ANTIGENIC RELATIONSHIPS AMONG TYPE-3 FIMBRIAE OF ENTEROBACTERIACEAE REVEALED BY IMMUNOELECTRONMICROSCOPY

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SUMMARY. Antigenic relationships among type-3 fimbriae of 31 strains, representing 19 species and eight genera of Enterobacteriaceae, were investigated by immunoelectronmicroscopy in tests with 17 different type-3 fimbrial antisera. At least nine antigenically distinct groups among type-3 fimbriae were distinguished. The constituent membership of some groupings did not conform with current views on the taxonomy of Enterobacteriaceae.

INTRODUCTION

The early definitive study of Duguid (1959) established that some strains of *Klebsiella* and *Serratia marcescens* formed a haemagglutinin which agglutinated the erythrocytes of different animal species only after the red cells had been treated with tannic acid. That haemagglutinin, which was associated with the presence of thin fimbriae of about 5nm external diameter (Thornley and Horne, 1962), was subsequently designated the MR/K (mannose-resistant and klebsiella-like) haemagglutinin by Duguid and Old (1980); the associated thin fimbriae were subsequently classified as type-3 fimbriae (Duguid *et al.*, 1966). Similar MR/K-like haemagglutinins and associated type-3 fimbriae have now been described in many strains of most genera of the tribes Klesielleae and Proteae (Duguid and Old, 1980; Adegbola and Old, 1982, 1983 and 1985; Old and Adegbola, 1982, 1983 and 1984a; Old *et al.*, 1983). Furthermore, thin, type 3-like fimbriae have been found on a few strains of *Salmonella* and *Yersinia*, genera in which type-3 fimbriae are uncommon (Rohde *et al.*, 1975; Aleksić *et al.*, 1976 and 1978; Adegbola *et al.*, 1983a).

Considering their wide distribution throughout the Enterobacteriaceae there is remarkably little information about the antigenic properties of type-3 fimbriae. Accordingly, the technique of immunoelectronmicroscopy was used in the present comparative study to establish the serological relationships among type-3 fimbriae of different genera of Enterobacteriaceae.

MATERIALS AND METHODS

Bacteria. The sources of the 33 strains of Enterobacteriaceae used in this study are given in table I. The identity of each strain was confirmed by conventional methods and in tests with

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TABLE I
Strains of enterobacteria

Species	Donor or source	Strain no.	Type-3 fimbriae	Other fimbriae (and associated haemagglutinins)
<i>Enterobacter aerogenes</i>	NCIB	11460	+	none
<i>E. aerogenes</i>	NCTC	10006	—	thin fimbriae (MS-HA)
<i>E. aerogenes</i>	NCTC	10336	—	thin fimbriae (MS-HA)
<i>E. gergoviae</i>	BR	9	+	none
<i>E. gergoviae</i>	CDC	558/79	+	none
<i>E. intermedium</i>	CDC	1163/81	+	type-1 fimbriae (MS-HA)
<i>E. intermedium</i>	CDC	9011/82	+	none
<i>Klebsiella oxytoca</i>	JPD	K70/1	+	none
<i>K. oxytoca</i>	JGB	U30	+	none
<i>K. pneumoniae</i> ss. <i>aerogenes</i>	JPD	K4/20	+	none
<i>K. pneumoniae</i> ss. <i>aerogenes</i>	JPD	K5/1	+	none
<i>K. pneumoniae</i> ss. <i>aerogenes</i>	JPD	K69/1	+	none
<i>Morganella morganii</i>	NCTC	232	+	thick fimbriae (MR/P-HA)
<i>M. morganii</i>	NCTC	2815	+	thick fimbriae (MR/P-HA)
<i>Proteus mirabilis</i>	NCTC	3177	+	thick fimbriae (MR/P-HA)
<i>P. mirabilis</i>	NCTC	10374	+	thick fimbriae (MR/P-HA)
<i>P. vulgaris</i>	NCTC	10740	+	none
<i>Providencia alcalifaciens</i>	JLP	T1747	+	thick fimbriae (non-HA)
<i>Prov. rettgeri</i>	JLP	75	+	none
<i>Prov. stuarti</i>	NCTC	10318	+	thick fimbriae (non-HA)
<i>Prov. stuarti</i>	BWS	68027B	+	thick fimbriae (non-HA)
<i>Salmonella enteritidis</i>	SA	45-36/71	+	type-1 fimbriae (MS-HA)
<i>S. enteritidis</i>	SA	236-36/73	+	type-1 fimbriae (MS-HA)
<i>S. typhimurium</i>	SA	162-36/72	+	type-1 fimbriae (MS-HA)
<i>S. typhimurium</i>	SA	221-36/73	+	type-1 fimbriae (MS-HA)
<i>S. manombo</i> SGII	SA	1427K	+	type-1 fimbriae (MS-HA)
<i>S. arizonae</i> SGIII	SA	548-59	+	type-1 fimbriae (MS-HA)
<i>Serratia marnorubra</i>	NCTC	3804	+	type-1 fimbriae (MS-HA) and thin fimbriae (MR/P-HA)
<i>Serr. marnorubra</i>	PADG	864(2)	+	thin fimbriae (MR/P-HA)
<i>Serr. plymuthica</i>	NCTC	8015	+	none
<i>Serr. plymuthica</i>	NCTC	9493	+	none
<i>Yersinia enterocolitica</i>	GW	IP551	+	none
<i>Y. intermedia</i>	GW	021.K1+	+	none

