The Effect of Plasmid-determined and Other Characteristics on the Survival of *Escherichia coli* in the Alimentary Tract of Two Human Beings

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INTRODUCTION

Little is known about the influence that different characteristics of bacteria have on their ability to survive in the alimentary tract of healthy human beings. A recent investigation (Smith & Huggins, 1976) in which two human beings consumed mixtures of pairs of cultures of an *Escherichia coli* strain showed that the ColV plasmid promoted survival in the alimentary tract but the ColE plasmid had no observable effect. These studies have now been extended to obtain information on the influence of other bacterial characteristics, both plasmid and chromosomally determined, on survival; the results are reported in this paper.

In an investigation of this nature, the choice of the parent *E. coli* strain is particularly important. Because only poor selection methods exist for detecting many plasmid-determined characteristics, it must be a good plasmid recipient. It should also be capable of surviving in the alimentary tract long enough for adequate observations to be made on each characteristic but not excessively long, otherwise the number of characteristics that can be studied becomes limited. The *E. coli* strain chosen was a good recipient and usually persisted in the alimentary tract of the two human beings used in the investigation for about 6 to 10 d after oral inoculation.

METHODS

Infecting strain of E. coli. This was H209, an O9:K31:H4 strain that had been isolated from the faeces of a healthy human being; it was used as a spontaneous chromosomal mutant resistant to sodium nalidixate.

Preparation of plasmid-carrying derivatives of strain H209. These were prepared by the methods of Smith & Huggins (1976) for ColV and ColVI-K94 (Fredericq, 1963), Smith & Linggood (1971*b*) for Ent, K88 and Hly, Smith (1974) for Vir, Smith & Parsell (1975) for Suc and Raf, Ørskov & Ørskov (1973) for H₂S, and Smith (1970) for R1, R100, de-repressed F, repressed F and derepressed I. F and I were linked to a tetracycline resistance determinant to aid the selection of H209 organisms that had acquired them. The Ent⁺ H209 strain synthesized heat-labile (LT) and heat-stable (ST) enterotoxin; its plasmid had been obtained from a strain of *E. coli* enteropathogenic for pigs. The Hly⁺ Tra⁻ strain had been obtained by transferring a non-conjugative Hly plasmid from an *E. coli* K12 strain to H209 with F and then eliminating F by sodium lauryl sulphate treatment (Tomoeda *et al.*, 1968).

Preparation of chromosomal mutants of strain H209. The mutant that did not produce the K31 antigen (K31⁻) was obtained by visual examination of six plates of MacConkey agar that had been inoculated with the stock H209 culture in a manner that yielded numerous discrete colonies after incubation at 37 °C for 24 h. One colony darker than the rest was shown by Dr B. Rowe of the Central Public Health Laboratory to have the antigenic structure O9:K31⁻:H4. The colicin V-resistant mutant was prepared by the method of Smith & Huggins (1976). All other chromosomal mutants were obtained by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Glover, 1968).

Comparison of the ability of different derivatives of strain H209 to survive in the alimentary tract. Unless stated otherwise, each of the different plasmid-carrying and chromosomal mutant strains of H209 was compared with the Nal^r parent strain from which they were all derived, except for Fla⁻ and Ent⁺ derivatives which were compared with a spectinomycin-resistant mutant of the Nal^r parent strain. Broth cultures of the

derivative to be studied and of H209 were incubated in a shaking water bath at 37 °C for 24 h. The two cultures were then centrifuged at 2500 g for 30 min and the deposits were resuspended in broth to the original volume. They were mixed together in the proportion of 1 ml to 9 ml, one loopful was inoculated on to two plates of MacConkey agar and the remainder (approximately 10^{10} viable organisms) was quickly consumed by one of the human beings; this proportion was chosen because it had been successfully employed in our earlier investigation (Smith & Huggins, 1976). At daily intervals thereafter, approximately 1 g of the faeces of the human beings was shaken with 10 ml distilled water in a Griffin bottle shaker, SGL 200 (stroke 37.5 mm, speed 275 min^{-1}) for 10 min; then 0.3 ml samples were inoculated on to plates of MacConkey agar containing $20 \mu\text{g}$ sodium nalidixate ml^{-1} . Fifty of the *E. coli* colonies that grew on the plates were examined to see whether they consisted of organisms of the plasmid-carrying or mutant derivative of H209 or of H209 itself; 50 colonies from the inoculum were similarly examined. In the earlier stages of each experiment only two plates of medium were inoculated with each faecal specimen but in the final stages as many as six plates were inoculated to obtain the necessary 50 colonies. The ColV and the ColVI-K94 plasmids were both F-like, the former being repressed and the latter de-repressed; strains carrying the latter plasmid synthesized colicin Ia in addition to colicin V. Because strain H209 is moderately sensitive to colicin V, the strains carrying these two plasmids were compared with a mixture of H209 and a colicin V-resistant derivative of it, instead of only with the sensitive strain; there was no difference in the results.

