Comparative Esterase Electrophoretic Polymorphism of *Escherichia coli* Isolates Obtained from Animal and Human Sources

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To determine whether enzyme electrophoretic polymorphism in *Escherichia coli* populations was influenced by environmental background, the mobilities of four electrophoretically variable esterases (A, B, C and I) were examined. The distinction between isolates was established by significant differences in the electrophoretic distribution and the genetic diversity coefficient of individual esterases. Principal components analysis on each population and on all strains revealed three groups of allozymes. The first, characterized by slow electrophoretic mobilities of esterase B, was frequently observed in strains obtained from human extra-intestinal infections and rarely in commensal organisms. The second, characterized by fast mobilities of esterases A and B, was frequently found in animal isolates. The third, characterized by prominence of the most common mobilities of esterases B and A, was recovered in all populations. These results were confirmed by discriminant analysis. Among the 610 strains investigated, 316 electrophoretic types (distinctive combinations of allozymes of the four varieties of esterases) were distinguished, illustrating high esterase polymorphism.

INTRODUCTION

Escherichia coli is a common member of the intestinal flora of warm-blooded vertebrates. Its widespread distribution in vertebrates may explain its high degree of DNA heterogeneity (Brenner *et al.*, 1969, 1972; Brenner & Falkow, 1971), and the extent of its enzyme electrophoretic polymorphism (Milkman, 1973; Selander & Levin, 1980; Ochman *et al.*, 1983; Whittam *et al.*, 1983). For ecological and medical reasons, it is relevant to determine whether enzyme electrophoretic polymorphism may be influenced by the nature of the host and whether it may be used to distinguish pathogenic from commensal strains.

Electrophoretic analyses of natural populations of diploid organisms have shown that esterases, which hydrolyse multiple substrates, are often more variable than enzymes which utilize single substrates (Gillespie & Langley, 1974). In haploid organisms, esterases show electrophoretic diversity, giving reliable information for both specific and subspecific differentiation of enterobacteria (Goullet, 1981; Goullet & Picard, 1984*b*). Among *E. coli* strains studied by us, four varieties of esterases, designated as A, B, C and I, appeared to be electrophoretically variable (Goullet, 1973, 1980; Goullet & Picard, 1984*a*). These enzymes, which hydrolyse β -naphthyl acetate, were easily differentiated from each other by variation of their hydrolytic activity on α -naphthyl acetate and α - and β -naphthyl butyrates, and in their sensitivity to heat and to di-isopropyl fluorophosphate.

The aim of this work was to evaluate the influence of the environment on esterase polymorphism of *E. coli* by comparing electrophoretic mobilities of esterases A, B, C and I in isolates obtained from three distinct sources: animal and human gastro-intestinal tracts and extra-intestinal infections in humans.

Abbreviations: AIS, animal intestinal strains; E_i , electrophoretic type; GDC, genetic diversity coefficient; HES, human extra-intestinal infection strains; HIS, human intestinal strains; M_F , electrophoretic mobility; WE_i , weighted electrophoretic type.

Table 1. *Source of the bacterial strains*

	Group	No. of strains	Recovered from:	Geographical source (France)
AIS*	1†	102	Stools from horse, cow, calf, pig, goat, sheep, rabbit, chicken	Acheres La Forêt (Seine et Marne); Fontainebleau (town) (Seine et Marne); Braine (Aisne); Marcy (Loir et Cher); Mereau (Cher)
	2	45	Stools from cow, sheep, rabbit, pig, chicken, rat	Experimental farm, Commentry (Allier)
	3	64	Stools from deer, roebuck, rabbit, hare, fox	Forest of Fontainebleau (Seine et Marne)
HIS*	4†	85	Stools from healthy man	Paris
	5	31	Stools from healthy man	Paris
	6	26	Stools from non-infected patient	Beaujon hospital, Clichy
HES*	7†	66	Extra-intestinal infections‡	Durance hospital, Avignon
	8†	81	Extra-intestinal infections‡	St Antoine hospital, Paris
	9†	44	Urinary tract infection	Clinical biology laboratory, Paris
	10	66	Extra-intestinal infections‡	Beaujon hospital, Clichy

* AIS, animal intestinal strains; HIS, human intestinal strains; HES, human extra-intestinal infection strains.

† Random collection.

‡ Urinary tract infections, genital infections, lung infections, deep abscess, cholecystitis, septicaemia.

