	11	1		
61									3		1	2
62						3		2		1		
63							1					
65										1	4	
68				11						3	2	
69												
70				14	13	3				11	8	11
72					10	3				1	6	5
73					2					2		
74											1	
75	25	16	6								2	3
78		5	14									
81		2										

using electrophoresis of 35 enzymes (Selander *et al.*, 1987) was obtained by electrophoresis of the four esterases. For strain 54 this differentiation was only confirmed by a distinct pI value.

Distribution of esterase allozymes in the three subspecific groups (Table 2)

For esterase A, allozyme A-75 was detected in all strains of group I and in the majority of strains of group II; allozyme A-78 was prevalent in strains of group III. For esterase B, allozyme B-68 was observed only in strains of group I; allozyme B-70 was more frequent in groups I and II; allozymes B-72 and B-73 were detected principally in group II strains. Allozymes B-57, B-60 and B-62 were detected only in group III. For esterase C, allozyme C-57 was prevalent in strains of group II and allozyme C-60 in strains of groups I and III; allozymes C-40 and C-44 were observed only in group I strains. For esterase I, allozyme I-70 was prevalent in all three groups.

Fig. 1. MCA of the ECOR strains using the four esterases. Plot of score of strains for the first two principal factors: □, group I; ▲, group II; ●, group III.

Genetic diversity

The GDCs (Nei, 1978, Selander *et al.*, 1986) determined in the 72 strains were as follows: 0.497 for esterase A; 0.742 for esterase B; 0.804 for esterase C; and 0.771 for esterase I. Mean GDCs of the 72 strains and of each of the three subspecific groups were higher for the esterases (for the 72 strains, 0.703; group I, 0.485; group II, 0.636; and group III, 0.621) than for the other enzymes (for the 72 strains, 0.465; group I, 0.239; group II, 0.332; and group III, 0.322) (Ochman & Selander, 1984).

Multiple correspondence analyses

MCA using data for the four esterases. The first two factors accounted for 16.8% of the total variance. Projections of different allozymes on the first two axes revealed that the first factor separated allozymes B-57, B-62, A-78, C-61, C-60 and I-75 from allozymes B-68, C-40 and I-73, whereas allozymes B-72, A-81, A-0, C-57 and C-55 were distinguished by the second factor. Projections of the 72 ECOR strains according to the four esterases correlated well with their allocation to the three subspecific groups defined by Ochman & Selander (1984) (Fig. 1): all strains of group I were clustered; among the strains of group II, only two strains (35 and 36) were recovered with the strains of group I and two strains (26 and 27) with those of group III; for the strains of group III, two strains (53 and 59) were recovered with strains of group I and six (strains 58 and 68 to 72) with those of group II.

MCA using both allozymes of the four esterases and the 11 other enzymes. The first two factors accounted for 14.4% of the total variance. Projections of different allozymes on the first two axes revealed that the first factor separated allozymes B-57, C-61, I-75, 6PG3, PEZ-2 and A-78 from allozymes G6P-1, 6PG-19, C-40 and B-68; the second factor distinguished between allozymes MDH-5, PGI-9, IDH-3, GOT-2, B-72, A-81 and C-55. Projections of the 72 ECOR strains on the basis of the 15 enzymes distinguished four groups (Fig. 2): groups A and B1 were distinguished along the first axis; groups B2 and D were separated by the second axis, whereas strains of group C failed to cluster. The dendrogram obtained from this analysis clearly classified the majority of strains to the six new groups defined by Selander *et al.* (1987) (Fig. 3).

Fig. 2. MCA of the ECOR strains using allozymes of the four esterases and of 11 other enzymes. Plot of score of strains for the first two principal factors: $\square$, group A; $\blacksquare$, group B1; $\bullet$, group B2; $\circ$, group C; $\blacktriangle$, group D; $\triangle$, group E.

Two-dimensional plotting of electrophoretic data for esterase B

A two-dimensional representation of the electrophoretic data (Goullet & Picard, 1985) was obtained by plotting the isoelectric points against electrophoretic mobility for esterase B detected in the 72 ECOR strains and, for comparison, in the collection of 610 strains constituted by AIS, HIS and HES. Of the 13 variants of esterase B defined by their pI and M_F values in ECOR strains (Table 1), 12 belonged to the 41 variants previously described (Picard *et al.*, 1987) while a new variant (pI 4.9/ $M_F \approx 72$) was detected in strain 47. Strains of groups A, B1, C, D and E were characterized by electrophoretic pattern B₁ (from $M_F \approx 66$ to $M_F \approx 74$ and from pI 4.5 to pI 4.9) (Goullet & Picard, 1986*b*) (with the exception of strains 66 and 67 of group B1). All strains of group B2 were distributed in pattern B₂ (from $M_F \approx 57$ to $M_F \approx 66$ and from pI 4.8 to pI 5). The most frequent variant, (pI 4.6/ $M_F \approx 70$), was detected in the three subspecific groups and in new groups A, B1, C, D and E. Strains of group A were distributed into only two variants, the major variant and variant pI 4.75/ $M_F \approx 68$, which were previously considered to be related by a single amino acid substitution (an acidic amino acid replaced by a neutral amino acid) (Picard *et al.*, 1987). Strains of groups B1, C and D were considerably more heterogeneous than strains of group A and displayed variants ranging from $M_F \approx 57$ to $M_F \approx 73$ and from pI 4.5 to pI 5. Four strains of group C and six of group D showed the major variant. Only one strain of the two groups had variant pI 4.75/ $M_F \approx 72$, whereas six of the 13 strains of group B1 had this variant (which we consider to be separated from the major variant by at least two successive mutations: Picard *et al.*, 1987). Of the 16 strains of pattern B₂, six produced an α -haemolysin and eight a MRHA (four produced both virulence factors). In contrast, of the 56 strains showing pattern B₁, no strain produced an α -haemolysin and only three a MRHA.

