Genetic structures of the B₂ and B₁ *Escherichia coli* strains responsible for extra-intestinal infections

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Escherichia coli strains causing human extra-intestinal infections may be divided into two groups, B₁ and B₂, according to the electrophoretic patterns of carboxylesterase B. This study compares the restriction fragment length polymorphism (RFLP) of ribosomal DNA (rDNA) for 45 B₁ strains and 45 B₂ strains to examine the genetic structure of B₂ strains and to distinguish them from B₁ strains. The isolates were chosen for diversity in their allozymes of esterases, B, A, C and I, their production of virulence factors (α -haemolysin, mannose resistant haemagglutinin and cytotoxic necrotizing factor) and certain O antigens, and their pathological and geographical origins. DNA was digested with *Hind*III and *Bam*HI restriction enzymes and analysed by Southern blotting. The resulting rDNA RFLP patterns of B₂ strains were distinct from those of the B₁ strains. Moreover, the B₂ strains appeared to be less heterogeneous than the B₁ strains. The B₂ strains gave 13 ribotypes (resulting from the combination of the rDNA RFLP patterns obtained with *Hind*III and *Bam*HI digestions) while the B₁ strains gave 32 ribotypes. Correspondence analysis of the data showed that several clusters of strains were identified in the B₂ strains by particular ribotypes, certain associations of esterase B and A electrophoretic variants, O serotypes and virulence factor production. In contrast, these parameters appeared to be unrelated in the B₁ strains, reflecting their heterogeneity. These findings, which differentiate two levels of genetic heterogeneity within *E. coli* pathogenic isolates, indicate that the B₂ strains constitute a phylogenetically distinct group within the species.

Introduction

We have previously established that *Escherichia coli* isolates from extra-intestinal infections may be divided into type B₁ (fast moving) and type B₂ (slow moving) according to the electrophoretic pattern of carboxylesterase B (Goullet & Picard, 1986*a, b*, 1990). Type B₂ strains express P-fimbriae, haemolysin and lethality in mice more frequently than type B₁ strains (Goullet & Picard, 1986*b*; Goullet *et al.*, 1986; Picard & Goullet, 1988, 1989). Moreover, B₂ strains carry chromosomal P-fimbriae and haemolysin determinants whereas B₁ strains lack these loci (Johnson *et al.*, 1991). To substantiate the

study of the genetic structure of the two groups of strains within the species, analysis of DNA regions remote from the loci encoding the virulence factors and esterase B is required.

Restriction fragment length polymorphism (RFLP) of ribosomal DNA (rDNA) (Grimont & Grimont, 1986) provides precise information on bacterial intra- and interspecies differentiation. The classification obtained with this method is in close agreement with that obtained by enzyme polymorphism in bacteria (Picard-Pasquier *et al.*, 1990; Picard *et al.*, 1991*a*; Denamur *et al.*, 1991; Wood *et al.*, 1992).

The present work was carried out to analyse the genetic structure of the B₂ and B₁ strains using rDNA polymorphism and to estimate the homogeneity of the two groups of strains by comparing these results with profiles obtained from esterase polymorphism (Goullet, 1973; Goullet & Picard, 1986*a*), O serotyping (Orskov & Orskov, 1985; Hacker *et al.*, 1983), and the production of mannose resistant haemagglutinin (MRHA),

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Abbreviations: CA, correspondence analysis; CNF1, cytotoxic necrotizing factor; Hly, α -haemolysin; *M_F*, electrophoretic mobility; MRHA, mannose resistant haemagglutinin; rDNA, ribosomal DNA; RFLP, restriction fragment length polymorphism.