METHODS

Bacterial strains. The sources of the strains are shown in Table 1. All isolates were cultured in Fernbach flasks containing 500 ml L broth (Lennox, 1955) without glucose. 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 PAGE (7%, w/v, acrylamide; Tris/glycine buffer pH 8.6), estimation of electrophoretic mobility (M_F) and esterase staining have been described previously (Goullet & Picard, 1985).

Each M_F variant was designated as an allozyme. 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). In principal components analysis, the weighted electrophoretic type (WE_i) designated the number of strains showing the same electrophoretic type.

Statistical analyses. Principal components analysis, discriminant analysis and analysis of two-way frequency tables (χ^2 test) were done using the Biomedical Programs of the University of California (Dixon, 1981). The electrophoretic data were converted into binary code by considering each allozyme as a variable, occurring in either of two states, i.e. present = 1 or absent = 0.

RESULTS AND DISCUSSION

The electrophoretic relationships between strains were established by multiple runs, comparing esterase bands side by side on the same gel. The M_F values were used only comparatively. A total of 59 allozymes were detected (including null allozyme): 7, 13, 18 and 21 for esterases A, B, C and I, respectively.

Distribution of esterase mobilities

Esterase A showed six allozymes ranging from $M_F \approx 71$ to $M_F \approx 83$ (Table 2), of which 3 occurred most frequently, i.e. those with $M_F \approx 75$ (designated A-75), $M_F \approx 78$ (A-78) and $M_F \approx 81$ (A-81). These included 48.2%, 29% and 13% of the strains, respectively. For each of these three allozymes, the proportion of organisms varied according to their origin. Thus, 68.3% of human intestinal strains (HIS) had the allozyme A-75, 37.7% of strains derived from human extra-intestinal infections (HES) had A-78 and 25.6% of animal intestinal strains (AIS) had A-81. Allozyme A-75 was the most frequently observed among the three populations. The ratio between this allozyme and allozymes A-78 and A-81 served to distinguish the three populations.

Table 2. *Electrophoretic distribution of esterases A, B, C and I in the three populations*

The values given are the percentage of strains with each allozyme; particularly significant values are shown in **bold type**. NA, Null allozyme; AIS, animal intestinal strains; HIS, human intestinal strains; HES, human extra-intestinal infection strains.

M_F	Esterase A			Esterase B			Esterase C			Esterase I		
	AIS	HIS	HES	AIS	HIS	HES	AIS	HIS	HES	AIS	HIS	HES
44							2.8					
45												
46							2.8	2.1				
47							0.5					
48							11.8	14.1	4.7			
49												
50							1.4	2.1	0.8			
51												
52							1.4	2.8	0.8			
53							3.3	6.3	5.4			0.4
54								5.6	0.4			
55							43.1	8.5	12.1			0.4
56							0.5		0.4			
57				1.4	2.8	29.2	13.7	7.0	8.6	0.5		0.8
58											0.7	0.4
59							3.8	6.3	2.3	0.5		0.8
60				0.5	5.6	9.7	8.1	21.8	28.8		9.9	5.4
61							0.5	0.7	0.8			0.8
62					1.4	4.7	0.5	2.1	4.7	1.4	2.8	5.1
63						0.4			0.8	1.9	4.2	1.6
64										0.9		0.4
65										0.5		0.8
66				2.4	0.7	1.9			0.8	2.8	4.9	
67						0.4						1.2
68				11.4	16.2	4.7				11.8	8.5	8.9
69												
70				58.3	61.3	33.1				38.9	23.2	31.1
71			1.2									
72			0.8	11.4	3.5	9.7				10.9	6.3	5.4
73				1.9	2.8	2.3				4.3	4.2	4.3
74				5.7	1.4	0.4					2.1	2.7
75	37.4	68.3	45.9	0.5						1.4	5.6	3.1
76												
77												
78	28.0	14.8	37.7									2.7
79												
80												
81	25.6	8.5	5.1									
82												
83	2.4		0.4									
NA	6.6	8.5	8.9	6.6	4.2	3.5	5.7	20.4	28.8	24.2	27.5	19.8

Thus, the ratio A-81/A-75 was higher for animal strains (0.68) than for human strains (0.12 for HIS and 0.11 for HES), whereas the ratio A-78/A-75 was higher for HES (0.82) than for HIS (0.21). Five strains originating from wild animals and one HES had an unusual mobility ($M_F \approx 83$) of esterase A.