CDC=Centers for Disease Control, Atlanta, Georgia, USA; NCIB=National Collection of Industrial Bacteria, Aberdeen; NCTC=National Collection of Type Cultures, Central Public Health Laboratory, London; SA=Dr S. Aleksić, National *Salmonella* Zentrale Hygienisches Institut, Hamburg, West Germany; JGB=Dr J. G. Barr, Royal Victoria Hospital, Belfast; JPD=Professor J. P. Duguid, Dundee Medical School, Dundee; PADG=Dr P. A. D. Grimont, Service des Entérobactéries, Unité INSERM, Institute Pasteur, Paris, France; JLP=Dr J. L. Penner, University of Toronto, Ontario, Canada; BR=Mr B. Reid, API Laboratory Products Ltd, Basingstoke; BWS=Dr B. W. Senior, Dundee Medical School, Dundee; GW=Dr G. Wauters, Université Catholique de Louvain, Brussels, Belgium.

MS-HA=mannose-sensitive haemagglutinin; MR/P-HA=mannose-resistant and proteus-like haemagglutinin (see Duguid and Old, 1980); non-HA=non-haemagglutinating.

commercial API 20E kits (API Laboratory Products Ltd, Basingstoke, Hampshire). The fimbrial haemagglutinins produced by these strains are also described in table I.

Media and culture methods have been described before (Old and Adegbola, 1982; Adegbola and Old, 1983).

Erythrocytes and haemagglutination tests. The preparation of erythrocyte suspensions, performance of haemagglutination tests and the characterisation of MS-HA, MR/K-HA and MR/P-HA (see Duguid and Old, 1980) have been described in full elsewhere (Adegbola and Old, 1982 and 1983; Old and Adegbola, 1982).

Preparation of fimbrial antisera. Antisera were raised in adult rabbits by the method of Adegbola and Old (1982). Type-3 fimbrial antisera were prepared against MR/K-HA⁺ suspensions of: *Enterobacter aerogenes* NCIB 11460 and *E. intermedium* CDC 1163/81; *Klebsiella oxytoca* K70/1, *K. pneumoniae* ss. *aerogenes* K5/1 and K69/1; *Morganella morganii* NCTC 232 and NCTC 2815; *Proteus mirabilis* NCTC 3177 and NCTC 10374; *Providencia*

rettgeri JLP 75, *Prov. stuarti* NCTC 10318 and BWS 68027B; *Serratia plymuthica* NCTC 8015 and NCTC 9493; and *Yersinia enterocolitica* GW IP551.

(i) From each of the 15 crude antisera, homologous *O* antibodies were removed by repeated absorptions at 37°C for 1 h with dense bacterial suspensions of the vaccine strains that had been grown on nutrient agar at 30° or 37°C for 48 h, boiled at 100°C for 1 h to destroy all flagellar (*H*) antigens and fimbrial antigens, and washed twice before resuspension in saline to $c. 5 \times 10^{11}$ bacteria/ml. Absorption was repeated until a 1 in 20 dilution of antiserum showed no agglutination of a boiled bacterial suspension of the absorbing strain. This procedure yielded antiserum specific for the type-3 fimbriae on strains of *Klebsiella* that were non-flagellate and produced only type-3 fimbriae.

