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Naturally occurring strains of *Salmonella paratyphi B* unable to form adhesive (type-1) fimbriae

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SUMMARY

The production of MSHA^{+}* (type-1 fimbriate) recombinants was observed in transductional crosses between different pairs of naturally occurring strains of *Salmonella paratyphi B*. MSHA^{+} recombinants were readily produced in transductions from MSHA^{+} donor strains to MSHA^{-} (type-2 fimbriate or non-fimbriate) recipient strains, and less frequently between some pairs of MSHA^{-} strains. The genetic evidence suggests that there are at least three different clones among MSHA^{-} strains of *S. paratyphi B*. Relationships between the different strains and their possible origins are discussed.

1. INTRODUCTION

In enterobacteria, different types of fimbriae with different adhesive and haemagglutinating properties have been described (Duguid & Old, 1980), the most common being type-1 fimbriae (Duguid, Anderson & Campbell, 1966). Although their function remains undefined, their presence is associated with properties of some interest, e.g. phenotypically type-1 fimbriate bacteria adhere to a wide range of cellular substrates including erythrocytes and epithelium (Duguid & Gillies, 1957; Duguid, 1959; Duguid *et al.* 1966). Adhesion to erythrocytes affords a ready, simple means for detecting type-1 fimbriate bacteria which agglutinate guinea-pig or fowl erythrocytes (Duguid *et al.* 1955), a reaction which is mannose-sensitive (MS), i.e. inhibited by D-mannose and related carbohydrates (Duguid & Gillies 1957; Old, 1972). Furthermore, when type-1 fimbriate bacteria are incubated statically in air in liquid media, the production of type-1 fimbriae is associated with the promotion of early, pellicular growth (Duguid & Gillies, 1957; Old *et al.* 1968). The finding that both haemagglutinating and pellicle-forming activities are specifically inhibited by D-mannose (Old *et al.* 1968; Old & Duguid, 1970) suggests that each is dependent on the same surface structure of the type-1 fimbriae (Duguid & Old, 1980).

* MSHA = D-mannose inhibitable agglutination of fowl or guinea-pig erythrocytes by type-1 fimbriate bacteria.

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In some serotypes of *Salmonella*, e.g. *S. paratyphi B*, *S. pullorum-gallinarum* and *S. typhimurium*, type-2 fimbriae have been described which in number, dimension and antigenic specificity resemble type-1 fimbriae, yet lack the latter's haemagglutinating properties (Duguid *et al.* 1966, 1975; Old & Payne, 1971). For example, a study of 135 strains of *S. paratyphi B* with the electron microscope and by haemagglutination (HA) tests showed: that 106 type-1 fimbriate strains were MS haemagglutinating ($MSHA^+$) and that 10 non-fimbriate and 19 type-2 fimbriate strains were $MSHA^-$ (Duguid *et al.* 1966). Type 2 fimbriate salmonellae which are non-adhesive in HA tests are also unable to form fimbrial pellicles in static cultures (Old *et al.* 1968).

In *S. typhimurium*, type-1 fimbriate transductants are readily selected from motile recipients by culture of transductant-recipient mixtures in aerobic static broth (Old & Duguid, 1971); under these conditions, aerotactic migration combined with the ability of type-1 fimbriate bacteria to grow in a surface pellicle with access to atmospheric oxygen enables the type-1 fimbriate (adhesive) transductants to outgrow parental non-adhesive bacteria (Old & Duguid, 1970, 1979). In a study with *S. typhimurium*, the occurrence of recombination to type-1 fimbriation was used to show that fertile bacteria bearing different *fim* mutations at different intragenic sites had descended from different, ancestral mutant bacteria (Old & Duguid, 1979).

The *fim* gene(s) in *S. paratyphi B* is similarly transmissible with phage P22 (Old *et al.* 1968). The purpose of this paper is to report transduction studies in which different pairs of $MSHA^-$ strains of *S. paratyphi B* were crossed in order to establish whether recombination to type-1 fimbriation occurred.

2. MATERIALS AND METHODS

(i) *Bacteria.* Strains of *S. paratyphi B* were naturally occurring isolates from the series previously examined for fimbriae and HA characters (Duguid *et al.* 1966). They were phage typed at the Division of Enteric Pathogens, Colindale, London; biotypes were determined by, and the biotype nomenclature followed that detailed in, the system of Duguid *et al.* (1975). Their phage types, biotypes, source, origin and type of fimbriation are detailed (Table 1). Two strains were type-1 fimbriate, 10 strains were type-2 fimbriate and 4 strains were non-fimbriate. Five strains were represented by replicate isolates (see Table 1), each from a distinct epidemic focus. The results for the replicate isolates are given collectively for each of the 5 strains (Table 2).

