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Esterase Electrophoretic Pattern Relatedness Between *Shigella* Species and *Escherichia coli*

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Esterases of 57 strains of *Shigella dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei* and 26 strains of *Escherichia coli*, including the Alkalescens Dispar group, were compared by polyacrylamide-agarose gel electrophoresis. Six types of esterase bands differing in their ability to hydrolyse synthetic substrates and in their sensitivity to heat and to di-isofluoropropyl phosphate were defined. Individual activities and sensitivities of these bands and the apparent molecular weight of the major esterase, estimated to be 58000 by polyacrylamide gradient gel electrophoresis, were identical for both *Shigella* species and *E. coli*. One esterase with a molecular weight of 104000 was found in some strains of *E. coli*. Variations in the number and mobility of bands among *Shigella* strains defined different esterase patterns (zymotypes) which appeared to be distinct for each species.

INTRODUCTION

Previous investigations have shown that *Escherichia coli*, *Proteus* and *Providencia*, *Salmonella*, *Levinea*, *Serratia*, *Klebsiella* and *Enterobacter* are characterized by distinct electrophoretic patterns of their esterases (Goullet, 1973, 1975, 1977, 1978, 1980; Goullet & Richard, 1977). In the present work, esterases of 57 strains of *Shigella dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei*, and 26 new strains of *E. coli*, including the Alkalescens Dispar group, were analysed by polyacrylamide-agarose gel and polyacrylamide gradient gel electrophoresis.

METHODS

The names and sources of the bacterial strains are given in Table 1.

Growth conditions, preparation of extracts, protein estimation, inhibition by di-isofluoropropyl phosphate (DFP), heat inactivation, polyacrylamide-agarose gel electrophoresis, estimation of electrophoretic mobility (M_F value), polyacrylamide gradient gel electrophoresis and esterase staining were all as described in the preceding paper (Goullet, 1980).

Disc electrophoresis was carried out according to the method of Davis (1964) at 5 °C in the Pharmacia electrophoresis apparatus GE-4. Each result is the mean ± standard deviation of at least six runs.

RESULTS

Characterization of esterases

Polyacrylamide-agarose zymogram analysis using synthetic substrates gave reproducible banding patterns for each of the strains examined. The greatest numbers of bands were

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Table 1. *Strains of Shigella and E. coli examined*

