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Correlation between esterase electrophoretic polymorphism and virulence-associated traits in extra-intestinal invasive strains of *Escherichia coli*

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SUMMARY

The electrophoretic variations of carboxylesterase B and of esterases A, C and I, the presence of mannose resistant haemagglutinin, α-haemolysin, cytotoxic necrotizing factor type 1 (CNF1) and certain O antigens were compared in 150 strains of *Escherichia coli* responsible for extra-intestinal infections. Electrophoretic mobilities of outer membrane proteins (OMP) were also studied for strains belonging to O4, O6, O7, O8 and O75 serogroups. Fast migrating allozymes of carboxylesterase B (pattern B_1) were correlated with slow migrating allozymes of esterase C, serogroups O7 and O8, lack of virulence factor, and particular OMP patterns, whereas slow migrating allozymes of carboxylesterase B (pattern B_2) were correlated with fast migrating allozymes of esterase C, serogroups O2, O4, O6, O18 and O75, virulence factor production, and distinct OMP patterns. Allozymes of esterases A and I were not clearly correlated with the distribution of virulence factors. The pattern B_2 was more strongly associated with CNF1 than with α-haemolysin and mannose resistant haemagglutinin. These results substantiate the view that the electrophoretic pattern B_2 of carboxylesterase B identified most of the highly pathogenic strains implicated in extra-intestinal infection of humans.

INTRODUCTION

Many studies have established that strains of *Escherichia coli* which cause extra-intestinal infections (EII) in humans possess several phenotypic traits which were rarely found in strains isolated from normal intestinal flora. Four of these traits,

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Table 1. *Characteristics of the strains*

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozyme§ B (M_F)	A (M_F)	C (M_F)	I (M_F)	Virulence factor‖ MRHA	Hly	CNF1
B_1 strains										
VANC9	S	C	O1	72	75	60	00	–	+	–
SA667	S	F	O1	70	81	62	70	+	–	–
AV748	U	F	O1	70	75	62	68	–	–	–
VANC12	D	C	O1	70	75	48	68	+	–	–
AV755	U	F	O1	70	75	00	00	+	–	–
SA641	U	F	O1	68	78	59	70	–	–	–
AV741	D	F	O1	68	00	60	70	–	–	–
SA637	S	F	O2	72	00	63	68	+	+	–
SA592	S	F	O2	70	78	57	72	+	+	+
SA604	U	F	O2	70	78	53	72	+	–	–
AUST35	U	A	O4	70	75	57	60	–	–	–
SA640	U	F	O4	70	00	57	73	–	–	–
SA595	U	F	O6	70	75	62	72	–	–	–
TIM31	U	F	O7	72	78	00	70	+	–	–
TIM30	U	F	O7	72	00	60	68	–	–	–
TIM36	U	F	O7	70	81	00	62	–	–	–
TIM27	U	F	O7	70	75	57	70	+	–	–
MELBR1	S	A	O7	70	75	57	68	+	–	–
AV2842	S	F	O7	70	75	57	00	–	–	–
AV757	U	F	O7	70	75	00	70	+	–	–
TIM29	D	F	O7	70	75	00	66	+	–	–
TIM28	U	F	O7	68	75	50	00	–	–	–
SA697	U	F	O8	73	78	55	70	–	–	–
SA658	U	F	O8	73	78	53	70	–	–	–
SA668	S	F	O8	72	78	55	70	–	–	–
AV2845	S	F	O8	70	78	55	74	–	–	–
AV2838	U	F	O8	70	78	55	70	–	–	–
AV2815	U	F	O8	68	81	53	70	–	–	–
TIM24	U	F	O8	68	78	50	00	–	–	–
AV2814	D	F	O8	66	75	48	70	–	–	–
JAP15	U	J	O18	72	75	60	68	+	+	–
TIM971	U	F	O18	70	75	53	00	+	–	–
TIM983	U	F	NT	74	00	59	70	–	–	–
SA648	U	F	NT	73	81	00	68	–	–	–
TIM977	U	F	NT	73	78	00	70	–	+	–
SA652	U	F	NT	72	81	57	00	–	–	–
TIM982	U	F	NT	72	81	55	74	–	–	–
SA598	U	F	NT	72	81	52	73	–	–	–
AV743	S	F	NT	72	78	60	70	–	–	–
AV738	U	F	NT	72	78	60	00	–	–	–
SA686	D	F	NT	72	78	55	73	–	–	–
AV768	U	F	NT	72	78	55	68	–	–	–
AV2809	U	F	NT	72	78	53	72	–	–	–
SA680	U	F	NT	72	78	00	68	–	–	–
TIM981	U	F	NT	72	78	00	00	–	–	–
SYDNEY76	U	A	NT	72	75	50	00	–	–	–
JAPON9	D	J	NT	72	75	00	62	–	–	–
TIM975	U	F	NT	72	00	00	68	–	+	–
TIM976	U	F	NT	72	00	00	60	–	–	–
TIM22	U	F	NT	70	81	53	70	+	–	–
VANC14	U	C	NT	70	81	57	68	–	–	–