(ii) *Bacteriophages.* In most experiments the generalised transducing phage P22 was used; in a few experiments, other A-type phages (Boyd, 1950) were employed. Phages were maintained as lysogenized cultures of *S. typhimurium* S375, indicator strain Q1 of Boyd (1956). Preparation, titration and preservation of phage lysates followed the methods of Old & Duguid (1971, 1979). Satisfactory transducing lysates contained $1–10 \times 10^9$ phages/ml.

(iii) *Media.* Nutrient Broth No. 2 (used in 8–10-ml amounts in cotton wool-stoppered tubes) and Nutrient Agar CM3 were Oxoid preparations.

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(iv) *Antisera.* Salmonella agglutinating sera – *O*4, *H*b and *H* 1, 2 – were commercial products (Wellcome Reagents Ltd, Beckenham).

(v) *Detection of fimbriate bacteria.* Bacteria with type-1 fimbriae were detected by the presence of MSHA of guinea-pig erythrocytes in a rocked-tile test (Dunguid *et al.* 1955, 1966).

Table 1. *Strains of* Salmonella paratyphi B *used in transduction experiments*

Strain no. (number of isolates)	Phage* type	Biotype	Date of isolation	Source	Place of origin	Type of fimbriae
S1076 (1)	1 var. 1	3b	pre-1965	Man	Australia	1
S2422 (1)	3aI var. 4	11bghz	1965	Environment	U.K.	1
S66 (1)	1	7bg	1939	Un	Denmark	2
S11 (1)	3a	3bgh	1954	Man	U.K.	2
S1309 (2)	3a	3bgh	pre-1960	Man	Australia	2
S1313 (1)	3b	11bhi	pre-1960	Man	Australia	2
S1361 (1)	3b	3bgh	1962	Egg	China	2
S1363 (1)	Taunton	3bgh	1961	Egg	Denmark	2
S2419 (1)	3aI var. 1	3bg	1965	Environment	U.K.	2
S2435 (1)	Beccles var. 3	3bgh	1965	Man	U.K.	2
S953 (3)	Taunton	3bgh	1961	Man	U.K.	2
S2453 (4)	Taunton	11bghi	1961-5	Man	U.K.	2
S2410 (4)	3a var. 2	3bghz	1965	Man	U.K.	0
S2413 (4)	3a var. 4	3bghz	1965	Man	U.K.	0
S2418 (1)	3aI var. 1	11bhi	1965	Man	U.K.	0
S2421 (1)	3aI var. 4	3bghz	1965	Man	U.K.	0

* For phage-type designations – see Anderson (1964).
Un = unspecified.
0 = non-fimbriate.

(vi) *Transduction experiments.* The technique was similar to that described for *S. typhimurium* by Old & Duguid (1979). A log-phase broth culture of recipient bacteria (*c.* 1×10^8 bacteria/ml) and P22 lysate from a donor strain were mixed (multiplicity of 1–5) and the phage-bacteria mixture incubated at 37 °C for 4 days without disturbance under microaerophilic conditions (i.e. in an anaerobic jar, from which *c.* 90% of the air had been removed) which are even more favourable for selection of type-1 fimbriate bacteria than static, aerobic culture (Old, 1963).

'No-phage' control cultures were included in each experiment to detect the spontaneous origin of type-1 fimbriate mutants from recipient bacteria.

Transduction test and control broth cultures were examined for the presence of $MSHA^+$ (type-1 fimbriate) bacteria in tests with centrifuged bacteria from 4 day-old broth cultures. From each $MSHA^+$ culture, an $MSHA^+$ clone was isolated, confirmed serologically as *S. paratyphi B* and biotyped.

(vii) *Statistical analysis.* Pooled results from different categories of crosses were analysed by the χ^2 test using Yates's correction, and P values obtained from standard tables.

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3. RESULTS

The aggregated results of the transduction and 'no-phage' control tests with the different groups of donor and recipient strains of *S. paratyphi B* are given (Table 2). It should be noted that type-1 fimbriate ($MSHA^+$) bacteria were not detected in the 'homologous phage' control cultures of the recipient strains, and that only three type-2 fimbriate and two non-fimbriate strains rarely back-mutated to the $MSHA^+$ state (in *c*. 3% of their cultures, Table 2).