Esterase B, which was recently purified and designated as carboxylesterase B (Goullet *et al.*, 1984), showed 12 allozymes, divided among two groups (Table 2). (i) a Gaussian distribution was observed for 450 strains (74% of the total) with $M_F \approx 66$ to $M_F \approx 75$. The most frequently occurring allozyme was $M_F \approx 70$; in this case the number of strains producing B-70 was higher in AIS and HIS (58 and 61%) than in HES (33%). Esterase B appeared to be more symmetrically distributed (from $M_F \approx 66$ to $M_F \approx 75$) in AIS than in HIS and HES, and migrated more

Table 3. *Pearson χ^2 calculated for each esterase*

Esterase	Pearson χ^2	Degree of freedom	P-value
B	175	24	0.999
A	88	12	0.999
C	205	34	0.999
I	78	40	0.999

Table 4. *Genetic diversity (H) of esterases A, B, C and I in each of the five random groups of E. coli*

$H = 1 - \sum x_i^2$ where x_i is the frequency for each allozyme.

	A	B	C	I	Mean
AIS: group 1	0.721	0.617	0.629	0.691	0.66
HIS: group 4	0.438	0.525	0.832	0.812	0.65
HES: group 7	0.609	0.782	0.731	0.798	0.73
HES: group 8	0.618	0.771	0.801	0.819	0.75
HES: group 9	0.665	0.745	0.792	0.813	0.75

frequently ($M_F \simeq 72$ for HES and $M_F \simeq 68$ for HIS). The faster migrating allozymes of esterase B ($M_F > 72$) were obtained predominantly from AIS. (ii) There was an increasing slope from $M_F \simeq 63$ to $M_F \simeq 57$ clearly separated from the Gaussian distribution by a free interval of two M_F units. A striking result was that this increasing slope was concerned principally with HES. The proportion of strains producing B-57 were 29% for HES, 2.8% for HIS and 1.4% for AIS. In the following paper (Goullet & Picard, 1986) we describe the resolved electrophoretic distribution of carboxylesterase B in a random collection of strains of human origin using a two-dimensional electrophoretic profile (Goullet & Picard, 1985) and demonstrate that production of α -haemolysin and mannose resistant haemagglutinin were significantly higher in pathogenic strains showing slow mobilities ($M_F \simeq 63$ to $M_F \simeq 57$) of esterase B.

Esterase C showed 17 allozymes ($M_F \simeq 44$ to $M_F \simeq 66$) (Table 2). More than 51% of the strains were included in the three allozymes $M_F \simeq 48$ (9.3% of the total), $M_F \simeq 55$ (22% of the isolates) and $M_F \simeq 60$ (20% of the strains). For each of these three allozymes, the distribution of the organisms varied with the origin. Thus, 43% of AIS had allozyme C-55, 14% of HIS had C-48 and 28.8% had C-60. A higher percentage of null allozyme was observed for HES.

Esterase I showed 20 allozymes (Table 2), divided as follows. (i) There was a Gaussian distribution from $M_F \simeq 65$ to $M_F \simeq 78$, including 392 strains of diverse origin but with 70.6% AIS. These had a frequent allozyme $M_F \simeq 70$. (ii) There was an increasing slope from $M_F \simeq 64$ to $M_F \simeq 60$. Here allozyme I-60 was recovered exclusively in human strains, and principally in HIS.

Pearson χ^2 test. For each of the four esterases, a table of frequency of the various allozymes for each populations (AIS, HIS and HES) was analysed. The Pearson χ^2 value calculated from each contingency table appeared to be highly significant at $P = 0.999$ (Table 3).

Genetic diversity in random groups

Genetic diversity coefficients (GDCs) (Nei, 1975; Selander & Levin, 1980) determined for the five random groups of organisms (Table 1) have been included in Table 4. Mean GDCs of AIS (group 1, corresponding to six mammalian species and one bird species) and of HIS (group 4) were very similar: 0.66 and 0.65, respectively. These values were lower than those of the HES groups 7, 8, 9, i.e. 0.73, 0.75 and 0.75. The individual GDC of each esterase was distinct from AIS to HIS. The similarity between the mean GDCs of AIS and of HIS was explained by the fact that increasing values of GDCs of esterases A and B from HIS to AIS were compensated by decreasing values of GDCs of esterases C and I. The GDCs correlated with the electrophoretic distribution (Table 2). Thus, the higher GDCs of esterases A and B in AIS reflected the higher

Principal components analysis

Animal intestinal strains. The first two principal components accounted for 9% of the total variance. Projections of different allozymes (Fig. 1a) on the first two axes revealed that the first component opposed allozymes A-81, C-55 and B-72 to allozyme A-75, and that allozymes I-73, A-83 and B-70 were distinguished by the second component. Projections of *WE_t* (Fig. 1b) distinguished one group (I) displayed by component 2 with high mobility of esterase A consisting of electrophoretic types recovered from wild animal strains, and another group (II) opposed to other strains by component 1 and exhibiting allozyme B-72.