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Table 1 (*cont.*)

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozyme§ B (M_F)	A (M_F)	C (M_F)	I (M_F)	Virulence factor‖ MRHA	Hly	CNF1
AUST38	U	A	NT	70	81	57	60	−	−	−
SA678	U	F	NT	70	81	55	70	−	−	−
SA635	U	F	NT	70	81	53	70	−	−	−
AV2830	U	F	NT	70	81	55	68	−	−	−
SYDNEY62	S	A	NT	70	78	65	65	−	−	−
SYDNEY72	D	A	NT	70	78	60	66	−	−	−
VANC20	U	C	NT	70	78	57	70	−	+	−
VANC1	D	C	NT	70	78	57	68	−	−	−
VANC13	U	C	NT	70	78	57	67	+	+	−
AV751	U	F	NT	70	78	55	72	−	−	+
TIM25	U	F	NT	70	78	00	62	−	−	−
SA703	S	F	NT	70	75	62	00	−	−	−
AUST22	U	A	NT	70	75	60	74	−	−	−
AV745	U	F	NT	70	75	60	70	−	−	−
AUST5	D	A	NT	70	75	60	65	−	+	−
AV765	U	F	NT	70	75	60	00	+	−	−
VANC15	U	C	NT	70	75	59	65	−	−	−
AV2841	U	F	NT	70	75	57	62	−	−	−
AV2817	U	F	NT	70	75	55	70	−	−	−
VANC11	D	C	NT	70	75	50	60	−	−	−
AV2833	U	F	NT	70	75	48	73	−	−	−
AV2840	U	F	NT	70	75	48	70	−	−	−
SA633	U	F	NT	70	75	48	00	−	−	−
FARMG3	U	US	NT	70	75	44	59	−	−	−
TIM980	U	F	NT	70	75	00	68	+	−	−
SA662	U	F	NT	70	75	00	62	+	−	−
SA685	U	F	NT	70	75	00	60	+	−	−
SA605	U	F	NT	68	75	57	70	−	−	−
TIM968	U	F	NT	68	75	55	75	−	−	−
B_2 strains										
SYDNEY64	U	A	O1	63	78	62	68	+	−	−
AV19	U	F	O1	63	78	00	61	+	−	−
FARMG9	U	US	O1	62	78	60	00	+	−	+
AV1	S	F	O1	62	00	60	70	+	−	−
MELBR140	U	A	O2	63	75	60	60	+	−	−
SA85	U	F	O2	62	78	62	70	+	−	−
SA86	U	F	O2	62	78	60	70	+	+	−
MELBR7	U	A	O2	62	78	60	60	+	−	−
SA76	S	F	O2	60	78	60	70	+	+	−
AUST8	D	A	O2	57	78	61	68	−	+	+
AV4	D	F	O2	57	78	00	78	+	+	−
SA79	U	F	O2	57	75	62	75	+	−	−
AV59	U	F	O2	57	75	60	73	−	+	+
AV10	U	F	O2	57	75	60	00	+	+	+
AV90	S	F	O4	62	75	55	70	+	+	+
AV70	S	F	O4	62	75	55	61	−	+	+
SA29	U	F	O4	60	78	57	70	+	+	+
SA88	U	F	O4	60	78	55	70	−	−	−
AUST34	S	A	O4	60	78	00	60	+	+	+
MELBR141	D	A	O4	60	75	60	60	+	+	+
MELBR145	D	A	O4	60	75	59	59	+	+	+
VANC19	D	C	O4	60	75	57	68	+	+	+