RESULTS AND DISCUSSION

Of the 14 plasmid-carrying strains, only the ColV⁺ strain survived better in the alimentary tract of the two human beings than did the parent strain (Tables 1 and 2). Except in the experiment in human being no. 1 in which it formed the larger proportion of the inoculum, the ColVI-K94⁺ strain survived less well than the parent strain; this was especially noticeable in human being no. 2 and it was confirmed in repeat experiments. In human being no. 1 it was also compared directly with the ColV⁺ strain. Of the 50 isolates examined from the inoculum, 44 were ColVI-K94⁺. The corresponding figures for the 50 isolates obtained from the faeces on days 1 to 9 were 28, 13, 7, 7, 3, 1, 0, 0 and 0, respectively. In both of two additional tests carried out in human being no. 2, a ColVI-K94⁺ derivative of an O78:K80 strain, B188, also survived less well than B188 itself; a ColV⁺ derivative of B188 had previously been shown to survive much better in the alimentary tract of both human beings than B188 itself (Smith & Huggins, 1976). Strain H209 carrying the repressed F plasmid survived as well as the parent strain, but the forms carrying the de-repressed F and I plasmids survived less well, suggesting that the de-repressed state hinders survival. This may also account for the strain containing the ColVI-K94 plasmid surviving less well than the strain containing the ColV plasmid, because the former plasmid is de-repressed whereas the latter is repressed. Other genetic differences between the two plasmids could also be responsible; there is no definite evidence, for that matter, implicating colicin V itself in survival in the alimentary tract (Smith & Huggins, 1976).

The K88⁺ strain survived less well than the parent strain and the remaining plasmid-carrying strains probably survived equally well. These included the R1⁺ and R100⁺ strains which had been included in the present study because they increased the resistance of *E. coli* K12 to the bactericidal activity of serum (Reynard & Beck, 1976); Anderson (1974) had found that R⁺ strains usually survived less well in the human alimentary tract than their R⁻ counterparts. In human being no. 1, the survival of an *E. coli* K12 strain of about 3 d was not increased by the presence of an LT Ent plasmid (Smith & Linggood, 1971a) or an ST Ent plasmid (Smith & Linggood, 1972) in that strain.

Of the chromosomal mutants, the O9⁻:K31⁻, *thy* and *his* strains survived less well in both human beings than the parent strain and the K⁻ and *lac* strains probably survived equally well. Similar results were obtained when the Fla⁺/Fla⁻ experiment in human being no. 1 was repeated; the evenness of the distribution of the Fla⁺ and Fla⁻ organisms in stool specimens was established by examining five discrete portions of each stool on each occasion. The median (with the range in parentheses) of the number of 50 isolates that were Fla⁺ in the five portions of the stool examined on each of these 7 d was 30 (26 to 36), 28 (26 to 38),

Table 1. Recovery of *E. coli* from faeces of human being no. 1 after ingestion of a mixture of a plasmid-carrying or mutant form of H209 and the parent form*

Form	Number in inoculum (of 50 isolates)	Number in faeces (of 50 isolates) on the following days after ingestion														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ColV ⁺	5	1	5	14	17	14	48	46	50	50	50	—	—	—	—	—
ColVI-K94 ⁺	6	1	0	0	0	1	0	0	0	...	0	—	—	—	—	—
ColVI-K94 ⁺	48	32	37	39	50	50	50	50	50	50	50	50	50	50	50	50†
Ent ⁺	5	2	5	3	1	1	—	—	—	—	—	—	—	—	—	—
Ent ⁺	47	46	50	47	49	47	47	49	47	48	50	—	—	—	—	—
K88 ⁺	45	44	39	22	30	20	19	4	2	7	0	—	—	—	—	—
Hly ⁺ (Tra ⁻)	45	43	39	39	43	31	43	45	45	36	—	—	—	—	—	—
Vir ⁺	4	6	6	4	4	3	6	4	4	—	—	—	—	—	—	—
Suc ⁺	5	2	0	1	0	2	0	1	0	1	1	1	—	—	—	—
Raf ⁺	5	5	4	2	1	0	1	1	2	0	2	0	—	—	—	—
H ₂ S ⁺	5	6	...	7	3	1	0	1	...	3	...	3	—	—	—	—
R1 ⁺	4	4	3	3	3	11	3	0	0	5	0	—	—	—	—	—
R100 ⁺	7	2	3	9	4	5	8	7	4	2	4	8	1	4	—	—
F ⁺ de-repressed	49	44	42	33	27	24	14	—	—	—	—	—	—	—	—	—
F ⁺ repressed	46	48	45	48	50	49	50	48	48	...	46	50	—	—	—	—
I ⁺ de-repressed	44	41	9	12	24	4	1	—	—	—	—	—	—	—	—	—
Fla ⁻	9	4	13	3	1	1	2	10	4	—	—	—	—	—	—	—
Fla ⁻	46	28	26	32	47	45	46	50	49	48	49	50	50	50	50	50†
K31 ⁻	45	46	38	38	47	48	46	45	45	44	...	44	—	—	—	—
O9 ⁻ :K31 ⁻	42	25	23	21	11	14	4	3	4	—	—	—	—	—	—	—
lac	45	...	46	48	49	49	50	50	50	50	49	50	—	—	—	—
thy	46	44	37	25	18	1	0	0	0	0	—	—	—	—	—	—
his	40	32	27	23	27	3	4	...	2	—	—	—	—	—	—	—

..., No faecal specimen available; —, < 50 colonies of the ingested organisms on the culture plates.