Table 1. *Characteristics of the E. coli strains*

No.	Source designation	Source of isolate*	Country†	Serogroup‡	Esterase electrophoretic types§ (E_{tBACI})			
					B (M_F)	A (M_F)	C (M_F)	I (M_F)
1	SYDNEY64	U	A	O1	63	78	62	68
2	AV19	U	F	O1	63	78	00	61
3	FARMG9	U	USA	O1	62	78	60	00
4	AV1	S	F	O1	62	00	60	70
5	MELBR140	U	A	O2	63	75	60	60
6	SA85	U	F	O2	62	78	62	70
7	SA86	U	F	O2	62	78	60	70
8	MELBR7	U	A	O2	62	78	60	60
9	SA76	S	F	O2	60	78	60	70
10	AUST8	D	A	O2	57	78	61	68
11	AV4	D	F	O2	57	78	00	78
12	SA79	U	F	O2	57	75	62	75
13	AV59	U	F	O2	57	75	60	73
14	AV10	U	F	O2	57	75	60	00
15	AV90	S	F	O4	62	75	55	70
16	AV70	S	F	O4	62	75	55	61
17	SA29	U	F	O4	60	78	57	70
18	SA88	U	F	O4	60	78	55	70
19	AUST34	S	A	O4	60	78	00	60
20	VANC19	D	C	O4	60	75	57	68
21	TIM43	U	F	O4	60	75	55	70
22	TIM40	U	F	O4	60	75	54	70
23	TIM7	U	F	O4	60	75	53	00
24	SA33	U	F	O4	60	75	00	00
25	SA23	U	F	O6	57	78	60	73
26	AUST2	S	A	O6	57	78	55	00
27	AV18	D	F	O6	57	78	00	62
28	AUST6	U	A	O6	57	75	62	62
29	AUST14	U	A	O6	57	75	61	00
30	MELBR134	U	A	O6	57	75	60	65
31	AUST44	U	A	O6	57	75	57	00
32	AV13	S	F	O6	57	75	00	62
33	AV51	D	F	O6	57	72	00	60
34	AUST3	U	A	O15	57	78	44	68
35	TOR126	U	C	O18	62	00	60	00
36	JAP7	U	J	O18	60	78	00	62
37	AUST13	S	A	O18	57	78	61	70
38	SA26	D	F	O18	57	78	60	75
39	AUST51	U	A	O75	57	78	61	74
40	SA1	S	F	O75	57	78	60	78
41	MELBR8	S	A	O75	57	75	60	78
42	MELBR4	U	A	O75	57	75	60	75
43	AV65	U	F	U	60	75	62	67
44	AUST36	U	A	U	60	75	60	68
45	SA12	U	F	U	57	75	00	59
46	SA667	S	F	O1	70	81	62	70
47	AV748	U	F	O1	70	75	62	68
48	AV755	U	F	O1	70	75	00	00
49	SA641	U	F	O1	68	78	59	70
50	AV741	D	F	O1	68	00	60	70
51	SA637	S	F	O2	72	00	63	68
52	SA592	S	F	O2	70	78	57	72
53	SA604	U	F	O2	70	78	53	72
54	AUST35	U	A	O4	70	75	57	60
55	SA640	U	F	O4	70	00	57	73
56	SA595	U	F	O6	70	75	62	72
57	TIM31	U	F	O7	72	78	00	70
58	TIM30	U	F	O7	72	00	60	68
59	TIM36	U	F	O7	70	81	00	62
60	TIM27	U	F	O7	70	75	57	70
61	AV2842	S	F	O7	70	75	57	00
62	AV57	U	F	O7	70	75	00	70
63	TIM29	D	F	O7	70	75	00	66
64	TIM28	U	F	O7	68	75	50	00

Table 1. (cont.)

No.	Source designation	Source of isolate*	Country†	Serogroup‡	Esterase electrophoretic types§ (E_{iBACI})			
					B (M_F)	A (M_F)	C (M_F)	I (M_F)
65	SA697	U	F	O8	73	78	55	70
66	SA658	U	F	O8	73	78	53	70
67	SA668	S	F	O8	72	78	55	70
68	AV2845	S	F	O8	70	78	55	74
69	AV2838	U	F	O8	70	78	55	70
70	AV2815	U	F	O8	68	81	53	70
71	TIM24	U	F	O8	68	78	50	00
72	AV2814	D	F	O8	66	75	48	70
73	JAP15	U	J	O18	72	75	60	68
74	TIM971	U	F	O18	70	75	53	00
75	TIM977	U	F	U	73	78	00	70
76	AV743	S	F	U	72	78	60	70
77	AV738	U	F	U	72	78	60	00
78	SA686	D	F	U	72	78	55	73
79	AV768	U	F	U	72	78	55	68
80	AV2809	U	F	U	72	78	53	72
81	SA680	U	F	U	72	78	00	68
82	SYDNEY76	U	A	U	72	75	50	00
83	TIM975	U	F	U	72	00	00	68
84	TIM976	U	F	U	72	00	00	60
85	VANC13	D	C	U	70	78	57	67
86	AUST5	D	A	U	70	75	60	65
87	TIM980	U	F	U	70	75	00	68
88	AV765	S	F	U	70	75	60	00
89	SA685	U	F	U	70	75	00	60
90	SA605	U	F	U	68	75	57	70

* S, septicaemia; U, urinary tract infection; D, diverse extra-intestinal infection (including lung infection, surgical infection).

† A, Australia; C, Canada; F, France; J, Japan; USA, United States of America.

‡ U, unserotypable.

§ B, esterase B; A, esterase A; C, esterase C; I, esterase I.

α -haemolysin (Hly) and cytotoxic necrotizing factor type 1 (CNF1) (Caprioli *et al.*, 1983, 1987; De Rycke *et al.*, 1990).

Methods

Bacterial strains. Ninety extra-intestinal *E. coli* strains isolated from urinary tract infections, septicaemia and other extra-intestinal infections in France, the USA, Canada, Japan and Australia were studied (Goullet & Picard, 1990) (Table 1). These strains were chosen for their diversity in the production of virulence factors (MRHA, Hly and CNF1) and in pathogenic serogroup (O1, O2, O4, O6, O7, O8, O15, O18 and O75) (Le Minor & le Coueffic, 1975; Vosti, 1979; De Rycke *et al.*, 1987; Hacker *et al.*, 1983; Orskov & Orskov, 1985; Goullet *et al.*, 1993). Each strain also exhibits a distinct electrophoretic type for esterases B, A, C and I (E_{iBACI}) (Goullet & Picard, 1986a). Forty-five strains produce a type B₂ carboxylesterase B ($M_F \approx 57$ to $M_F \approx 63$) (Goullet & Picard, 1986b). A further 45 strains produce a type B₁ carboxylesterase B ($M_F \approx 68$ to $M_F \approx 72$).