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Table 1 (*cont.*)

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozyme§ B (M_F)	A (M_F)	C (M_F)	I (M_F)	Virulence factor‖ MRHA	Hly	CNF1
TIM43	U	F	O4	60	75	55	70	+	+	+
TIM40	U	F	O4	60	75	54	70	+	+	+
TIM7	U	F	O4	60	75	53	00	+	+	+
SA33	U	F	O4	60	75	00	00	+	−	+
SAND5	U	US	O6	60	78	60	57	+	−	+
SA23	U	F	O6	57	78	60	73	−	+	+
AUST2	S	A	O6	57	78	55	00	−	−	+
AV18	D	F	O6	57	78	00	62	+	−	+
AUST6	U	A	O6	57	75	62	62	+	+	+
AUST14	U	A	O6	57	75	61	00	+	+	+
MELBR134	U	A	O6	57	75	60	65	+	+	+
AUST44	U	A	O6	57	75	57	00	+	+	+
AV13	S	F	O6	57	75	00	62	+	+	+
AV51	D	F	O6	57	72	00	60	+	+	+
MELBR17	U	A	O15	62	75	59	65	+	−	−
AUST3	U	A	O15	57	78	44	68	−	+	−
TOR126	U	C	O18	62	00	60	00	+	−	+
JAPON7	U	J	O18	60	78	00	62	+	+	+
AUST13	S	A	O18	57	78	61	70	+	+	−
SA26	D	F	O18	57	78	60	75	+	+	−
FARMG2	U	US	O75	57	78	62	78	+	−	−
AUST51	U	A	O75	57	78	61	74	+	+	−
SA1	S	F	O75	57	78	60	78	+	−	−
MELBR8	S	A	O75	57	75	60	78	−	+	+
MELBR4	U	A	O75	57	75	60	75	+	+	+
BERK6	S	US	NT	62	81	57	00	−	−	−
SA75	S	F	NT	62	78	59	70	+	−	−
AV16	D	F	NT	62	78	00	70	−	−	−
AV60	U	F	NT	62	78	00	60	−	−	−
NEWZ95	S	NZ	NT	62	75	60	60	−	−	−
SA71	S	F	NT	62	75	59	70	+	+	−
MELBR144	U	A	NT	60	81	60	68	−	−	−
AV65	U	F	NT	60	75	62	67	−	−	−
AUST36	U	A	NT	60	75	60	68	−	−	−
JAPON16	D	J	NT	60	75	00	60	+	+	−
SA22	U	F	NT	60	75	00	58	+	−	−
NEWZ120	U	NZ	NT	57	78	62	75	−	−	−
TIM4	U	F	NT	57	78	60	72	−	+	+
SA74	U	F	NT	57	78	60	70	+	+	+
SA3	U	F	NT	57	78	60	68	+	+	−
SA34	U	F	NT	57	78	60	00	−	+	−
AV35	D	F	NT	57	78	00	72	−	−	+
SA38	U	F	NT	57	78	00	60	−	−	−
SA37	U	F	NT	57	75	62	00	+	+	+
SA44	U	F	NT	57	75	60	70	−	−	−
SA13	U	F	NT	57	75	60	62	+	+	+
SA20	U	F	NT	57	75	00	60	−	+	+
SA12	U	F	NT	57	75	00	59	−	−	−

* U, urinary tract infection; S, septicaemia; D, diverse.