(ii) Other antisera were rendered specific for type-3 fimbriae by removing homologous *H* antibodies with suspensions of the vaccine strains rich in *H* antigen. Thus, each vaccine strain was grown in several 50-ml volumes of nutrient broth in 1000-ml tissue-culture bottles laid flat on their sides and left at ambient temperature ($c. 20^\circ\text{C}$) for about 18 h. The resultant growth, which is poorly fimbriate at that temperature, was harvested and washed twice before resuspension in saline to give $c. 5 \times 10^{11}$ bacteria/ml. These suspensions, rich in *H* antigen, were used repeatedly to absorb homologous antiserum at 37°C for 1 h, until a 1 in 20 dilution of the serum did not show floccular agglutination of an unboiled flagellate suspension of the absorbing strain. By this procedure, antisera specific for type-3 fimbriae were obtained against type-3 fimbriate strains of *E. aerogenes*, *E. intermedium* and *Serr. plymuthica*.

(iii) For antiserum against the type-3 fimbriae of *Y. enterocolitica*, absorption of *H* antibodies was similar to that for *Enterobacter* except that the flagella-rich bacterial suspensions were prepared from a heterologous strain of *Y. enterocolitica* (strain 88) which was flagellate and of the same serotype (O10) as the vaccine strain, but non-fimbriate. The resultant antiserum did not at a dilution of 1 in 20 agglutinate strain 88; and it did not coat the flagella of the vaccine strain, as shown by electronmicroscopy.

(iv) In addition to type-3 fimbriae, strains of *Morganella*, *Proteus* and *Providencia* often possess other types of fimbriae. These are usually thick, and in *Morganella* and *Proteus* (Old and Adegbola, 1982; Adegbola *et al.*, 1983*b*) they are associated with MR/P-HA (see table I); but in *Providencia* (Adegbola, 1981; Old and Scott, 1981) they are not associated with any known haemagglutinating activity. Because it was not possible to obtain cultures of *Morganella* or *Proteus* that were phenotypically MR/K-HA⁺ and MR/P-HA⁻ (Old and Adegbola, 1982), or cultures of *Providencia* that had lost their non-haemagglutinating thick fimbriae (Adegbola, 1981), fimbrial antisera against these strains contained antibodies against both thick and thin fimbriae. This did not interfere, however, with the assessment of coating of type-3 fimbriae because observation could be restricted to thin fimbriae.

(v) In addition, fimbrial antisera prepared against *S. arizonae* SA 548-59 and *S. enteritidis* SA 45-36/71 were gifts from Dr S. Aleksic.

Electronmicroscopy. The techniques have been described in full elsewhere (Adegbola and Old, 1982; Old and Adegbola, 1982). For preparations of most bacteria, uranyl acetate 0.3% w/v in deionised water (pH 4.6) was used (Adegbola and Old, 1982). However, for preparations of cultures of *Morganella*, *Proteus* and *Providencia*, methylamine tungstate (pH 6.5), a commercial preparation from EMscope Laboratories Ltd, Ashford, Kent, was used because some fimbriae of some strains in these genera are acid-labile (Adegbola, 1981; Old and Adegbola, 1982).

Immunoelectronmicroscopy. Fimbriate bacteria were also stained by previously described methods (Adegbola and Old, 1982) after interaction with different fimbrial antisera. Degrees of antibody coating of thin fimbriae were recorded on an arbitrary scale from 0 (no coating) to + + + (maximum, strong coating) as described by Adegbola and Old (1982).

RESULTS

Haemagglutinins and fimbriae

When cultured in appropriate conditions, e.g., serial broth cultures at 20° or 30°C, 29 of the 33 strains listed in table I formed a haemagglutinin (MR/K-HA) active

against tanned ox-erythrocytes and demonstrable in rocked-tile tests (for details, see Adegbola and Old, 1982 and 1983; Old and Adegbola, 1982, 1983 and 1984a; Old *et al.*, 1983). Another two of the 33 strains, namely *S. manombo* (subgenus II) and *S. arizonae* (subgenus III), when cultured similarly, formed an MR/K-like HA which also was active against tanned ox-erythrocytes but required a settling, rather than a rocked-tile, test for its demonstration (Adegbola *et al.*, 1983a).