rDNA RFLP analysis. Bacterial DNA preparation, gel electrophoresis of restricted DNA, Southern transfer and hybridization were

performed as described previously (Picard-Pasquier *et al.*, 1989). DNA (3 µg) was digested with *Hind*III and *Bam*HI restriction enzymes (Boehringer), analysed by electrophoresis on submarine ethidium-bromide-containing 0.8% agarose gels and transferred to Hybond-N (Amersham) nylon membranes. DNA fragment-size marker MII (Boehringer) was used. Ribosomal 16+23S RNA from *E. coli* (Boehringer) was used as a probe and labelled by random oligopriming using a mixture of hexanucleotides (Pharmacia) and a reverse transcriptase (BRL) in the presence of 0.35 mM-DIG-11-dUTP (Boehringer) (Bingen *et al.*, 1992). DNA was hybridized overnight at 65 °C according to the Amersham instructions. Filters were washed with 0.15 M-NaCl, 0.015 M-sodium citrate pH 7.3 containing 0.1% SDS. Chemiluminescence was detected as described in the protocol provided by Boehringer. Membranes were incubated with an antidigoxigenin-antibody linked to alkaline phosphatase and the alkaline phosphatase substrate AMPPD3. Autoradiographs of the filters were prepared by exposure to X-Omat film (Kodak) for 90 min at room temperature.

Statistical analysis. Data can be summarized by a two-way table of 90 rows (the strains) and 89 columns corresponding to (i) the rDNA fragment obtained after *Hind*III (H) and *Bam*HI (B) digestion, (ii) the number of allozymes detected in all strains (including the null allozyme) for each esterase, (iii) nine studied O serogroups and a column for the

Fig. 1. *E. coli* rDNA RFLP patterns. Diagram of the 31 *Hind*III restriction patterns of DNA after hybridizing to *E. coli* rDNA probe. The number of strains exhibiting each pattern is indicated.

absence of O serogroup detected, and (iv) the presence or absence of MRHA, Hly and CNF1. The value of each cell is 1 (present) or 0 (absent).

Two correspondence analyses (CA) (Lebart *et al.*, 1984; Lerman, 1981; Greenacre, 1984; Tenenhaus & Young, 1985) were conducted, one with the rDNA RFLP data obtained after *Hind*III digestion, one including all data. For the latter CA, the distances restricted to the vector subspace defined by the first six factors were used to draw a dendrogram resulting from cluster analysis. These computations were performed using a computer Data General MV10000 running with software from F. Tekaiä, Informatique Scientifique de l'Institut Pasteur, Paris, France.

CA is an eigenvector method of ordination similar to principal component analysis. It differs from the latter in the use of a covariance matrix based on X^2 distances rather than a covariance matrix based on Euclidean distances. Both techniques describe the dispersion and shape of a cloud of n objects or p variates in a multidimensional space by replacing the original data set by a new set of orthogonal linear coordinates in a space of significantly lower dimension. The explained variances of the elements of the data set are in decreasing order of magnitude with respect to these new coordinates. Furthermore, the variables used for CA are categories, whereas those used for principal component analysis are quantitative or continuous variables. For each CA the hierarchical classification was based on the first six factors of the analysis. The number corresponds to the structural and stable portions of the variance, whereas the rest of the variance is interpreted as random noise and thus is discarded in the hierarchical classification.

Results

rDNA polymorphism

*Hind*III digestion produced four to eleven rDNA fragments with sizes of 1.4 to 22.5 kb (Fig. 1). Their combination enabled 31 rDNA RFLP patterns (h_1 to h_{31}) to be distinguished within the 90 *E. coli* strains. *Bam*HI digestion produced five to eight rDNA fragments with

sizes of 6 to 22.5 kb (Fig. 2). Their combination enabled 20 rDNA RFLP patterns (b_1 to b_{20}) to be distinguished within the strains.

Comparison of rDNA polymorphism with esterase B typing

*Hind*III rDNA RFLP patterns. Projection of the strains in the plane defined by the first two principal axes of CA computed from the *Hind*III rDNA RFLP patterns indicated that the B_2 and the B_1 strains were clearly separated (Fig. 3). Only two B_1 strains (SA592 and AV755) and two B_2 strains (TOR126 and AV19), exhibiting rDNA patterns h_9 and h_{10} , were not distinguished. The B_2 strains exhibited 10 distinct *Hind*III rDNA RFLP patterns and the B_1 strains showed 23 patterns (Fig. 1). Among the B_1 strains, a particular subgroup of *Hind*III rDNA patterns h_{21} to h_{28} lacked the 22.5 and 21 kb rDNA fragments, had a particular pattern of five fragments from 13 to 6 kb, and also had 3.1 and 2.6 kb fragments in several strains.

*Bam*HI rDNA RFLP patterns. Six *Bam*HI rDNA RFLP patterns were detected in the B_2 strains and 15 patterns in the B_1 strains (Fig. 2). The B_2 rDNA patterns were distinct from the B_1 ones (with the exception of the B_1 strain SA592, which had a B_2 rDNA pattern). Most (39) of the 45 B_2 strains showed a particular rDNA pattern (b_1) with a 7.2 kb fragment. A total of 44 ribotypes were detected among the 90 strains by combining the results from *Hind*III and *Bam*HI digestion. The B_1 strains exhibited 32 ribotypes and the B_2 strains only 13.