(i) *Transduction from* $MSHA^+$ (*type*-1 *fimbriate*) *donors*

Phage propagated on the $MSHA^+$ (type-1 fimbriate) strains S1076 and S2422 produced type-1 fimbriate recombinants from each of the recipient strains (Table 2). The donor strains were equally competent in their donation of *fim* and together gave positive results in 133/163 tests with the 13 recipients (Table 2). That the type-1 fimbriate bacteria detected were recombinants seems likely, for spontaneous $MSHA^+$ mutant bacteria were obtained from these same recipients in only 6/402 'no-phage' control tests ($P < 0{\cdot}001$). It was not possible to determine the proportion of type-1 fimbriate transductants originally produced in the phage-recipient bacteria mixtures because the $MSHA^+$ bacteria could be detected by HA testing only after their selective outgrowth after 96 h incubation microaerophilically after which time they had formed *c*. 10–50% of the population. The frequency of transduction of *fim* might have been low, however, for negative results were obtained in a proportion of the tests with competent pairs of donors and recipients.

(ii) *Transduction from* $MSHA^-$ *donors to* $MSHA^-$ *recipients*

Some transductional crosses from among the different pairs of $MSHA^-$ strains gave $MSHA^+$ recombinants. However, the naturally occurring type-2 or non-fimbriate strains, in which the *fim* gene was in a mutated form, and which were relatively stable in their $MSHA^-$ character, were less effective as donors of *fim* than type-1 fimbriate strains (Table 2). Thus, even the type-2 fimbriate strain S66, which was an unusually competent donor of *fim* with each of the other eight type-2 fimbriate strains as recipients, gave fewer positive crosses yielding $MSHA^+$ recombinants (in 12/62 tests) than when competent type-1 donors were used (Table 2). Strain S66 was also a most competent recipient in tests with the other seven type-2 strains as donors, being transduced to $MSHA^+$ in 15/49 tests.

The different classes of $MSHA^- \times MSHA^-$ crosses will now be presented.

(*a*) *Type-2 fimbriate* × *non-fimbriate*. $MSHA^+$ recombinants were obtained from seven of nine type-2 fimbriate recipients in tests with three non-fimbriate donors and from three of four non-fimbriate recipients in tests with eight type-2 fimbriate donors at frequencies greater than those at which $MSHA^+$ bacteria were recovered from 'no-phage' control tests with these same type-2 fimbriate strains ($P < 0{\cdot}05$) and non-fimbriate strains ($P < 0{\cdot}01$) as recipients. The apparent unreactivity of

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Table 2. *Transduction of type-1 fimbriation between strains of* S. paratyphi B *of different fimbrial types*

Fimbrial type of donor and donor strain no.	Number of tests in which MSHA$^+$ (type-1 fimbriate) bacteria were obtained/number of tests made with MSHA$^-$ recipient strains that were: type-2 fimbriate									non-fimbriate			
	S1309	S1313	S1361	S2419	S1363	S11	S953	S2435	S66	S2410	S2413	S2418	S2421
Type-1 fimbriate													
S1076	8/10	4/6	5/8	1/6	5/6	5/6	13/14	5/6	5/6	9/13	7/8	2/2	1/2
S2422	8/8	2/4	3/6	2/2	6/6	5/6	10/10	2/2	6/6	10/10	7/8	·	2/2
Type-2 fimbriate													
S1309	0/18	0/11	0/9	0/9	0/9	1/11	0/23	0/9	2/9	4/34	0/22	0/4	0/8
S1313	0/10	0/4	0/4	0/4	0/4	0/4	0/12	0/4	1/4	1/15	0/16	0/1	0/3
S1361	0/10	0/8	0/4	0/4	0/4	0/4	0/12	0/4	0/4	0/14	0/12	0/1	0/3
S2419	0/8	0/7	0/3	0/3	0/3	0/3	0/9	0/3	3/3	4/12	0/6	0/1	0/1
S1363	0/10	0/5	0/5	0/5	0/5	0/5	0/15	0/5	1/5	1/17	0/16	0/2	0/4
S11	0/14	0/7	0/5	0/5	2/9	0/7	0/19	1/5	2/9	4/13	1/16	0/2	0/4
S953	0/34	0/15	0/11	0/11	2/15	1/13	0/42	3/11	6/15	8/35	1/43	0/4	0/10
S66	1/14	1/6	1/4	1/4	1/8	2/6	4/16	1/4	0/8	0/20	7/16	0/2	4/8
Non-fimbriate													
S2410	1/26	0/14	1/12	0/6	1/12	1/12	2/18	4/16	0/22	0/45	3/40	0/4	2/14
S2413	0/34	0/13	0/13	1/5	0/13	0/13	0/15	3/21	0/29	1/41	0/49	0/4	0/13
S2421	0/8	0/4	0/4	1/2	0/4	0/4	0/6	0/2	0/4	1/29	1/16	0/2	0/4
'*No-phage*' *control*	1/39	0/20	0/21	0/19	0/22	1/22	0/58	1/21	0/25	2/70	1/64	0/4	0/17