All 33 strains formed thin, non-channelled fimbriae (putatively type-3 or type 3-like), though *E. aerogenes* strains NCTC 10006 and NCTC 10336 are known not to form MR/K-HA (Adegbola and Old, 1985).

Immunoelectronmicroscopy

Haemagglutinating bacteria of the 31 strains that formed MR/K-HA (or MR/K-like HA), and were rich in type-3 fimbriae, were tested by immunoelectronmicroscopy (IEM) with each of the 17 antisera to type-3 fimbriae (table II). Several broad areas of serological relatedness were observed.

(i) Fimbrial antisera against five type-3 fimbriate strains (*K. oxytoca*, *K. pneumoniae* ss. *aerogenes* (2 strains), *Y. enterocolitica* and *S. enteritidis*) coated strongly the thin fimbriae of homologous and heterologous strains of the same species as the vaccine strains. Furthermore, the thin fimbriae of strains of *E. gergoviae*, *Y. intermedia* and *S. typhimurium* were also coated strongly in tests with these five type-3 fimbrial antisera.

Type-3 fimbrial antibody coated all the thin fimbriae present on bacteria from MR/K-HA⁺ cultures of each of these strains and did so to the same degree (+ + +) irrespective of whether the haemagglutinating cultures of these strains of *Enterobacter*, *Klebsiella*, *Salmonella* and *Yersinia* showed MR/K-HA activity alone or together with other kinds of haemagglutinating activities (see table I). The strains so far described, therefore, produced type-3 fimbriae which seemed to be serologically related to one another, though not necessarily identical.

Type-3 fimbrial antiserum against the thin fimbriae of the MR/K-HA⁺ strain of *E. aerogenes* NCIB 11460 strongly coated the thin fimbriae of the homologous vaccine strain as well as the type-3 fimbriae of MR/K-HA⁺ strains of *Klebsiella*, *Salmonella* (subgenus I) and *Yersinia*, just as the type-3 fimbrial antisera of strains of these latter genera coated the thin fimbriae of *E. aerogenes* strain NCIB 11460. Morphologically similar thin fimbriae observed on *E. aerogenes* strains NCTC 10006 and NCTC 10336, and originally thought by us to belong to the type-3 group, were not coated by any of the type-3 fimbrial antisera tested in this series though they were coated by 'thin fimbrial' antisera prepared against strains of *E. aerogenes* other than strain NCIB 11460 (Adegbola and Old, 1985) suggesting heterogeneity among the thin fimbriae of *E. aerogenes* strains.

(ii) The thin, type 3-like fimbriae on bacteria from haemagglutinating (MR/K-like-HA⁺) cultures of *S. manombo* and *S. arizonae* (subgenera II and III respectively) were coated by the type 3-like fimbrial antiserum against *S. arizonae* strain 548/59, but not by the other 16 fimbrial antisera tested (table II). Likewise, that *S. arizonae* fimbrial antiserum did not coat the thin fimbriae of any other species examined in this series.

(iii) Type-3 fimbrial antisera prepared against MR/K-HA⁺ strains of *E. intermedium* and *Serr. plymuthica* coated the thin fimbriae present on homologous and

TABLE II
Serological relationships among type-3 fimbriae of eight genera of Enterobacteriaceae revealed by immunoelectronmicroscopy

Species		Degree of antibody coating of type-3 fimbriae of stated species in IEM tests with type-3 fimbrial antiserum prepared against															
Strain no.																	
<i>K. oxytoca</i>	K70/1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>K. oxytoca</i>	U30	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>K. pneumoniae</i>	K4/20	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>ss. aerogenes</i>	K5/1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>E. aerogenes</i>	K69/1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>E. gergoviae</i>	11460	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>E. gergoviae</i>	558/79	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Y. enterocolitica</i>	551	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Y. enterocolitica</i>	021.K.1+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. intermedia</i>	45-36/71	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. enteritidis</i>	236-36/73	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. enteritidis</i>	162-36/72	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. typhimurium</i>	721-36/73	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. typhimurium</i>	1427/K	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. manombo</i>	548-56	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. arizonae</i>	1163/81	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>E. intermedium</i>	9011/82	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>S. intermedium</i>	9493	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Serr. plymuthica</i>	3804	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Serr. marnorubra</i>	864(2)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Serr. marnorubra</i>	232	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>M. morganii</i>	2815	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>P. mirabilis</i>	3177	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>P. mirabilis</i>	10374	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>P. vulgaris</i>	10740	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Prev. alcalifaciens</i>	T1747	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Prev. rettgeri</i>	75	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Prev. stuarti</i>	10318	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
<i>Prev. stuarti</i>	68027B	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