Code	Organism	Strain*	Serotype	Biotype†	Origin‡
1	*Sh. dysenteriae*	12.77	1		Lisbon
2	*Sh. dysenteriae*	15.77	1		Dakar
3	*Sh. dysenteriae*	2.64	2		Laval
4	*Sh. dysenteriae*	3.76	2		Yaoundé
5	*Sh. dysenteriae*	4.77	2		Besanççon
6	*Sh. dysenteriae*	5.77	2		Bobo-Dioulasso
7	*Sh. dysenteriae*	CIP 53.127	3		Saigon
8	*Sh. dysenteriae*	17.77	3		Dakar
9	*Sh. dysenteriae*	CIP 59.2	4		Dakar
10	*Sh. dysenteriae*	6.77	4		Bobo-Dioulasso
11	*Sh. dysenteriae*	30.73	5		Brussels
12	*Sh. dysenteriae*	31.73	7		Brussels
13	*Sh. dysenteriae*	5.76	9		Marseille
14	*Sh. flexneri*	9.76	1		Marseille
15	*Sh. flexneri*	8.77	1		Paris
16	*Sh. flexneri*	401.77	1		Dakar
17	*Sh. flexneri*	18.70	2		Fort Lang
18	*Sh. flexneri*	20.76	2		Martigues
19	*Sh. flexneri*	416.77	2		Dakar
20	*Sh. flexneri*	446.77	2		Rabat
21	*Sh. flexneri*	6.77	3		Tourcoing
22	*Sh. flexneri*	418.77	3		Dakar
23	*Sh. flexneri*	3.75	4		Gonesse
24	*Sh. flexneri*	6.74	6	88	Avignon
25	*Sh. flexneri*	3.77	6	88	Chartres
26	*Sh. flexneri*	445.77	6	88	Rabat
27	*Sh. flexneri*	302.78	6	Newcastle	Paris
28	*Sh. flexneri*	354.78	6	Newcastle	Paris
29	*Sh. flexneri*	14.79	6	Manchester	Colmar
30	*Sh. flexneri*	66.79	6	Manchester	Reims
31	*Sh. boydii*	CIP 54.73	1		Bobo-Dioulasso
32	*Sh. boydii*	32.70	1		Paris
33	*Sh. boydii*	CIP 54.74	2		Saigon
34	*Sh. boydii*	16.76	2		Bangui
35	*Sh. boydii*	CIP 54.76	4		Saigon
36	*Sh. boydii*	5.77	4		Bobo-Dioulasso
37	*Sh. boydii*	CIP 56.36	5		Addis Ababa
38	*Sh. boydii*	7.77	5		Bobo-Dioulasso
39	*Sh. boydii*	21.77	5		Brest
40	*Sh. boydii*	CIP 56.35	8		Addis Ababa
41	*Sh. boydii*	CIP 57.43	9		Bonn
42	*Sh. boydii*	2.77	9		Rabat
43	*Sh. boydii*	CIP 599	10		Brazzaville
44	*Sh. boydii*	12.77	10		Australia
45	*Sh. sonnei*	46.70		a	Chambéry
46	*Sh. sonnei*	2.77		a	Bourges
47	*Sh. sonnei*	13.77		a	Tarbes
48	*Sh. sonnei*	110.77		a	Rabat
49	*Sh. sonnei*	191.77		a	La Réunion
50	*Sh. sonnei*	217.77		a	Barcelona
51	*Sh. sonnei*	5.75		d	Caen
52	*Sh. sonnei*	4.76		d	Limoges
53	*Sh. sonnei*	15.77		d	Concarneau
54	*Sh. sonnei*	18.77		d	Blois
55	*Sh. sonnei*	20.75		g	Bagnolet
56	*Sh. sonnei*	586.77		g	Dakar
57	*Sh. sonnei*	1.78		g	Paris
58	*E. coli*	30.77	O26:B6		Faeces, Gonesse
59	*E. coli*	87.77	O26:B6		Faeces, Ville d'Avray
60	*E. coli*	CIP 52.170	O55:B5		NCTC 8959
61	*E. coli*	18.77	O124:B17		Faeces, Gonesse
62	*E. coli*	CIP 6224	O125:B15		NCTC 8623
63	*E. coli*	15.77	O125:B15		Faeces, Gonesse
64	*E. coli*	29.77	O126:B16		Faeces, Gonesse
65	*E. coli*	51.77	O142:K86		Faeces, Colmar
66	*E. coli*	4.77			Faeces, Lille
67	*E. coli*	22.77			Faeces, Dijon
68	*E. coli*	45.77			Blood, Tangier
69	*E. coli*	180.77			Urine, Saint-Etienne

Table 1.—*continued*

Code	Organism	Strain*	Serotype	Biotype†	Origin‡
70	*E. coli* A–D§	3.76	O1		Pus, Meaux
71	*E. coli* A–D	4.77	O1		Urine, Bordeaux
72	*E. coli* A–D	11.77	O1		Urine, Paris
73	*E. coli* A–D	15.77	O1		Faeces, Colmar
74	*E. coli* A–D	6.78	O2		Strasbourg
75	*E. coli* A–D	24.78	O3		Switzerland
76	*E. coli* A–D	8227.70	O4		Argentina
77	*E. coli* A–D	3.77	O5		Faeces, Bordeaux
78	*E. coli* A–D	7.77	O5		Faeces, Pontoise
79	*E. coli* A–D	16.77	O5		Pus, Alençon
80	*E. coli* A–D	18.77	O5		Faeces, Paris
81	*E. coli* A–D	CIP 5340	O6		Kauffmann collection
82	*E. coli* A–D	CIP 5328	O7		Kauffmann collection
83	*E. coli* A–D	CIP 5338	O9		Kauffmann collection

* The strains were provided by Dr M. Toucas and by Dr C. Richard from the Collection du Service des Entérobactéries de l'Institut Pasteur de Paris (Professeur L. Le Minor). CIP, Collection de l'Institut Pasteur, Paris. In addition, *E. coli* strains K12, LM111, HB10 and HB13, previously analysed by Goullet (1973), were used as standards. Bacteria were grown in L broth (Goullet, 1973) and harvested during the stationary phase of growth.

† Biotypes of *Sh. sonnei* according to Szturm-Rubinstein (1964).

‡ NCTC, National Collection of Type Cultures.