Fig. 2. *E. coli* rDNA RFLP patterns. Diagram of the 20 *Bam*HI restriction patterns of DNA after hybridizing to *E. coli* rDNA probe. The number of strains exhibiting each pattern is indicated.

Fig. 3

Fig. 4

Fig. 3. Correspondence analysis of the 90 *E. coli* strains performed on the rDNA data obtained by digestion with *Hind*III. A plot of rDNA pattern score obtained in B₂ strains (●), in B₁ strains (○) and in B₁ and B₂ strains (◐) for factors F₁ and F₂ is shown. A particular group of B₁ rDNA patterns (see Results) is indicated.

Fig. 4. Correspondence analysis of the 90 *E. coli* strains performed on the rDNA data obtained by digestion with *Hind*III and *Bam*HI, the esterase electrophoretic data, the O serogroups and the virulence factors. A plot of scores of the B₂ strains (●) and the B₁ strains (○) for factors F₁ and F₂ (which accounted respectively for 12% and 6% of the variance) is shown. For clarity, when several strains are projected on the same point in the plane, only one is indicated. Particular groups 1, 2, 3 of the B₁ and B₂ strains (see Results) are indicated.

Statistical analysis

The first two principal axes defined by the CA including all data accounted for 18% of the total variance. Projection of rDNA fragments, esterase allozymes,

virulence factors and O serogroups on the first two axes indicated that the first axis F₁ separated the B₂ allozymes of esterase B, several rDNA fragments obtained after digestion with *Hind*III and after digestion with *Bam*HI, the production of virulence factors and serogroups O2,

Fig. 5a. For caption see facing page.

O4, O6, O18 and O75 from B₁ allozymes of esterase B, lack of virulence factor production and serogroups O7, O8 and non-typable O strains.

Projection of the strains onto plane F₁, F₂ (Fig. 4) indicated that the B₂ strains were clearly separated from the B₁ strains by the first axis F₁. The B₂ strains also appeared to be more homogeneous. However, two groups could be identified: (i) the strains having esterase B allozymes B63 and B62 and ribotypes h₆-b₁ (group 1), and (ii) the strains having serotypes O4 and O6 and ribotypes h₁-b₁ and h₂-b₁ (group 2). The two strains which did not fit the rest of the B₂ strains are strains AV19 and AUST3. Strain AV19 exhibited a *Hind*III rDNA RFLP pattern h₁₀ as a B₁ strain and was distinguished on the positive values of the second axis F₂ whereas strain AUST3 was projected near the origin of the axes. These two strains produced a single virulence factor. The B₁ strains were more heterogeneous. A third particular group (group 3) having *Hind*III rDNA profiles h₂₁ to h₂₈ and h₃₀ was identified on the negative values of axis F₂.

The dendrogram computed from the distance of strains obtained in the subspace of the CA indicated that the B₁ and the B₂ strains were clearly differentiated into two major lineages at a distance of 312 (Fig. 5). Several clusters appeared within the B₂ strains (Fig. 5a): cluster A corresponded to O1 and O2 strains exhibiting ribotype h₆-b₁ and allozymes B63, B62 and A78; cluster B corresponded to O4 strains having ribotypes h₁-b₁ and h₂-b₁ and esterase B60, esterase A78 or A75; cluster D corresponding to O6 strains having ribotypes h₁-b₁ or h₂-b₁, esterase B57 and esterase A75; cluster C corresponded to O18 strains having ribotype h₇-b₁ and esterases B57 and A78; cluster E corresponded to O75 strains exhibiting ribotypes h₂-b₁, h₄-b₁ and h₇-b₁ and esterase allozymes B67 and A78. The genetic homogeneity of the B₂ strains tended to increase with the virulence factor production. Strains producing none, one or two virulence factors showed more ribotypes than the strains having three virulence factors (Table 2). The frequency of virulence factor production varied from one cluster to another: 22 factors for the eight strains of

Fig. 5. Dendrogram prepared from the distances restricted to the vector subspace defined by the factors of the CA performed on all data. (a) Major lineage of B_2 strains; (b) major lineage of B_1 strains. For each strain (numbered as in Table 1), the O serogroup, the ribotype, the zymotype of esterases B and A and the production of virulence factors are indicated. Particular clusters A, B, C, D and E of the B_2 or B_1 strains (see Results) are indicated.

Table 2. Comparisons of the numbers of ribotypes, strains and virulence factors among the B_2 and B_1 strains of *E. coli*

r = No. of strains/no. of ribotypes.

	No factors	One factor	Two factors	Three factors
B_2	$r = 2$ (4 strains, 2 ribotypes)	$r = 1.6$ (10 strains, 6 ribotypes)	$r = 1.8$ (15 strains, 8 ribotypes)	$r = 4$ (16 strains, 4 ribotypes)
B_1	$r = 1.3$ (27 strains, 21 ribotypes)	$r = 1.3$ (14 strains, 11 ribotypes)	$r = 1$ (3 strains, 3 ribotypes)	$r = 1$ (1 strain, 1 ribotype)

cluster D, 25 for the ten strains of cluster B, 8 for the four strains of cluster E and 9 for the seven strains of cluster A. The B_1 strains were more heterogeneous (Fig. 5b).