0 = no coating; + = poor coating; ++ = strong coating.
See table I for genus abbreviations.

heterologous strains of these two species but did not coat the thin fimbriae of any strain of the other species tested, including MR/K-HA⁺ strains of *Serr. marinorubra*.

(iv) The thin fimbriae present on bacteria from MR/K-HA⁺ cultures of *Serr. marinorubra* were coated, albeit poorly (+), by the type-3 fimbrial antisera raised against strains of *E. aerogenes* (strain NCIB 11460 only), *Klebsiella*, *Salmonella* (subgenus I) and *Yersinia*, described above in (i).

(v) Each of the type-3 fimbrial antisera prepared against MR/K-HA⁺ cultures of *M. morgani* (2 strains), *P. mirabilis* (2 strains), *Prov. rettgeri* and *Prov. stuarti* (2 strains) coated strongly the thin fimbriae of homologous and heterologous strains of the same species only (table II).

(vi) The thin fimbriae of *P. vulgaris* and *Prov. alcalifaciens*, against which fimbrial antisera were not prepared, were not coated in tests with any of the 17 type-3 fimbrial antisera available.

DISCUSSION

Apart from observations that the type-1 and type-3 fimbriae of *Klebsiella* are antigenically distinct (Nowotarska and Mulczyk, 1977; Korhonen *et al.*, 1983), there is little information about the antigenic properties of the type-3 fimbriae of Enterobacteriaceae. In this study we used immunoelectronmicroscopy (IEM), in which type-3 fimbriae were specifically labelled by attachment of type-3 fimbrial antibody, to assess the degrees of relatedness among type-3 fimbriae present on strains from eight genera. Previously, IEM has been used successfully to establish serological relationships among different kinds of sex pili (Lawn and Meynell, 1970) and, in intrageneric studies, to identify different kinds of fimbriae present on multi-haemagglutinating strains of different species of *Enterobacter*, *Klebsiella* and *Serratia* (Adegbola and Old, 1982 and 1983; Old and Adegbola, 1983 and 1984a; Old *et al.*, 1983). The data from this and our other IEM studies are summarised in table III from which it is clear that the antigenic relationships are complex and often inconsistent with current taxonomy.

In taxonomic terms, the broadest group (*a*, table III) comprised the five type-3 fimbriate species of *Klebsiella* (Old and Adegbola, 1984) along with representatives of *Salmonella*, *Yersinia* and *E. aerogenes*. The types of fimbriae (and associated haemagglutinins) commonly found on most strains of *Salmonella* and *Yersinia* are, respectively, type-1 fimbriae (and MS-HA) and MR/Y fimbriae (and broad-spectrum MR/Y-HA) (Duguid *et al.*, 1966; MacLagan and Old, 1980; Old and Adegbola, 1984b). It has long been known, however, that occasional strains of *Salmonella* subgenus I possess thin fimbriae, antigenically like those found on strains of *Y. enterocolitica* possessing K1 antigen (Rohde *et al.*, 1975; Aleksić *et al.*, 1976 and 1978; Aleksić and Aleksić, 1979); the knowledge that these fimbriae were also MR/K-HA-associated and antigenically related to those of *Klebsiella* is more recent (Adegbola *et al.*, 1983a). Furthermore, the thin fimbriae rarely found on occasional strains of *Salmonella* of subgenera II and III are unlike the type-3 fimbriae of *Salmonella* subgenus I in antigenicity (Aleksić *et al.*, 1978), haemagglutinating activity (Adegbola *et al.*, 1983a) and surface hydrophobicity (Dr S. Aleksić, personal communication). We have previously designated these fimbriae as type 3-like (Adegbola *et al.*, 1983a) and their antigenic distinctness is clear.