§ A–D, Alkalescens Dispar.

detected with β-naphthyl acetate as substrate. Six anodic bands varying in sharpness and colour intensity (Fig. 1), which were designated as F, A, B, I, C and S by Goullet (1973), were found in extracts of both *Shigella* and *E. coli* strains. Band C included a minor component C′. Esterase band characteristics, summarized in Table 2, were identical for the different species. Three other anodic bands, designated as A′, B′ and D, were observed only in some strains of *E. coli*. Band A′ differed from band A by its heat resistance at 60 °C and band B′ differed from band B by its inactivation at this temperature. Esterase D was a prominent band hydrolysing acetate esters and inactivated by heating for 10 min at 50 °C.

Esterases A, B and D, which were readily detectable by α-naphthyl acetate or indoxyl acetate, were investigated by polyacrylamide gradient gel electrophoresis. In these conditions, bands A and B each exhibited identical mobilities for both *Shigella* species and for *E. coli*. Comparison of electrophoresis for 400, 900, 1500, 2000 and 2500 V h (Fig. 2) showed that asymptotic migration, necessary to determine molecular weight (Rodbard *et al.*, 1971), was obtained with esterases B and D whereas esterase A migrated out of the gel. Band B from both *Shigella* species and *E. coli* showed the same molecular size. Using bovine serum albumin and *E. coli* alkaline phosphatase as reference proteins, the apparent molecular weights of esterases B and D were estimated to be 58000 ± 1000 and 102000 ± 2500, respectively.

Distribution of esterase bands

Electrophoretic relationships were established between the strains by numerous replicate runs comparing esterase bands in adjacent positions on the same polyacrylamide-agarose gel. The M_F values were used only comparatively. Bands F ($M_F \approx 97$) and S ($M_F \approx 25$) from different strains showed similar electrophoretic mobility with the exception of band F ($M_F \approx 93$) from strain 74, whereas bands A, B, I and C varied in mobility. Different esterase patterns, hereafter called zymotypes, were distinguished by variations in the number and mobility of bands.

Shigella flexneri. Strains of serotypes 1, 2, 3 and 4 were distributed in two related zymotypes f_1 and f_2 (Fig. 3). In zymotype f_1, bands B and I overlapped. Characterization of bands was then achieved by differential DFP inhibition. In zymotype f_2, band B was not

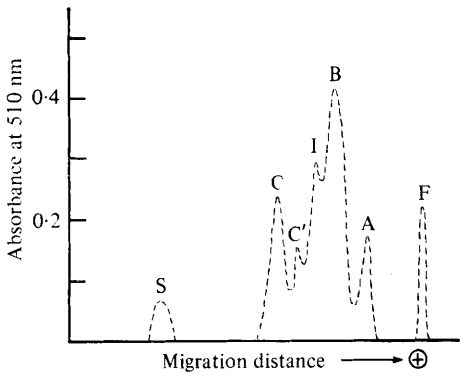

Fig. 1.

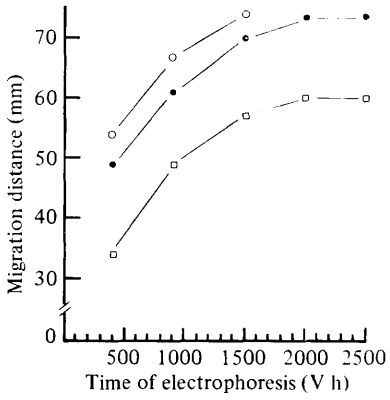

Fig. 2

Fig. 1. Polyacrylamide gel spectrophotometric profile of *Shigella* esterases (strain 25) stained with β-naphthyl acetate and scanned at 510 nm. Bands are designated as previously (Goullet, 1973). C′ is the faster migrating component of band C.

Fig. 2. Plots of mobilities of esterase A, B and D (strain 70) against time of electrophoresis in a polyacrylamide gradient gel: ○, esterase A; ●, esterase B; □, esterase D.

Table 2. *Characteristics of esterases of Shigella species and E. coli*

Band*	Substrates hydrolysed† αNA	αNB	IA	βNA	βNB	Heat denaturation	Inhibitory concn of DFP (M)	Apparent molecular weight‖
F	−	−	−	+	−	60 °C	10^{-4}	
A	++	+	+	+	−	60 °C	10^{-4}	
B	+++	++	++	++	+	R 60 °C‡	10^{-4}	58000±1000
C	−	−	−	+	−	60 °C	R 10^{-3}§	
I	+	−	±	+	−	60 °C	R 10^{-3}§	
S	−	−	−	+	±	R 60 °C‡	10^{-3}	
D	+++	−	++	+++	−	50 °C	10^{-3}	102000±2500

* Band D was detected in some strains of *E. coli* only.