† A, Australia; C, Canada; F, France; J, Japan; NZ, New Zealand; US, United States.

‡ NT, not typed with panel of sera used in this experiment.

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

‖ MRHA, mannose resistant haemagglutinin; Hly, α-haemolysin; CNF1, cytotoxic necrotysing factor; + and − indicate the presence or absence of factor.

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the production of mannose resistant haemagglutinin (MRHA), α-haemolysin (Hly), cytotoxic necrotizing factor type 1 (CNF1) [1, 2] and certain O antigens are frequently found together in pathogenic isolates. MRHA and Hly are simultaneously expressed in many strains and have been shown to be chromosomally linked [3, 4]. These two virulence factors or Hly plus CNF1 were more prevalent among strains belonging to a restricted number of serogroups [5–11].

In *E. coli*, four varieties of esterases designated A, B, C and I, differing in their ability to hydrolyse synthetic substrates and in their sensitivity to heat and to diisopropyl fluorophosphate, were found to be electrophoretically polymorphic [12, 13]. Esterase B (carboxylesterase B) [14], the principal component of this set of enzymes, exhibits two patterns of electrophoretic mobility: B_1 (fast mobilities) and B_2 (slow mobilities). We have demonstrated that strains having carboxylesterase pattern B_2 are considerably more frequent in *E. coli* from EII than in *E. coli* obtained from the stools of healthy individuals, and that B_2 strains produce more MRHA and Hly than do B_1 strains [15, 16]. The strong association between electrophoretic pattern B_2 and these virulence factors was found regardless of the geographical origins of EII and patient characteristics [16] and was confirmed by the prevalence of P-fimbriae and Hly genetic determinants in B_2 strains [17].

To substantiate the relationship between esterase polymorphism and virulence-associated traits, we have extended the analysis of strains causing EII by studying the electrophoretic variations of esterases A, C and I, determining CNF1 production, serotyping O antigens 1, 2, 4, 6, 7, 8, 15, 18 and 75, which are commonly detected in pathogenic strains [7, 18] and studying the outer membrane protein (OMP) patterns of some serotypes. This work compares the electrophoretic polymorphism of the four *E. coli* esterases with presence of MRHA, Hly, CNF1 and the O antigens listed above, in two representative panels of 80 B_1 and 70 B_2 strains.

MATERIALS AND METHODS

Bacterial strains

The 80 B_1 and the 70 B_2 strains (Table 1) were isolated from urinary tract infections, septicaemia and other EII in France ($n = 101$) and other parts of the world ($n = 49$). These strains were chosen from the 705 strains previously analysed [16] for their distinct combination of allozymes for the four esterases.

Electrophoretic mobility

The mobilities (M_F values) of esterases were determined as previously described [19]. Each M_F variant was designated as an allozyme.

Determination of the O serogroup

This determination was limited to the identification of O serogroups 1, 2, 4, 6, 7, 8, 15, 18 and 75, which are frequently associated with extra-intestinal human diseases [7, 18]. The bacteria grown in L broth were heated to 120 °C for 1 h to destroy capsular material. An aliquot (2 μl) of heat-treated bacterial suspension was pipetted on to a nitrocellulose filter. The filters were dried, saturated with skimmed milk, and incubated for 1 h at 25 °C with rabbit *E. coli* O antisera (Difco) diluted 1/200 in gelatin buffer, pH 7·5. The filters were washed and the rabbit O

antibodies were detected using goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase, which reacted with hydrogen peroxide (substrate) and 4-chloronaphthol (chromogen). This serological O grouping was also evaluated by SDS–PAGE of extracts obtained by heating bacterial suspensions at 60 °C for 20 min [20]. After silver staining, the lipopolysaccharide (LPS) patterns of the strains were compared on a same gel with reference strains for O antigens. Strains showing a LPS pattern different to that of the reference strain for the O serogroup were discarded and the immunological result considered false positive.

Determination of cytotoxic necrotyzing factor

CNF1 toxin was detected by its multinucleating effect on cell cultures as described by de Rycke and co-workers [21].