Strains derived from human extra-intestinal infections. The first two principal components accounted for 8.6% of the total variance. Projection of different allozymes on the first two axes showed that component 1 opposed allozymes A-78, B-57 and C-60 to allozymes A-75 and B-70.

Fig. 2. Principal components analysis of the HIS. (a) Plot of score of allozymes for the first two principal components: symbols as in legend to Fig. 1. (b) ▽, Plot of score of weighted electrophoretic types for the first two principal components. For clarity, some of the allozymes from the various esterases which were projected near the origin of the axes have been omitted from the Figure.

Fig. 3. Principal components analysis of the 610 *E. coli* strains. (a) Plot of score of allozymes for the first two principal components: symbols as in legend to Fig. 1. (b) Plot of score of weighted electrophoretic types for the first two principal components: △, AIS; ▽, HIS; ▼, HES. For clarity, some of the allozymes from the various esterases which were projected near the origin of the axes have been omitted from the Figure.

This confirmed the electrophoretic distribution of esterase B (Table 2) which showed the clear-cut division into slow and fast mobilities. Weighted electrophoretic types were projected in a circle centred on the origin of the axes.

The 610 strains of E. coli. The first two principal components accounted for 8.1% of the total variance. Projections of different allozymes (Fig. 3a) on the first two axes revealed three major groups: group I_e, consisting of allozymes B-72, A-81 and C-55, opposed to group II_e, with allozymes B-70, A-75 and C-48, by component 1; and group III_e, with allozymes B-57, A-78,

Table 5. Numbers of strains recovered in the most frequently observed electrophoretic types distinguished within the three populations of *E. coli*

Population of <i>E. coli</i>	Allozyme B	E_{tBA}	E_{tBAC}
AIS: 211 (100%)	B-70: 123 (58.3%)	A-75: 53 (25%)	C-57: 14 (6.6%)
		A-78: 37 (17.5%)	C-55: 22 (10.5%)
		A-81: 21 (9.9%)	C-55: 15 (7.1%)
		A-81: 18 (8.5%)	C-55: 17 (8.1%)
HIS: 142 (100%)	B-72: 24 (11.4%) B-70: 87 (61.3%)	A-75: 64 (45%)	C-0: 13 (9.2%)
			C-48: 13 (9.2%)
			C-60: 17 (12%)
HES: 257 (100%)	B-70: 85 (33.1%) B-57: 75 (29%)	A-75: 53 (20.6%)	C-0: 13 (5%)
		A-75: 30 (11.7%)	C-0: 13 (5%)
			C-60: 14 (5.4%)
		A-78: 42 (16.4%)	C-60: 32 (12.45%)

C-60 and C-0, distinguished by component 2. Projections of WE_t (Fig. 3*b*) distinguished three groups. One group, I_s, of weighted electrophoretic types comprised allozymes B-72, A-81 and C-55 and was opposed by component 1 to a second group, II_s, of weighted electrophoretic types which comprised allozymes B-70 and A-75 and was composed of strains of the three populations; a third group, III_s, of 104 weighted electrophoretic types, displayed by component 2, was composed of 88 HES (32% of HES), 12 HIS (the same strains distinguished by principal components analysis of HIS) and four AIS, and comprised allozymes B-57, A-78 and C-60.

From the principal components analysis of AIS, HIS, HES and 610 strains of *E. coli*, three groups of allozymes were distinguished: (i) allozyme B-72 associated with A-81 and C-55; (ii) allozymes B-57 and B-60 associated with A-78, C-60 and C-0; and (iii) allozyme B-70 associated with A-75 and C-48. These three associations corresponded to three groups of strains: the first, frequently isolated from animals, exhibited the same M_F values of esterase B and A as *Shigella flexneri* strains of serotype 6 and some strains of *Shigella boydii* (Goulet, 1980; Goulet *et al.*, 1983); the second, characterized by slow mobilities of esterase B, was largely represented in HES and rarely in HIS and AIS; the third, characterized by prominence of the most common mobilities of esterases B and A ($M_F \approx 70$ and $M_F \approx 75$, respectively), was frequently recovered in the three populations.