However, cluster A corresponded to strains having ribotypes h_{21} - h_7 to h_{30} - h_7 , but exhibiting various serotypes and zymotypes.

Discussion

The *E. coli* strains responsible for extra-intestinal infections differ in several phenotypic traits from those present in the commensal intestinal flora (Caprioli *et al.*, 1983; Low *et al.*, 1984; Johnson, 1991; Evans *et al.*, 1981; Orskov & Orskov, 1985). Analyses of the genetic structure of *E. coli* populations indicate that the majority of virulent isolates represent a limited subset of the lineage found among the whole species (Achtman & Plushke, 1986). Several attempts have been made to delineate the virulent strains by multilocus enzyme electrophoresis (Selander *et al.*, 1986, 1987; Caugant *et*

al., 1983; Achtman *et al.*, 1986), or by analysing the phenotypic expression and/or genetic polymorphism of the virulence genes (Arthur *et al.*, 1990; Plos *et al.*, 1989). A group of pathogenic strains within *E. coli* is readily characterized by the electrophoretic polymorphism of a single enzyme, carboxylesterase B (Goullet & Picard, 1986*a, b*, 1990; Johnson *et al.*, 1991). The procedure is simple and easy to perform. Moreover, the strains producing pattern B₂ could be phylogenetically distinct from those producing type B₁ for two reasons. First, whereas on the basis of physicochemical considerations the molecular relationship between B₁ allozymes may be expressed in term of successive amino acid substitutions, no structural relationship between B₁ and B₂ allozymes can be drawn suggesting the occurrence of an important change in the balance of charge of the B₂ allozymes (Picard *et al.*, 1987). This difference could reflect major genetic events and consequently a significant phylogenetic distance between the strains of the two groups, as has also been seen in *Providencia alcalifaciens* (Goullet *et al.*, 1988; Picard *et al.*, 1991*a*). Second, the B₂ strains correspond to the phylogenetic group B2 delineated by allelic variation of 38 enzymes (Goullet & Picard, 1989; Herzer *et al.*, 1990; Selander *et al.*, 1987).

However, the above distinctions concern proteins, and a comparison of nucleic acid polymorphism is required to substantiate the genetic relatedness of the B₂ strains within the species. Several studies have indicated that a comparison of enzyme polymorphism and DNA polymorphism can be used to discriminate between lineages within *E. coli* (Arbeit *et al.*, 1990; Arthur *et al.*, 1990). By rDNA polymorphism, we have previously distinguished the haemolytic, haemagglutinating and lethal-for-mouse B₂ strains from the B₁ strains lacking these characteristics (Picard *et al.*, 1991*b*). The present study was carried out on a larger number of strains chosen to be representative of the whole diversity of B₂ and B₁ pathogenic isolates by their esterase polymorphism and their diversity in O serogroups and virulence factor production (see Methods). It unambiguously demonstrates that the B₂ and the B₁ strains are genotypically distinct because of the rDNA RFLP patterns obtained after digestion with *Bam*HI and *Hind*III (Figs 1–3). However, two B₁ and two B₂ strains cannot be distinguished after *Hind*III digestion and one B₁ strain (SA592) and one B₂ strain (TOR126) have the same B₂ ribotype (h₉-b₁) (Figs 1 and 2). Strain SA592 is the only strain in our panel of B₁ isolates that produces all three virulence factors. The clear delineation between the B₁ and B₂ strains confirms the fact that comparison of enzyme electrophoretic polymorphism and rDNA polymorphism can be used to differentiate between species or subspecies within several groups of bacteria: *Yersinia* (Picard-Pasquier *et al.*, 1990), *E. coli* (Arthur *et al.*, 1990; Arbeit *et al.*, 1990;

Picard *et al.*, 1991*b*), *Providencia alcalifaciens* (Picard *et al.*, 1991*a*), *Branhamella* (Denamur *et al.*, 1991) and *Neisseria meningitidis* (Woods *et al.*, 1992). Ribotyping is less discriminating than the electrophoretic typing of esterases, as 44 ribotypes were detected among the 90 distinct esterase electrophoretic types (*E*_{tBACI}). This has also been observed in *E. coli* (Arthur *et al.*, 1990; Picard *et al.*, 1991*b*) and in *Yersinia* (Picard-Pasquier *et al.*, 1990).