SDS–PAGE of outer membrane proteins [22]

Bacteria grown on Minca agar medium were collected in 2 ml of Tris buffer at 4 °C (Tris base, 0·05 M; EDTA, 1 mM, pH 7·8). After centrifugation for 15 min at 4500 ***g***, the pellet was suspended in 10 ml Tris buffer at 4 °C. Fifteen-second ultrasonication was done four times in ice using 15% continuous cycle (New Brunswick). After centrifugation for 20 min at 1200 ***g***, the supernatant was centrifuged at 50000 ***g*** for 1 h at 4 °C. The pellet was suspended in 10 mM Tris-buffer with 5 mM-$MgCl_2$, pH 8 containing 2% Triton X-100 to render the inner membrane soluble [23, 24]. After centrifugation at 50000 ***g*** for 1 h at 4 °C, the pellet was dissolved in buffer v/v (Tris base 0·0025 M, glycine 0·192 M, SDS 1%, pH 8·6).

Polyacrylamide gel electrophoresis was performed after addition of Laemmli buffer v/v and heating at 100 °C for 5 min. Ten percent polyacrylamide gel was used and electrophoresis was run at 20 mA for 5 h. Silver staining of the gel was done according to Oakley and colleagues [25].

Statistical analysis

The factorial analysis of correspondence method [26] was used with STAT-ITCF software [27] from a two-way table of 150 rows (the strains) and 62 columns corresponding to (i) the number of allozymes detected in all strains (including the null allozyme) for each esterase, (ii) 9 studied O serogroups and a column for the absence of O serogroup detected, (iii) the presence or absence of Hly, MRHA and CNF1. Pearson X^2 values were calculated between several data.

RESULTS

Distribution of esterase allozymes in B_1 and B_2 strains

Table 1 shows allozyme distribution of esterases B, A, C and I in the 80 strains of pattern B_1 and in the 70 strains of pattern B_2. The most frequently occurring allozyme of esterase B was $M_F \approx 70$ for B_1 strains and $M_F \approx 57$ for B_2 strains. For esterase A, the proportion of allozyme at $M_F \approx 81$ was higher for B_1 strains and the proportion of allozyme at $M_F \approx 78$ was higher for B_2 strains. The most frequently

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Table 2. *Correlation between carboxylesterase B electrophoretic patterns B_1 and B_2 and presence of MRHA, Hly and CNF1*

Detection of virulence factor*			Carboxylesterase B electrophoretic pattern No. of strains	
MRHA	Hly	CNF1	B_1 (80)	B_2 (70)
−	−	−	55 (68·75%)	12 (17·14%)
+	−	−	15 (18·75%)	12 (17·14%)
−	+	−	5 (6·25%)	2 (2·85%)
−	−	+	1 (1·25%)	2 (2·85%)
+	+	−	3 (3·75%)	9 (12·86%)
+	−	+	—	5 (7·14%)
−	+	+	—	7 (10%)
+	+	+	1 (1·25%)	21 (30%)

* + and − indicate the presence or absence of factor.

occurring allozyme of esterase C was $M_F \approx 57$ for B_1 strains and $M_F \approx 60$ for B_2 strains. The majority of B_1 strains had allozymes C of $M_F \approx 44$ to $M_F \approx 57$, while most B_2 strains had allozyme C with $M_F \approx 58$ to $M_F \approx 62$. Most B_1 strains had esterase I allozymes with mobilities of $M_F \approx 65$ to $M_F \approx 75$, whereas most B_2 strains had slower (M_F from 57 to 62) or faster mobilities (M_F from 67 to 78).