Electrophoretic types

Among the 610 strains, 316 distinct combinations of allozymes for the four esterases (electrophoretic types B, A, C, I: E_{tBACI}) were characterized. However, the allozymes which were found to be the most discriminating by principal components analysis were those of esterases B, A and C. As shown esterase I (Table 2) appeared to be a less discriminating enzyme for the precise origin of strains. Consequently, we have disregarded allozymes of this esterase, and examined only electrophoretic types B, A and C. We have considered within the three populations the electrophoretic types B, A, C (E_{tBAC}) which grouped individually at least 5% of strains (Table 5). Of the AIS, 32.4% were distributed in four distinct E_{tBAC} ; the electrophoretic type B-72, A-81, C-55 represented 8.1% of strains and only 1.4% and 2.8% of HES and HIS, and appeared to be an electrophoretic type more frequently recovered in animal strains. Of the AIS, 25% and 45% of the HES exhibited allozymes B-70 and A-75 but these two groups of strains were distinguished by the mobility of esterase C ($M_F \approx 57$ for AIS and $M_F \approx 48$ and 60 for HIS). Of the HES, 29% produced esterase B with $M_F \approx 57$; they were distributed in three E_{tBAC} including allozymes C-60 and C-0 exclusively, whereas these E_{tBAC} were rarely observed in AIS and HES (1.5% and 2.7%, respectively). This stereotyped distribution conferred a particular character to human pathogenic strains.

The GDCs of the four esterases, calculated according to the 316 E_{tBACI} , were 0.812 for esterase B, 0.706 for esterase A, 0.870 for esterase C and 0.848 for esterase I. The mean of these GDCs (0.81) was higher than the value (0.52) reported by Ochman *et al.* (1983) for allozymes of 12 enzymes studied in 1608 *E. coli* isolates; however, individual GDCs of esterases were similar to

Table 6. *Discriminant functions (F) used by discriminant analysis to classify the 610 observations within the three populations of E. coli*

Allozyme used	F	Selection order	Coefficient		
			HES	HIS	AIS
B-57	50.4	1	5.88	2.07	3.14
C-55	37.45	2	3.30	2.72	5.76
B-60	21.4	3	2.45	0.10	-0.92
C-57	12.87	7	3.53	2.47	4.82
I-60	11.42	5	-1.94	1.11	-1.13
B-62	9.80	4	4.49	1.57	1.24
C-54	9.51	9	2.32	6.48	2.49
C-44	8.69	10	4.38	3.97	8.72
A-75	7.15	8	3.00	4.02	3.11
B-72	6.56	11	3.36	1.70	2.28
C-48	5.69	14	2.31	2.42	3.72
I-70	5.52	12	2.51	1.72	2.56
C-46	5.39	15	2.55	3.69	5.77
A-81	5.08	13	1.06	2.07	2.29
C-0	4.96	6	3.77	2.65	3.03
B-74	4.19	16	2.12	2.32	4.22

Table 7. *Results of the classification according to discriminant analysis calculations from the 610 observations*

	Percentage of strains correctly classified	Calculated HES	Calculated HIS	Calculated AIS
HES strains (257)	59.5	153	60	44
HIS strains (142)	69.7	15	99	28
AIS strains (211)	69.7	21	43	147
Total strains		189	202	219

those of the four most polymorphic enzymes: alcohol dehydrogenase (0.729), 6-phosphogluconate dehydrogenase (0.715), β -galactosidase (0.893) and mannose phosphate isomerase (0.710).

The high polymorphism of *E. coli* esterases may be resolved further by using a two-dimensional electrophoretic profile (Goullet & Picard, 1985; Picard & Goullet, 1985).

Discriminant analysis

Using the 610 strains and the 59 allozymes, discriminant analysis was used to distinguish AIS, HES and HIS (Table 6 and 7). Sixteen allozymes were selected for discriminant functions (Table 6); the most significant of these were B-57, C-55, B-60, C-57 and I-60 (exclusively recovered in strains from human origin), and B-62. Application of the discriminant functions (corresponding to coefficients presented in Table 6) to the 610 strains enabled the correct classification of 59.5%, 69.7% and 69.7% of HES, HIS and AIS, respectively (Table 7). Whereas 40% of HES were classified within AIS and HIS, only 10% of AIS and HIS were classified in HES.

Both principal components analysis and discriminant analysis demonstrated that the three populations of *E. coli* strains examined in the present work may be distinguished by esterase electrophoretic polymorphism. The validity of these data was supported by the fact that the samples were very diverse in origin (see Table 1). It would be interesting to compare these results with those obtained with strains from other parts of the world.

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