* The inocula consisted of approximately 10¹⁰ viable NaI^r organisms, 10⁶ being of strain H209 and the remainder of one of its plasmid-carrying or mutant forms, or vice versa. The organisms were recovered from the faeces by culturing on MacConkey medium containing sodium nalidixate.† The ColVI-K94⁺ organisms disappeared from the faeces after 24 d; the Fla⁻ organisms disappeared after 15 d.

Table 2. Recovery of *E. coli* from faeces of human being no. 2 after ingestion of a mixture of a plasmid-carrying or mutant form of H209 and the parent form

Form	Number in inoculum (of 50 isolates)	Number in faeces (of 50 isolates) on the following days after ingestion														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ColV ⁺	1	5	...	27	...	24	49	50	50	...	50	...	50	...	50	...
ColVI-K94 ⁺	10	2	2	1	1	0	...	...	...	...	...	...	...	...	...	...
ColVI-K94 ⁺	43	5	3	1	0	0	0	0	...	...	...	...	...	...	...	...
Ent ⁺	2	17	...	9	14	14	8	10	7	...	...	...	...	...	...	...
K88 ⁺	43	...	48	43	47	50	28	0	0	0	...	...	...	...	...	...
Hly ⁺ (Tra ⁻)	4	2	0	0	0	0	0	0	0	0	...	...	...	...	...	...
Vir ⁺	7	4	4	7	4	4	1	1	...	...	...	...	...	...	...	...
Suc ⁺	2	0	0	0	0	0	0	0	0	0	0	0	...	...	...	...
Raf ⁺	6	10	4	5	2	0	0	...	...	...	...	...	...	...	...	...
H ₂ S ⁺	4	1	0	0	1	0	1	1	0	1	...	...	...	...	...	...
R1 ⁺	5	11	3	5	4	0	0	3	...	...	...	...	...	...	...	...
R100 ⁺	7	...	1	1	3	1	3	...	1	...	0	2	...	...	...	...
F ⁺ de-repressed	45	38	36	32	20	23	8	...	...	...	...	...	...	...	...	...
F ⁺ repressed	45	45	45	44	40	44	...	46	42	...	...	...	...	...	...	...
I ⁺ de-repressed	44	43	42	29	9	8	...	...	...	...	...	...	...	...	...	...
Fla ⁻	47	15	5	8	8	39	...	...	...	...	...	...	...	...	...	...
K31 ⁻	44	36	32	22	9	40	29	41	...	42	...	...	49	...	...	...
O9 ⁻ ; K31 ⁻	42	...	4	7	4	1	5	6	2	...	...	...	...	...	...	...
<i>lac</i>	45	46	46	44	45	45	46	48	49	48	49	50	50	...	...	...
<i>thy</i>	44	46	44	44	45	33	0	0	0	...	...	...	...	...	...	...
<i>his</i>	44	...	13	23	10	12	5	1	0	0	...	...	...	...	...	...

For other details, see Table 1.

34 (27 to 36), 36 (31 to 39), 37 (33 to 40), 38 (35 to 40) and 38 (31 to 41). One test was carried out in each human being in which the usual Nal^r parent strain was used in place of the Nal^r Spe^r strain, all isolates being individually tested for motility. Again, there was no evidence of Fla⁺ organisms becoming more common in the faeces than Fla⁻ ones. Of the 50 isolates from the inoculum given human being no. 1, 47 were Fla⁻. The corresponding figures for the isolates from faeces on days 1, 10 and 12, were 41, 45 and 50, respectively. Those for human being no. 2 were, for the inoculum, 47 and, for the faeces on days 1, 6 and 7, 48, 49 and 50, respectively. In fact the Fla⁻ state may have enhanced survival.

The conclusions that can be drawn from our experiments are, of course, limited because they were mainly performed on only two human beings and with only one strain of *E. coli*. The fact that this strain persisted for only a short period in the alimentary tract implies that any small differences in colonizing ability which could be important in the long term would have gone undetected. Despite the shortness of this period, it sometimes varied considerably from experiment to experiment indicating that to obtain meaningful comparisons, strains should be tested at the same time, as they were in the present study; comparisons made at different times could yield erroneous results.

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