DISCUSSION

As was observed in our previous analysis of AIS, HIS and HES (Goullet & Picard, 1986*a*), esterases are highly polymorphic, since the number of electrophoretic types (49) defined in the ECOR collection by electrophoretic analysis of the four esterases is similar to that (48) defined by analysis of the 11 other enzymes (Ochman & Selander, 1984). In addition, the findings obtained by the combination of conventional electrophoresis and IEF of esterases were complementary for strain differentiation to those obtained by electrophoresis of 35 enzymes,

Fig. 3. Dendrogram representation of the data obtained from the whole MCA of the ECOR strains using allozymes of the four esterases and of 11 other enzymes.

with 69 electrophoretic profiles finally being distinguished. Thus, differentiation between *E. coli* strains increased with both the diversity of enzymes examined and the diversity of electrophoretic criteria used.

MCA using both the 72 ECOR strains and the collection of 610 strains for electrophoretic variations of esterases showed that the ECOR strains displayed a wide distribution throughout the plane of the first two factors and consequently that the two collections of strains were analogous in terms of esterase electrophoretic polymorphism. However, only one ECOR strain, which was identified by the negative value of factor 1, exhibited allozymes B-72 and A-81,

whereas this association of allozymes was frequently observed in the collection of 610 strains, especially in 8.5% of the 211 AIS. Although allozymes of the four esterases produced by ECOR strains were detected earlier (with the exception of allozyme C-40), only 19 of the 49 E_{tBACI} revealed in the present study were observed among the 316 E_{tBACI} previously characterized (Goullet & Picard, 1986a).

Electrophoretic distribution of esterases was distinct for each of the three subspecific groups, as indicated by the distributions of electrophoretic types (Table 1) and allozymes (Table 2). Esterase polymorphism in groups I, II and III appeared to have similar features to that in HIS, AIS and HES. Strains of group I resembled HIS by the prevalence of the most common mobilities of esterase A ($M_F \approx 75$) and of esterase B ($M_F \approx 70$), by the relatively high proportion of allozymes B-68 and C-60 and by the high frequency of the association of allozymes B-70, A-75 and C-60 (displayed by nine ECOR strains and by 12% of the 142 HIS). Group I was electrophoretically more homogeneous than groups II and III both for the esterases and for the 11 other enzymes (see mean GDC values and Table 1). This homogeneity was substantiated by the occurrence of only two variants of esterase B. These were related to each other by a single amino acid substitution. The most frequent variant (pI 4.6/ $M_F \approx 70$) was also detected in the two other subspecific groups and in the new groups A, B₁, C, D and E. Its wide distribution in AIS, HIS and in HES, in *Shigella sonnei* (Goullet & Picard, 1987) and in the ECOR strains, which are representative of the genetic diversity in the species as a whole (Ochman & Selander, 1984), suggests that this major variant remained stable throughout the process of evolutionary differentiation within the *E. coli* genospecies.

Strains of group II resembled AIS by their higher proportion of allozymes B-72 and C-57, their lower proportion of allozyme C-60 and the presence of allozyme A-81. Thus, the ratio between the number of strains having allozyme C-57 and the number of strains having allozyme C-60 (C-57/C-60) was higher for group II (12) than for either group III (0.54) or group I (0.09). An analysis of 35 enzymes resulted in the distribution of 21 strains of group II into groups B₁ and D (Selander *et al.*, 1987). This was also found by MCA using a combination of allozymes of esterases and of 11 other enzymes (Figs 2 and 3). Five of the six strains of group B₁ having the variant pI 4.75/ $M_F \approx 72$ were of animal origin. This observation was corroborated by data obtained in previous work (Picard *et al.*, 1987) in which this variant was detected in 10.9% of AIS and but in only 2.8% of HIS and 0.39% of HES.

Strains of group III resembled HES by their relatively high proportions of allozymes B-57, A-78 and E_{tBAC} (seven ECOR strains and 12.5% of HES contained B-57, A-78 and C-60). Of the 22 strains in this group, 16 showed slow mobilities of esterase B corresponding to the electrophoretic pattern B₂ (see Results and Table 1), whereas six exhibited esterase mobilities corresponding to the electrophoretic pattern B₁ (Goullet & Picard, 1986b). Fourteen strains corresponding to pattern B₂ were clearly distinguished by MCA (Fig. 1). As previously observed, the strains with this latter pattern possessed α -haemolysin and MRHA more frequently than did all the strains with pattern B₁. Analysis of esterases corroborated the distinction of the 22 strains of group III which resulted from the analysis of 35 enzymes. Among the 16 strains having pattern B₂, 14 constitute group B₂, and two (strains 66 and 67) are in group B₁. Five of the six strains of pattern B₁ belonged to group C. The fact that no structural relationship could be drawn in terms of amino acid substitution between variants of pattern B₁ and variants of pattern B₂ (Picard *et al.*, 1987) and that group B₂ was exclusively composed of strains having pattern B₂ indicate that these strains, which are frequently implicated in extra-intestinal infections (Goullet & Picard, 1986b), correspond to a taxonomically distinct cluster within *E. coli*.

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