† αNA, α-naphthyl acetate; αNB, α-naphthyl butyrate; IA, indoxyl acetate; βNA, β-naphthyl acetate; βNB, β-naphthyl butyrate. +++, ++, +, decreasing intensities of esterase band; ±, very weak activity; −, no activity.

‡ Resistant to 60 °C.

§ Resistant to 10^{-3} M-DFP.

‖ Mean±S.D.; 6 runs.

detected. All strains of serotype 6 were grouped in zymotype f_3 which differed from the others in that bands A, B and C were faster moving.

Shigella sonnei. Esterases A, B and I showed the same mobilities in all strains of this serotype (Fig. 4). Band C was not detected in strains of biotype d and in one strain of biotype a.

Shigella boydii. Strains of serotypes 2 and 4 exhibited complete banding patterns (Fig. 5). Strains of other serotypes (zymotypes b_2, b_3, b_4, b_5) lacked two or three bands. In strains of serotypes 5 and 9, one additional faint band was detected by α-naphthyl acetate.

Shigella dysenteriae. Four zymotypes were defined by decreasing numbers of bands (Fig. 6). Band B was not detected. Zymotype d_3 (serotype 1) differed from other zymotypes in the mobilities of bands A and C.

Escherichia coli. There was considerable electrophoretic heterogeneity in the distribution of bands among the 26 *E. coli* strains (Fig. 7). Bands A and B were observed in most of the

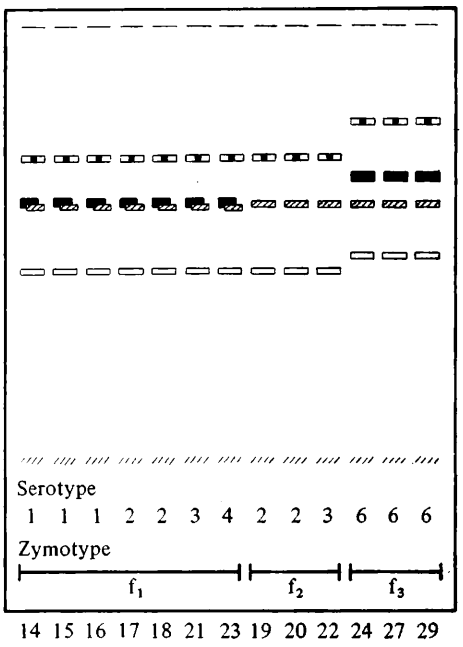

Fig. 3

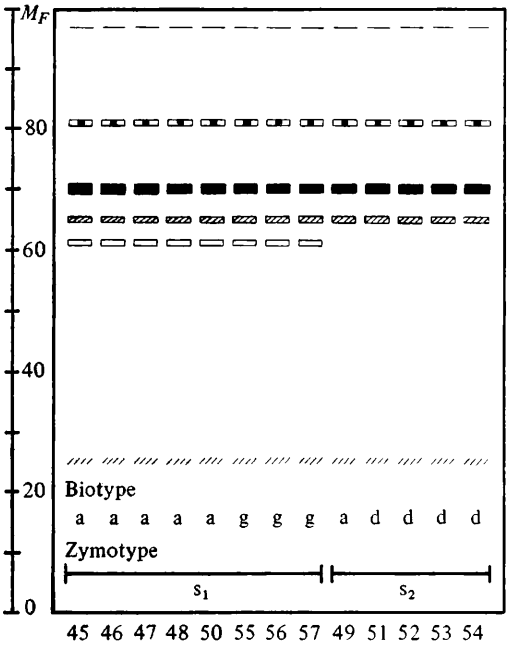

Fig. 4

Fig. 3. *Shigella flexneri.* Diagrammatic representation of esterase patterns of 13 strains (numbered as in Table 1) grouped according to zymotype. Horizontal slab polyacrylamide-agarose gel electrophoresis was performed using 7% (w/v) acrylamide and discontinuous Tris/glycine buffer, pH 8·7. ▯, A band; ▮, B band; ▨, I band; □, C band; ——, F band; ////, S band. Esterase patterns of strains 25, 26, 28 and 30 (serotype 6), not shown, were identical to that of strain 24.