· = cross not performed.

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S2418 probably reflects that it was seldom tested as a recipient and could not be tested as a donor.

(*b*) *Type-2 fimbriate* × *type-2 fimbriate.* In tests in which eight type-2 fimbriate strains as donors were crossed with each of nine type-2 fimbriate strains as recipients, MSHA$^+$ recombinants were detected in 37 out of a total of 631 tests and from 14 of 35 'different pair' crosses performed. Although seven and nine strains, respectively, were involved as donors or recipients in the positive 'different pair' crosses, the most reactive type-2 fimbriate strain (S66) was implicated in 27 of 37 positive tests and 8 of 14 positive 'different pair' crosses. As a donor, it was fertile with each of the other eight type-2 fimbriate strains; most of the reciprocal crosses were also positive (Table 2). Furthermore, back mutation to type-1 fimbriation was not demonstrated in any 'no-phage' or 'homologous phage' test with strain S66.

The 'different pair' crosses among the eight type-2 fimbriate strains other than S66 accounted for the remaining 10 positive tests in this section; but MSHA$^+$ bacteria were obtained only irregularly at a frequency not significantly greater than that in the 'no-phage' controls of these strains ($P > 0{\cdot}1$). Furthermore, although there were six 'different pair' crosses in which MSHA$^+$ bacteria were detected, none of the four reciprocal crosses that could be tested (strain S2435 did not propagate A-type phages) yielded positive results, and strains S11 and S2435, involved in five of the six positive 'different pair' crosses, occasionally reverted to type-1 fimbriation and MSHA positivity (Table 2).

(*c*) *Non-fimbriate* × *non-fimbriate.* MSHA$^+$ bacteria were recovered from some of the tests involving six 'different pair' non-fimbriate crosses but at a frequency no greater than that found in their 'no-phage' controls ($P > 0{\cdot}1$). Furthermore, strains S2410 and S2413 which occasionally reverted to type-1 fimbriation and MSHA positivity (Table 2) were involved as donor or recipient in each of the six apparently positive crosses.

(iii) *Phage-type Taunton strains*

Among strains of *S. paratyphi B* recovered from patients with paratyphoid B fever in the U.K., and formerly associated in many cases with imported egg products (Hobbs & Smith, 1955), were those of phage type Taunton (Anderson, 1964). In this collection, seven of the type-2 fimbriate isolates recovered from patients in the U.K. were of phage type Taunton and some were associated with egg products (Professor J. P. Duguid, personal communication). However, as judged by donor ability and revertibility to type-1 fimbriation, these seven isolates fell into two groups: (*a*) strain S953 (represented by three isolates) was stable in its MSHA$^-$ character and was not an efficient donor of *fim* to other MSHA$^-$ strains; (*b*) strain S2453 (represented by four isolates from distinct epidemic foci from 1961–5) was exceptionally unstable in its MSHA$^-$ character and back mutated to MSHA$^+$ in 15/78 'no-phage' tests. Hence it was of no value as a recipient in fimbriation transduction tests. As a donor of *fim* it was as effective as type-1

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fimbriate ($MSHA^+$) strains with each of the other 13 $MSHA^-$ strains as recipeints, giving $MSHA^+$ recombinants in 175/348 tests.

Because the exceptional instability of the $MSHA^-$ character of this type-2 fimbriate strain may have unduly influenced its behaviour as both donor and recipient, results for that strain are not included in Table 2.

(iv) *Characters of $MSHA^+$ transductants*

The isolated $MSHA^+$ transductant clones retained the other biotype characters of the recipient strains. Those examined with the electron microscope were richly fimbriated; since their HA reactions were MS, these were presumably type-1 fimbriae.