Correlation between esterase B allozymes and O serogroups

Forty-seven strains of pattern B_2 (67%) and only 32 strains of pattern B_1 (40%) fell in serogroups O1, O2, O4, O6, O7, O8, O15, O18 and O75 ($\chi^2 = 10{\cdot}81$; $P < 0{\cdot}01$) (Table 1). Thirty-seven strains (86%) belonging to serogroups O2, O4, O6 and O75 had the B_2 pattern. In contrast, all strains of serogroups O7 and O8 had the pattern B_1. Among the B_2 strains, serogroup O1 and O4 strains had allozyme B with an M_F from 60 to 63, whereas all serogroup O6 and O75 strains (with the exception of one strain of serogroup O6) had allozyme at $M_F \approx 57$. Among the B_1 strains, serogroup O7 and O8 strains appeared heterogeneous in esterase B allozyme distribution.

Correlation between electrophoretic patterns B_1 and B_2 and MRHA, Hly and CNF1

Table 2 shows the relationships between the electrophoretic patterns B_1 and B_2 and MRHA, Hly and CNF1. As was previously demonstrated for MRHA and Hly [15, 16], more B_2 pattern strains produced CNF1 than did B_1 pattern strains. The difference was 20-fold in terms of CNF1, 5-fold in terms of Hly and 2·8-fold in terms of MRHA. Thus, the B_2 pattern was more strongly associated with CNF1 than with Hly or MRHA. Since we have previously demonstrated that the percentage of pattern B_2 strains increases with the number of virulence factors while the percentage of type B_1 strains decreases [13, 16], we have compared in the present study the number of factors (MRHA, Hly and CNF1) in the B_1 and B_2 strains. The percentage of strains producing no factor was higher for pattern B_1 (68·75%) than for pattern B_2 (17·14%) ($\chi^2 = 39{\cdot}73$; $P < 0{\cdot}001$), whereas the

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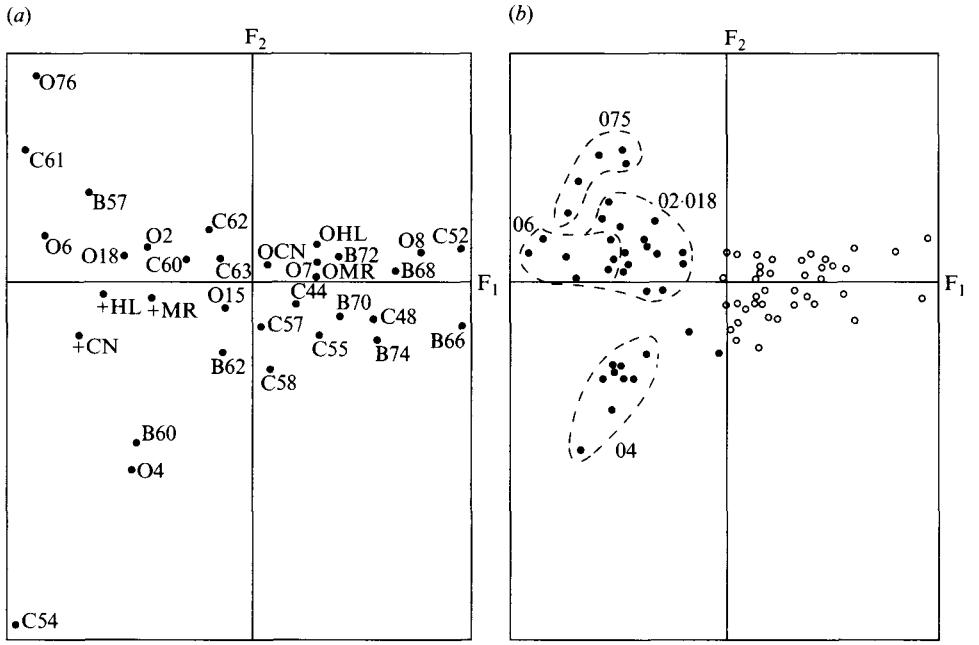

Fig. 1. Analysis of correspondance of the 150 *E. coli* strains. (*a*) The scores of allozymes of the four esterases plotted against virulence-associated traits for the first two factors F1 and F2. B57, esterase B allozyme at $M_F \approx 57$; +HL, production of Hly; OHL, absence of production of Hly; +MR, production of MRHA; OMR, absence of production of MRHA; +CN, production of CNF1; OCN, absence of production of CNF1; O1 to O75, serogroups O1, O2, O4, O6, O7, O8, O15, O18 or O75. Projections of esterase A and I allozymes were not indicated (see results). (*b*) Scores of strains for factors F1 and F2. ○, B_1 strains; ●, B_2 strains. In addition, serogroups O2, O4, O6, O18 and O75 were indicated for B_2 strains.

percentage of strains producing one factor were similar for the two patterns. More B_2 pattern strains produced two or three factors than did pattern B_1 strains. For strains producing two factors, the difference was 8-fold, while for strains producing the three factors the difference was about 30-fold.