The present work indicates a difference in the genetic structure of B₂ and B₁ isolates. Thus, the greater homogeneity of the B₂ strains is roughly indicated by the fewer rDNA RFLP patterns obtained after *Hind*III (10) and *Bam*HI (6) digestions and fewer ribotypes (13) than in the B₁ strains (23, 15 and 32 respectively). This observation is refined by comparing the rDNA polymorphism with other characters of the strains. The dendrogram computed from the data (Fig. 5*a*) indicates several clusters of strains within the major lineage of the B₂ group. The strains of each cluster display a single or very few serogroups, ribotypes and allozymes of esterases B and A, and have distinct numbers of virulence factors. The most virulent B₂ clusters are the O4 and O6 groups (clusters B and D), where the majority of strains produce the three virulence factors. The prevalence of these two serogroups in the strains responsible for septicaemia and their frequent production of virulence factors has been reported previously (Cherifi *et al.*, 1990; Low *et al.*, 1984; O'Hanley *et al.*, 1985; Caprioli *et al.*, 1987). The present work indicates that these strains, which all have few ribotypes (h₁-b₁ and h₂-b₁), are genotypically homogeneous. The other clusters of B₂ strains, such as the O1 and O2 serogroup strains (cluster A), the O75 (cluster E) or the O18 strains (cluster C) produce fewer virulence factors. Within each cluster, esterases I and C are more polymorphic (Table 1), as previously shown in several studies of the intraspecies differentiation of *E. coli* (Cherifi *et al.*, 1990; Picard *et al.*, 1988). The concordance between rDNA restriction profiles and enzyme electrophoretic type based on the study of 13 metabolic enzymes was reported by Arthur *et al.* (1990) in uropathogenic strains, particularly in phylogenetic group IV. Interestingly, group IV strains have a particular frequency of the *pap* operon and its phenotypic expression (18 for 20 strains), which is the same in the B₂ strains (Johnson *et al.*, 1991). In contrast, the B₁ strains appeared to be more heterogeneous. A group of B₁ strains is distinguished by particular rDNA RFLP patterns after digestion by *Hind*III (cluster A) (Figs 1, 3 and 5*b*), but this group is as heterogeneous as the rest of the B₁ strains in terms of esterase polymorphism, serogroup or virulence factor production.

Comparison of the electrophoretic polymorphism of esterases and rDNA polymorphism indicated that there

are three distance groupings between the esterase allozymes and rDNA RFLP patterns. First, the variant of esterases B₁ and those of B₂, which were unreliable in terms of amino acid substitution (Picard *et al.*, 1987), correspond to two groups of genetically distinguishable strains. This correlation, which was previously demonstrated for esterase β a of *P. alcalifaciens* (Goullet *et al.*, 1988; Picard *et al.*, 1991a), indicates that the study of physicochemical relationships within the allozymes of a given bacterial enzyme could constitute a valuable phylogenetic approach. Second, within the B₂ strains, some of the allozyme variations of esterase B correspond to major alterations in the rDNA patterns. Thus, allozymes B57 are found in the O6 serogroup strains which have rDNA RFLP patterns h₁ and h₂ (cluster D, Fig. 5a), whereas allozymes B62 and B63 occur in the O1 and O2 serogroups strains which have rDNA RFLP pattern h₆ (cluster A, Fig. 5a). The electrophoretic distance between allozymes B57 and B62 could encompass several successive amino acid substitutions (Picard *et al.*, 1987) and the difference between the rDNA RFLP patterns h₁ and h₆ concerns at least four rDNA fragments (Fig. 1). Consequently, this agreement between esterase B polymorphism and rDNA polymorphism could reflect a clear and/or ancient genetic divergence between the O6 strains of cluster D and the O1/O2 strains of cluster A. Lastly, some allozyme variations of esterases B and A correspond to minor changes in the rDNA pattern. For example, ribotypes h₁-b₁ and h₂-b₁ and allozymes A75 and A78 within the strains of cluster B occur independently. The difference in the rDNA RFLP patterns of h₁ and h₂ also correspond to the presence of a single 7 kb rDNA fragment (Fig. 1). These variations in the esterase A locus, or in the rDNA regions from one strain to another of the cluster B, could reflect minor and/or recent genetic events.

The correlation between enzymes and rDNA polymorphism revealed in this study confirms that the difference in terms of size and number of fragments in the rDNA RFLP patterns is a genotypic method for evaluating the distance between strains or groups of strains (Picard-Pasquier *et al.*, 1990; Picard *et al.*, 1991). The combination of these two types of polymorphism appears to provide a valuable, precise approach to the classification of bacterial strains that is suitable for taxonomical and epidemiological studies.