4. DISCUSSION

Transductional studies to determine the genetic relatedness among naturally occurring strains of *S. typhimurium* have provided much information about the evolution of the different biotypes of that serotype (Old & Duguid, 1979; May & Old, 1980; Old, Dawes & Barker, 1980). The present study, which attempted to examine similarly the genetic relationships among naturally occurring $MSHA^-$ (non-adhesive) strains of *S. paratyphi B* of different phenotypes (type-2 fimbriate and non-fimbriate), has clearly established that wild-type $MSHA^+$ recombinants were obtained from both non-fimbriate and type-2 fimbriate $MSHA^-$ recipients by transduction of *fim* from $MSHA^+$ donors. It has indicated also their production at a lower frequency from transductional crosses among some different pairs of $MSHA^-$ strains.

Thus, the recombination to $MSHA^+$ in crosses between the type-2 fimbriate strain S66 (class A) and the other eight type-2 fimbriate strains (class B) probably indicates that the sites of the *fim* mutation(s) in strains of classes A and B are independent. Furthermore, the readiness of production of $MSHA^+$ recombinants and the reliability of the indirect method for their demonstration suggest that the respective *fim* mutations in strains of classes A and B were probably not closely linked. Thus, S66, the earliest strain in this collection, might have arisen independently from an ancestral bacterium different from that of the class B type-2 fimbriate strains.

On the other hand, if the general absence of recombination to $MSHA^+$ in crosses among class B strains indicates that the sites of their *fim* mutations are identical or overlapping, the group B strains, though diverse in phage type and biotype (Table 1), might have descended from the same ancestral bacterium. The possibility cannot be discounted, however, that they carry further independent, closely linked *fim* mutations, recombining to $MSHA^+$ at a frequency not detectable in a system of low sensitivity.

Previous serological analysis indicating that the antigens of type-1 and type-2 fimbriae of *S. paratyphi B* were identical led to the suggestion that, as a result of mis-sense mutations, type-2 fimbriae were rendered non-adhesive but serologically

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cross-reacting by the incorporation of incorrect amino acids in the fimbrial protein (Old & Payne, 1971). If, indeed, the *fim* mutations rendering strains type-2 fimbriate are mis-sense mutations in the *fim* structural gene and if, as seems likely, S66 is genetically distinct from class B strains, the putative mis-incorporated amino acids in their respective fimbrial proteins might be different also. Other explanations involving, for example, mutation in control genes, should not be excluded.

The data seem inadequate to allow assessment as to whether the general absence of $MSHA^+$ recombinants from crosses among the four non-fimbriate strains (class C) indicates their genetic homogeneity or a lack of sensitivity in the chosen selection system.

The difficulty in interpreting the outcome of crosses among $MSHA^-$ strains is nowhere better illustrated than in those between the type-2 fimbriate strains of class B and the non-fimbriate strains of class C. It would seem reasonable to argue, for example, that the revertible, non-fimbriate strains such as S2410 and S2413 of class C, carry *fim* mutation(s) different from those in the generally stable $MSHA^-$, type-2 fimbriate strains of class B. The frequence at which positive ($MSHA^+$) cultures were obtained in crosses between strains of these classes might seem to be a reasonable measure of the sensitivity of the selection method used to detect $MSHA^+$ recombination between presumptive, non-identical alleles. Accordingly, because the recovery of $MSHA^+$ bacteria in these crosses ($B \times C$; and, $C \times B$) was, in each case, at a frequency slightly, but significantly, greater than that of the corresponding 'no-phage' controls ($P < 0{\cdot}05$), it is likely that many of the $MSHA^+$ cultures detected in these crosses arose from recombination to $MSHA^+$. Nevertheless, in view of the observed revertibility of strains like S2410 and S2413, it seems probable that one is working at the limits of sensitivity of this particular system.

Thus, results from the present study with $MSHA^-$ strains of *S. paratyphi B* have indicated the probable presence of at least three distinct clones represented by: the type-2 fimbriate strain S66 (class A); the other eight type-2 fimbriate strains (class B); and, the four non-fimbriate strains (class C), the latter recognizable also by their phenotype. If the genetic relationships between and among the strains of classes B and C are to be more fully defined, a more direct method for the selection and reliable demonstration of even rare recombinations to $MSHA^+$ will be required. In that way only will strains with independent, yet closely linked *fim* mutations in classes B and C be identified. Such a method, however, is not yet available.

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