Fig. 4. *Shigella sonnei.* Diagrammatic representation of esterase patterns of the 13 strains grouped according to zymotype. Key as in legend to Fig. 3.

strains. In strains 68 and 77 to 80, bands B and I overlapped. Band C was generally faint. Band A′ was found in strains 75, 76 and 83, and band B′ in strains 60, 74 and 83. Band D was found in strain 67 and in strains of serotypes 1, 2, 5 and 7 of the Alkalescens Dispar group. Bands A′, B′ and D were previously detected in *E. coli* strains LM26, LM55 and HB13, respectively (Goullet, 1973).

Identical mobilities of band A observed in polyacrylamide-agarose gel for some strains of *Shigella* and *E. coli* were confirmed by disc electrophoresis: *Sh. dysenteriae* strain 5, $M_F = 85{\cdot}5 \pm 1{\cdot}75$; *Sh. flexneri* strain 24, $M_F = 85{\cdot}5 \pm 2{\cdot}0$; *Sh. boydii* strain 35, $M_F = 85{\cdot}5 \pm 2{\cdot}5$; *Sh. sonnei* strain 45, $M_F = 85{\cdot}5 \pm 1{\cdot}5$; *E. coli* strain 72, $M_F = 85{\cdot}5 \pm 2{\cdot}5$; *E. coli* strain LM111, previously analysed (Goullet, 1973), $M_F = 85{\cdot}5 \pm 1{\cdot}5$.

DISCUSSION

Zymograms in polyacrylamide-agarose gel of cellular extracts of *Shigella* and *E. coli*, including the Alkalescens Dispar group, demonstrated several distinct types of esterase bands differing in their ability to hydrolyse synthetic substrates and in their sensitivity to heat and to DFP.

Individual characteristics of bands confirmed previous findings for *E. coli* strains (Goullet, 1973) and showed that extracts of some strains of *Shigella* and *E. coli* contained individual esterases which were apparently identical. This similarity is supported by the fact that the apparent molecular weight of esterase B was found in polyacrylamide gradient gel to be

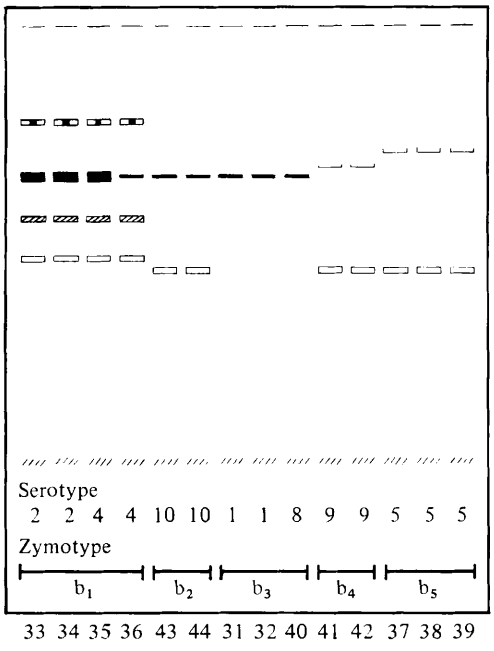

Fig. 5

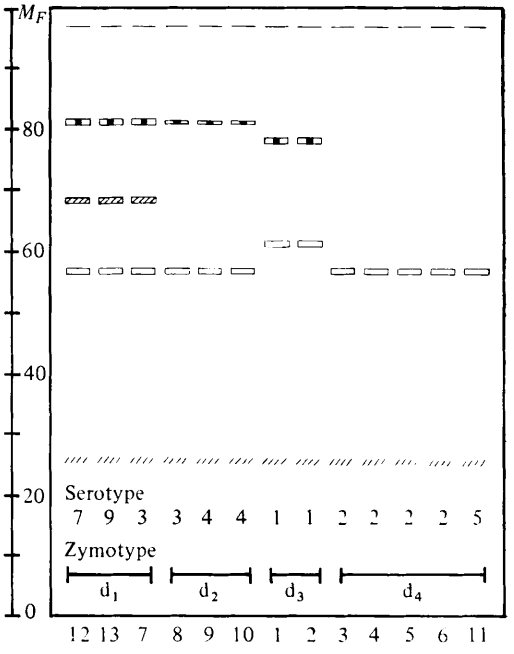

Fig. 6

Fig. 5. *Shigella boydii*. Diagrammatic representation of esterase patterns of the 14 strains grouped according to zymotype. Key as in legend to Fig. 3. For strains 31, 32, 36, 40, 43 and 44 the band B was faint. ⌙, faint band hydrolysing α-naphthyl acetate.