The classification of the thin fimbriae found on different species of *Enterobacter* has

TABLE III
Antigenic groups among type-3 fimbriae of *Enterobacteriaceae*

Group of type-3 fimbriae	Representative bacteria
a	<i>Salmonella</i> subgenus I <i>Yersinia enterocolitica</i> , <i>Y. frederikseni</i> , <i>Y. intermedia</i> <i>Klebsiella oxytoca</i> , <i>K. planticola</i> , <i>K. pneumoniae</i> ss. <i>aerogenes</i> , <i>K. terrigena</i> , <i>K. trevisani</i> <i>Enterobacter aerogenes</i> (strain NCIB 11460), <i>E. gergoviae</i>
b	<i>Salmonella</i> subgenera II and III
c	<i>E. intermedium</i> <i>Serratia ficaria</i> , <i>Serr. fonticola</i> , <i>Serr. grimesi</i> , <i>Serr. liquefaciens</i> , <i>Serr. marcescens</i> , <i>Serr. odorifera</i> , <i>Serr. plymuthica</i> , <i>Serr. proteamaculans</i>
d	<i>Serr. marinorubra</i>
e	<i>Morganella</i>
f	<i>Proteus mirabilis</i>
g	<i>Providencia rettgeri</i>
h	<i>Prov. stuarti</i>
not assigned	<i>P. vulgaris</i> and <i>Prov. alcalifaciens</i>

proved troublesome (Adegbola and Old, 1983 and 1985; Old and Adegbola, 1984a). Thus, in antigenic terms the type-3 fimbriae of *E. gergoviae* are *Klebsiella*-like and those of *E. intermedium* are *Serratia*-like (groups a and c, table III). However, whereas the thin fimbriae found on most strains of *E. aerogenes* (syn. *K. mobilis*) do not belong to the type-3 class and are associated with MS-HA activity, those on *E. aerogenes* strain NCIB 11460 (the properties of which strain do not identify it definitively as either *E. aerogenes* or *K. pneumoniae* ss. *aerogenes*; Adegbola and Old, 1985) are type 3-like and antigenically similar to those of *Klebsiella*.

Thus, there is considerable heterogeneity among the thin fimbriae not only of different *Enterobacter* species but even among those of different strains of the same species. Further evidence of heterogeneity among *Enterobacter* strains has been obtained from immunodiffusion studies of their glyceraldehyde-3-phosphate dehydrogenases and from principal-components analysis of data on DNA-relatedness; this has led some workers (Izard *et al.*, 1983; Trinel and Leclerc, 1983) to consider unjustified the inclusion, in *Enterobacter*, of species other than *E. cloacae* and *E. amnigenus*. Moreover, these two species form antigenically similar kinds of type-1 fimbriae (Old and Adegbola, 1984a).

The finding that the type-3 fimbriae of *Serr. marinorubra* belonged to an antigenic group different from that of the other eight species of *Serratia* is perhaps not surprising, for *Serr. marinorubra* is poorly related to other *Serratia* species (Grimont *et al.*, 1977a) and its esterases and proteases are also distinct (Grimont *et al.*, 1977b; Goullet, 1978).

Thus, the type-3 fimbriae of *Klebsiellae* were distributed through three antigenic groups. None of these was related to the type-3 fimbriae of *Proteeae*, which appeared to constitute at least five groups, each species-specific.

This IEM study has established the diversity of antigenic types among the type-3

fimbriae of Enterobacteriaceae and the complexity of their inter-relationships. Although it is not yet possible to decide whether the type-3 fimbriae of members within any group are identical or closely related, further experiments with fimbrial antisera absorbed on an intragroup basis may reveal minor antigenic differences among them. In time, receptor studies may also assist analysis of the type-3 fimbriae, just as in the reclassification of the fimbriae of *Escherichia coli* that were formerly classed as mannose-resistant and eluting (MRE) on the basis of the common property of their associated MRE haemagglutinins (Duguid, 1964; Duguid and Old, 1980). The classification of the fimbriae associated with tanned erythrocyte haemagglutinins is of similar convenience, and it too may require further assessment as information accumulates. This preliminary IEM study has indicated some points of taxonomic interest that require clarification; it provides a useful basis for future studies.

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