References

- ACHTMAN, M. & PLUSCHKE, G. (1986). Clonal analysis of descent and virulence among selected *Escherichia coli*. *Annual Review of Microbiology* **40**, 185–210.
- ACHTMAN, M., HEUZENROEDER, M., KUSECEK, B., OCHMAN, H., CAUGANT, D., SELANDER, R. K., VAISANEN-RHEN, V., KORHONEN, T. K., STUART, S., ORSKOV, F. & ORSKOV, I. (1986). Clonal analysis of *Escherichia coli* O2:K1 isolated from diseased humans and animals. *Infection and Immunity* **51**, 268–276.
- ARBEIT, R. D., ARTHUR, M., DUNN, R., KIM, C., SELANDER, R. K. & GOLDSTEIN, R. (1990). Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages: the application of pulsed field electrophoresis to molecular epidemiology. *Journal of Infectious Diseases* **161**, 230–235.
- ARTHUR, M., ARBEIT, R. D., KIM, C., BELTRAN, P., CROWE, H., STEINBACH, S., CAMPANELLI, C., WILSON, R. A., SELANDER, R. K. & GOLDSTEIN, R. (1990). Restriction fragment length polymorphisms among uropathogenic *Escherichia coli* isolates: *pap*-related sequences compared with *rrn* operons. *Infection and Immunity* **58**, 471–479.
- BINGEN, E., DENAMUR, E., LAMBERT-ZECHOVSKY, N., AUJARD, Y., BRAHIMI, N., GESLIN, P. & ELION, J. (1992). Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. *Journal of Infectious Diseases* **165**, 569–573.
- CAPRIOLI, A., FALBO, L., RODA, G., RUGGERI, F. M. & ZONA, C. (1983). Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. *Infection and Immunity* **39**, 1300–1306.
- CAPRIOLI, A., FALBO, V., RUGGERI, F. M., BALDASSARRI, L., BISICCHIA, R., IPPOLITO, G., ROMOLI, E. & DONELLI, G. (1987). Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. *Journal of Clinical Microbiology* **25**, 146–149.
- CAUGANT, D. A., LEVIN, B. R., LIDIN-JANSON, G., WHITTAM, T. S., SVANBORG-EDEN, C. & SELANDER, R. K. (1983). Genetic diversity and relationships among strains of *Escherichia coli* in the intestine and those causing urinary tract infections. *Progress in Allergy* **33**, 203–227.
- CHERIFI, A., CONTREPOIS, M., PICARD, B., GOULLET, PH., DE RYCKE, J., FAIRBROTHER, J. & BARNOUN, J. (1990). Factors and markers of virulence in *Escherichia coli* from human septicemia. *FEMS Microbiology Letters* **70**, 279–284.
- DE RYCKE, J., GUILLOT, J. F. & BOIVIN, R. (1987). Cytotoxins in non-enterotoxigenic strains of *Escherichia coli* isolated from feces of diarrheic calves. *Veterinary Microbiology* **15**, 137–150.
- DE RYCKE, J., GONZALEZ, E. A., BLANCO, J., OSWALD, E., BLANCO, M. & BOIVIN, R. (1990). Evidence for two types of cytotoxic necrotizing factor in human and animal clinical isolates of *Escherichia coli*. *Journal of Clinical Microbiology* **28**, 694–699.
- DENAMUR, E., PICARD-PASQUIER, N., MURA, C., PICARD, B., ORFILA, J. & KRISHNAMOORTHY, R. (1991). Comparison of molecular epidemiological tools for *Branhamella catarrhalis* typing. *Research in Microbiology* **142**, 585–589.
- EVANS, D. J., EVANS, D. G., HOHNE, C., NOBLE, M. A., HALDANE, E. V., LIOR, H. & YOUNG, L. S. (1981). Hemolysin and K-antigens in relation to serotype and hemagglutination type of *Escherichia coli* isolated from extraintestinal infections. *Journal of Clinical Microbiology* **13**, 171–178.
- GOULLET, PH. (1973). An esterase zymogram of *Escherichia coli*. *Journal of General Microbiology* **77**, 27–35.
- GOULLET, PH. & PICARD, B. (1986a). Comparative esterase electrophoretic polymorphism of *Escherichia coli* isolates obtained from animal and human sources. *Journal of General Microbiology* **132**, 1843–1851.
- GOULLET, PH. & PICARD, B. (1986b). Highly pathogenic strains of *Escherichia coli* revealed by the distinct electrophoretic pattern of carboxylesterase B. *Journal of General Microbiology* **132**, 1853–1858.
- GOULLET, PH. & PICARD, B. (1989). Comparative electrophoretic polymorphism of esterases and other enzymes in *Escherichia coli*. *Journal of General Microbiology* **135**, 135–143.
- GOULLET, PH. & PICARD, B. (1990). Electrophoretic type B₂ of carboxylesterase B for characterisation of highly pathogenic *Escherichia coli* strains from extra-intestinal infections. *Journal of Medical Microbiology* **33**, 11–16.
- GOULLET, PH., PICARD, B. & SEVALI GARCIA, J. (1986). Electrophoretic mobility of an esterase from *Escherichia coli* isolated from extra-intestinal infections. *Journal of Infectious Diseases* **154**, 727–728.
- GOULLET, PH., PICARD, B. & KRISHNAMOORTHY, R. (1988). An evaluation of allozyme amino-acid substitutions for the study of