Fig. 6. *Shigella dysenteriae*. Diagrammatic representation of esterase patterns of the 13 strains grouped according to zymotype. Key as in legend to Fig. 3.

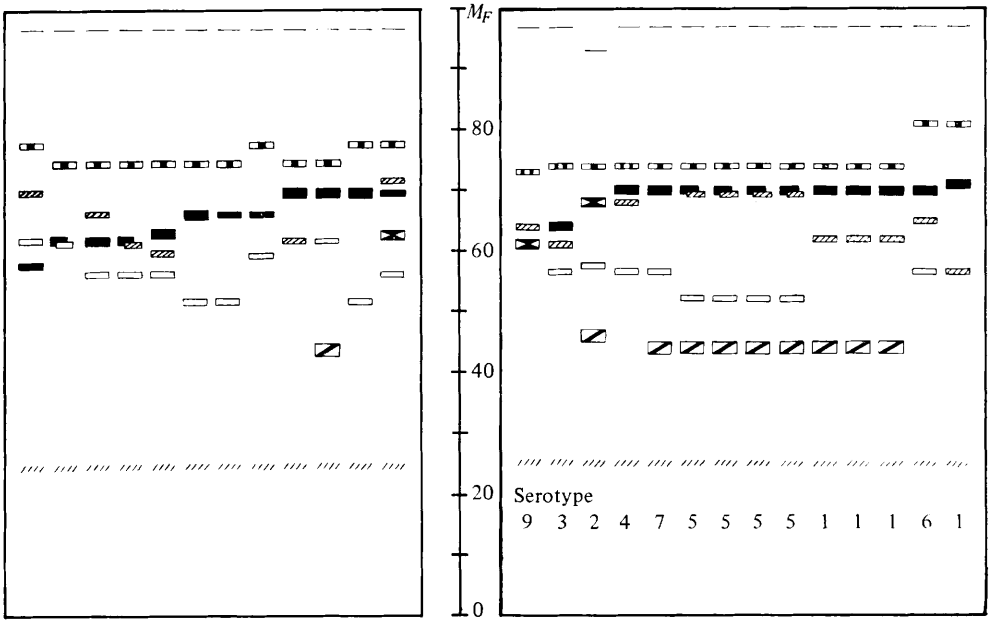

Fig. 7. *Escherichia coli*. Diagrammatic representation of esterase patterns of the 26 strains arranged in order of increasing mobility of band B. Key as in legend to Fig. 3. ▥, A′ band; ⧓, B′ band; ◪, D band. (*a*) *E. coli*; (*b*) *E. coli* Alkalescens Dispar group with serotype designation.

Table 3. *Identical mobilities for the same band in Shigella species and E. coli*

Band	M_F value*	Number of strains exhibiting the band: *Sh. dysenteriae* †(13)	*Sh. flexneri* (17)	*Sh. boydii* (14)	*Sh. sonnei* (13)	*E. coli* (12)	*E. coli* A–D (14)
A	81	6	7	4	13	0	2
	78	2	0	0	0	4	0
	75	0	10	0	0	8	0
B	72	0	7	9	0	0	0
	70	0	0	0	13	4	10
I	68	3	17	0	0	0	1
	65	0	0	4	13	0	1
C	61	2	0	0	8	0	0
	59	0	7	4	0	1	0
	57	11	10	7	0	4	4

* In polyacrylamide-agarose gel using 7% (w/v) acrylamide and discontinuous Tris/glycine buffer pH 8·7.
† For each species, the total number of strains tested is given in parentheses.

identical for both *Shigella* species and *E. coli*. The molecular weight of esterase A was too low to be determined in these conditions. Some strains of *Shigella* and *E. coli* contained other bands of identical mobilities (Table 3).

There were limited differences in esterase patterns between *Shigella* and *E. coli* and within *Shigella* species. Numerous strains of *Sh. dysenteriae* and *Sh. boydii* lacked two or three bands, whereas some strains of *E. coli*, mostly from the Alkalescens Dispar group, exhibited additional esterases A′, B′ or D. Variations in the number and mobility of bands A, B, I and C among *Shigella* strains defined different esterase patterns (zymotypes) which appeared distinct for each species. Strains of *Sh. flexneri* serotype 6 resembled strains of *Sh. boydii* serotypes 2 and 4 in the identical mobilities of their esterases A, B and C, which could provide a new argument for the inclusion of *Sh. flexneri* serotype 6 strains in *Sh. boydii* (Gekker *et al.*, 1965; Petrovskaya & Bondarenko, 1977).

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