Statistical analysis

Projections of different allozymes on the plan defined by the first two principal axes of the correspondance analysis (Fig. 1*a*) revealed that the first axis F1 separated the B_1 allozymes of esterase B migrating at $M_F \approx 74$, $M_F \approx 72$, $M_F \approx 70$, $M_F \approx 68$, $M_F \approx 66$ (designated B-74, B-72, B-70, B-68, B-66) and the slow migrating allozymes of esterase C (C-44, C-48, C-52, C-55, C-57) from the B_2 allozymes of esterase B (B-57, B-60) and the fast migrating allozymes of esterase C (C-60, C-61, C-62, C-63). Projections of esterase A and I allozymes were less clearly separated (data not shown). Projections of the virulence factors and the O serogroups revealed that the first axis separated lack of MRHA, Hly and CNF1 production and serogroups O7 and O8 or non-O typable strains from MRHA, Hly and CNF1 production and serogroups O2, O4, O6, O18 and O75. The second axis separated serogroups O75, O6 and O2 from serogroup O4. The B_2 strains were clearly distinguished from the B_1 strains by the first axis (Fig. 1*b*) whereas axis F2

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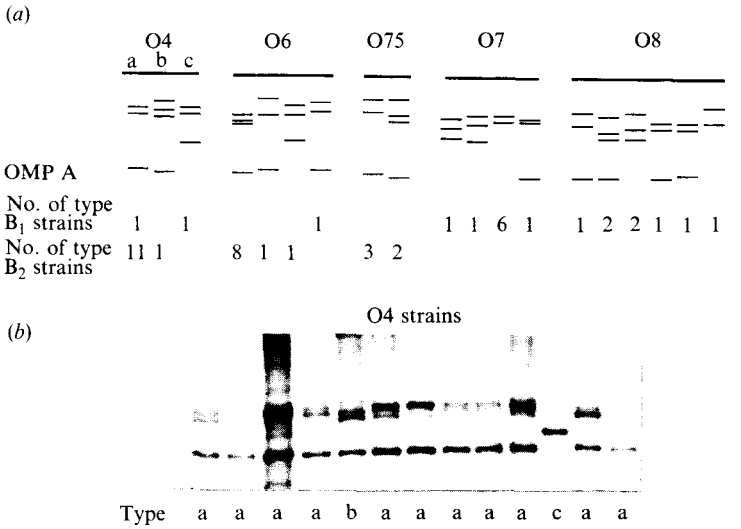

Fig. 2. (*a*) OMP patterns of the B_1 and B_2 strains belonging to serogroups O4, O6, O7, O8 and O75. (*b*) photograph of OMP patterns [numbered as in (*a*)] for O4 isolates.

allowed the separation of several subclusters within the B_2 strains, each of them having a distinct serogroup.

Characterization by the OMP patterns of the B_1 and B_2 strains belonging to serogroups O4, O6, O7, O8, and O75

In order to refine the subclustering delineated within B_1 and B_2 groups by O serogrouping, the OMP patterns of the 30 strains belonging to serogroups O4, O6, O75 (27 B_2 strains), and the 17 strains of serogroups O7 and O8 (all B_1 strains) were studied. Nineteen OMP patterns were evidenced (Fig. 2). A relation between OMPs and B_1 or B_2 patterns was observed for OMP A which is the fastest migrating outer membrane protein. In the 27 B_2 strains, 26 had a fast OMP A compared to only 8 in the 20 B_1 strains ($\chi^2 = 22{\cdot}6$; $P < 0{\cdot}001$). Also, the OMPs were more diversified in the B_1 strains which had 13 different patterns compared to 7 only for 27 B_2 strains. The diversity was also O-serogroup associated. Eight O8 *E. coli* strains revealed 6 different OMP patterns. At the opposite, O4 *E. coli* strains were less heterogenous, 12 of 14 strains having a same OMP pattern.