- molecular relationships in *Providencia*. *Annales de l'Institut Pasteur/Microbiology* **139**, 689–702.
- GOULLET, PH., PICARD, B., CONTREPOIS, M., DE RYCKE, J. & BARNOUN, J. (1993). Correlation between esterase electrophoretic polymorphism and virulence-associated traits in extra-intestinal invasive strains of *Escherichia coli*. *Epidemiology and Infection* (in the Press).
- GREENACRE, M. J. (1984). *Theory and Applications of Correspondence Analysis*. London: Academic Press.
- GRIMONT, F. & GRIMONT, P. A. D. (1986). Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Annales de l'Institut Pasteur/Microbiologie* **137B**, 165–175.
- HACKER, J., SCHROTER, G., SCHRETTENBRUNNER, A., HUGHES, C. & GOEBEL, W. (1983). Hemolytic *Escherichia coli* strains in the human fecal flora as potential urinary pathogens. *Zeitschrift für Bakteriologie, Mikrobiologie und Hygiene* **254**, 370–378.
- HERZER, P. J., INOUE, S., INOUE, M. & WHITTAM, T. (1990). Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. *Journal of Bacteriology* **172**, 6175–6181.
- JOHNSON, J. R. (1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clinical Microbiology Reviews* **4**, 80–128.
- JOHNSON, J. R., GOULLET, PH., PICARD, B., MOSELEY, S. L., ROBERTS, P. L. & STAMM, W. E. (1991). Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. *Infection and Immunity* **59**, 2311–2315.
- LE MINOR, S. & LE COUEFFIC, E. (1975). Étude sur les hémolysines des *Enterobacteriaceae*. *Annales de Microbiologie* **126**, 313–332.
- LEBART, L., MORINEAU, A. & WARWICK, K. M. (1984). Multivariate descriptive analysis: correspondence analysis and related techniques for large matrices. New York: Wiley-Interscience.
- LERMAN, I. C. (1981). *Classification et Analyse ordinale des Données*. Paris: Dunod.
- LOW, D., DAVID, V., LARK, D., SCHOOLNIK, G. & FALKOW, S. (1984). Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotypes O4 and O6 isolated from urinary tract infections. *Infection and Immunity* **43**, 353–358.
- O'HANLEY, P., LOW, D., ROMERO, I., LARK, K., VOSTI, K., FALKOW, S. & SCHOOLNIK, G. (1985). Gal–Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. *New England Journal of Medicine* **7**, 414–420.
- ORSKOV, I. & ORSKOV, F. (1985). *Escherichia coli* in extra-intestinal infections. *Journal of Hygiene* **95**, 551–575.
- PICARD, B. & GOULLET, PH. (1988). Correlation between electrophoretic types B₁ and B₂ of carboxylesterase B and host-dependent factors in *Escherichia coli* septicaemia. *Epidemiology and Infection* **100**, 51–61.
- PICARD, B. & GOULLET, PH. (1989). Correlation between electrophoretic types B₁ and B₂ of carboxylesterase B and sex of patients in *Escherichia coli* urinary tract infections. *Epidemiology and Infection* **103**, 97–103.
- PICARD, B., GOULLET, PH. & KRISHNAMOORTHY, R. (1987). A novel approach to study of the structural basis of enzyme polymorphism. Analysis of carboxylesterase B of *Escherichia coli* as model. *Biochemical Journal* **241**, 877–881.
- PICARD, B., GOULLET, PH. & CONTREPOIS, M. (1988). Clonal distribution of septicaemic *Escherichia coli* O78 isolates evidenced by enzyme electrophoretic polymorphism. *Annales de l'Institut Pasteur/Microbiologie* **139**, 239–242.
- PICARD, B., PICARD-PASQUIER, N., KRISHNAMOORTHY, R. & GOULLET, PH. (1991a). Correlation between DNA polymorphism and enzyme polymorphism argues in favour of the delineation of two species within *Providencia alcalifaciens*. *Research in Microbiology* **142**, 965–969.
- PICARD, B., PICARD-PASQUIER, N., KRISHNAMOORTHY, R. & GOULLET, PH. (1991b). Characterization of highly virulent *Escherichia coli* strains by ribosomal DNA restriction fragment length polymorphism. *FEMS Microbiology Letters* **82**, 183–188.
- PICARD-PASQUIER, N., OUAGHED, M., PICARD, B., GOULLET, PH. & KRISHNAMOORTHY, R. (1989). A simple sensitive method for analysing bacterial ribosomal DNA polymorphism. *Electrophoresis* **10**, 186–189.
- PICARD-PASQUIER, N., PICARD, B., HEERALAL, S., KRISHNAMOORTHY, R. & GOULLET, PH. (1990). Correlation between ribosomal DNA polymorphism and electrophoretic enzyme polymorphism in *Yersinia*. *Journal of General Microbiology* **136**, 1655–1666.
- PLOS, K., HULL, S. I., HULL, R. A., LEVIN, B. R., ORSKOV, I., ORSKOV, F. & SVANBORG-EDEN, C. (1989). Distribution of the P-associated-pilus (pap) region among *Escherichia coli* from natural sources: evidence for horizontal gene transfer. *Infection and Immunity* **57**, 1604–1611.
- SELANDER, R. K., KORHOENEN, T. K., VAISANEN-RHEN, V., WILLIAMS, P. H., PATTISON, P. E. & CAUGANT, D. A. (1986). Genetic relationships and clonal structure of strains of *Escherichia coli* causing neonatal septicemia and meningitis. *Infection and Immunity* **52**, 213–222.
- SELANDER, R. K., CAUGANT, D. A. & WHITTAM, T. S. (1987). Genetic structure and variation in natural populations of *Escherichia coli*. In *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology*, vol. 2, pp. 1625–1648. Edited by J. L. Ingraham and others. Washington DC: American Society for Microbiology.
- TENENHAUS, M. & YOUNG, F. W. (1985). An analysis and synthesis of multiple correspondence analysis, optimal scaling, dual scaling, homogeneity analysis and other methods for quantifying categorical multivariate data. *Psychometrika* **50**, 91–119.
- VOSTI, K. L. (1979). Relationship of hemagglutination to other biological properties of serologically classified isolates of *Escherichia coli*. *Infection and Immunity* **25**, 507–512.
- WOODS, T. C., HELSEL, L. O., SWAMINATHAN, B., BIBB, W. F., PINNER, R. W., GELLIN, B. G., COLLIN, S. F., WATERMAN, S. H., REEVES, M. W., BRENNER, D. J. & BROOME, C. V. (1992). Characterization of *Neisseria meningitidis* serogroup C by multilocus enzyme electrophoresis and ribosomal DNA restriction profiles (ribotyping). *Journal of Clinical Microbiology* **30**, 132–137.