DISCUSSION

The *E. coli* strains causing extra-intestinal infections were initially characterized using a limited number of virulence-associated traits, without considering the overall genetic diversity of bacterial populations. Since then, several studies have used enzyme or nucleic acid electrophoretic polymorphism to survey the genetic structure of *E. coli* clinical isolates [13, 15, 16, 28–31]. Electrophoretic polymorphism of esterases A, B, C and I was found to give reliable information on the overall genetic diversity of *E. coli* populations [13, 32]. Previous studies on strains causing human extra-intestinal infections focused on carboxylesterase B [15–17, 33, 34]. The present study show two correlations, one between the fast

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migrating allozymes of carboxylesterase B (B_1 pattern) and the slow migrating allozymes of esterase C, and the other between the slow migrating allozymes of carboxylesterase B (B_2 pattern) and the fast migrating allozymes of esterase C. Slow migrating esterase B allozymes and fast migrating esterase C allozymes are correlated with the distribution of MRHA, Hly and CNF1, whereas esterase A and I allozymes which show distinct distributions in B_1 and B_2 strains, are not clearly correlated with the distribution of these factors. Thus, allelic variations of esterases B and C converge to delineate two groups of pathogenic strains differing considerably in the proportions of their virulence-associated traits. B_2 strains may well be particularly aggressive because they frequently carry two or three of these factors (Table 2). According to Caprioli and co-workers [9, 35], the haemolytic isolates of *E. coli* may be divided in two distinct classes on the basis of their ability to produce CNF1. The results of our study indicate that few strains produced Hly alone and they were not significantly associated with either the B_1 or the B_2 patterns, while 96·5% of haemolytic strains producing CNF1 or both CNF1 and MRHA belonged to B_2 electrophoretic patterns. Previous studies have established an association of serogroups O4 and O6 with Hly and MRHA or P-adhesin [4, 7, 8, 36], or with Hly and CNF1 [9, 10, 35]. The classification of strains according to their carboxylesterase B electrophoretic pattern used in this study provides a more detailed discrimination of pathogenic isolates, since 15 of the 22 strains of serogroups O4 and O6 having pattern B_2 were positive for the three factors, five were positive for two factors and one was positive for one factor. In contrast, the three strains of these serogroups having pattern B_1 lacked these factors (Table 1). On the other hand, the strains of serogroups O7 and O8 with low virulence factor levels were all in type B_1. The present results are consistent with our earlier studies showing association of pattern B_2 with Hly, CNF1 and serogroups O4 or O6 [37, 38]. Several subclusters can also be identified within B_1 and B_2 strains by their esterase B allozymes, O serogroups and OMP patterns; the factorial plan of the CA (Fig. 1*b*) indicates that most of the B_2 strains are clustered according to serogroups and certain allozymes of esterases B and C. The divergent molecular structure of B_2 allozymes [39], which correlates with allelic variation of esterase C, is in agreement with the fact that the strains showing pattern B_2 correspond to a distinct phylogenetic group within the ECOR strains [40] as detected by the allelic variations of 38 enzymes [32, 41]. We have recently demonstrated that the restriction fragment length polymorphism (RFLP) of ribosomal DNA patterns of B_2 strains having MRHA, Hly and virulence in mice are clearly distinct from those of B_1 strains lacking these virulent characters [31]. The findings of esterase and ribosomal DNA polymorphism studies, coupled with virulence-associated traits, converge to demonstrate that the electrophoretic pattern B_2 of carboxylesterase B identifies most of the highly pathogenic strains implicated in extra-intestinal infections in humans.

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