Transmissible Substrate-utilizing Ability in Enterobacteria

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SUMMARY

Three of 152 strains of *Escherichia coli* transmitted their ability to utilize sucrose (Sac⁺) to other strains by conjugation. The transfer factor of one of them and of a Sac⁺ *Salmonella thompson* strain was thermosensitive. The raffinose-utilizing ability of 27 of 163 *E. coli* strains was also transmissible. Transmissible raffinose-utilizing ability was a feature of porcine enteropathogenic strains possessing the K88 antigen. The determinants controlling raffinose utilization (Raf) and K88 antigen production were commonly transmitted together from these strains; so also was the determinant controlling enterotoxin production, but to a lesser extent. It was not possible to transfer lactose-utilizing ability from 320 strains of *E. coli*, salicin-utilizing ability from 12 strains of *E. coli* or dulcitol-utilizing ability from 99 strains of *E. coli* and 88 strains of salmonellae.

Sucrose- and raffinose-utilizing ability were transmitted separately to several *Salmonella* sp., including *Salm. typhi*, to *Shigella flexneri* and *Sh. sonnei* and to a variety of strains of *E. coli*.

A strain of *Salm. typhimurium* in which Sac had been established and a strain of *E. coli* in which Raf had been established survived less well in the alimentary tract of chickens than their Sac⁻ or Raf⁻ parent strains.

INTRODUCTION

Because we found that the sucrose-utilizing ability of four epidemiologically-related strains of *Salmonella thompson* received from Professor S. S. Kasitiya of the University of Quebec was controlled by a transmissible plasmid (unpublished observation) we decided to determine the incidence in enterobacteria, and especially in *Escherichia coli*, of strains whose ability to utilize substrates sometimes used in their identification was plasmid-controlled. Experiments were also conducted to establish whether plasmid-determined substrate-utilizing ability conferred survival value on organisms inhabiting the alimentary tract.

When this work was nearing completion, Ørskov & Ørskov (1973) reported that six of eleven strains of *E. coli* that transmitted ability to produce hydrogen sulphide also transmitted ability to ferment raffinose, and Le Minor *et al.* (1973) reported that of eleven *Salmonella* strains that fermented sucrose, three, *Salm. agona*, *Salm. ohio* and *Salm. bovis morbificans*, transmitted this property.

METHODS

Bacterial strains. The epidemiologically-unrelated strains examined for transmissible substrate-utilizing ability were maintained on Dorset egg medium at 5 °C. Five strains were used as prospective recipients. These were: (i) *Escherichia coli* K12 Proto, a lac⁺ prototrophic strain that could not utilize sucrose, salicin or raffinose, either resistant to nalidixic acid (nal^r) and spectinomycin (spe^r) or to nalidixic acid and ampicillin (amp^r). (ii) *Escherichia coli* K12 Auxo, a nal^r spe^r lac⁻ strain that required proline, histidine and tryptophan and could

not utilize dulcitol, salicin, sucrose or raffinose. (iii) *Escherichia coli* D282, a *nal^r* prototrophic O8:K⁻:H⁻ strain that did not possess a transfer factor (Tra⁻), could not utilize raffinose, sucrose, dulcitol or salicin and was a good recipient of the plasmid controlling production of the K88 antigen (Ørskov & Ørskov, 1966). (iv) *Escherichia coli* P104, a *nal^r* Tra⁻ prototrophic O139:K82 strain that could not utilize raffinose, sucrose, dulcitol or salicin. (v) *Salmonella typhimurium* 576, a *nal^r* Tra⁻ strain of phage type 36.

Synthetic medium. This contained (g/l): K₂HPO₄, 7; KH₂PO₄, 3; (NH₄)₂SO₄, 1; MgSO₄·7H₂O, 0·1; NaCl, 5; agar 15. Lactose, sucrose, raffinose, dulcitol or salicin (5 g/l) and, when required, growth factors (25 mg/l) and antibiotics (20 mg/l) were added immediately before the medium was poured into Petri plates.

Substrate-utilizing tests. Broth cultures were streaked on to plates of synthetic medium containing the substrate under test and the plates incubated at 37 °C for 48 h. Strains that utilized the substrate as energy source grew as well-developed colonies; those that did not, either did not grow or grew as very tiny colonies. When an impression was required of the numbers of fermenting and non-fermenting organisms in a culture it was streaked on to plates of MacConkey agar base (Difco) containing 0·5 % of the substrate; after incubation at 37 °C for 24 h the proportion of fermenting to non-fermenting colonies was estimated.

The identification of strains whose substrate-utilizing ability was transmissible. Nutrient broth (Oxoid No. 2) was inoculated with 0·02 ml volumes of broth cultures of an enterobacterial strain that utilized lactose, sucrose, raffinose, or salicin (prospective donor strain) and one of the *nal^r* prospective recipient strains. The recipient strains employed for lactose-utilization transmission were *E. coli* K12 Auxo and *Salm. typhimurium* 576; for sucrose-, raffinose- and salicin-utilization transmission was *E. coli* K12 Proto; and for dulcitol-utilization transmission were *E. coli* K12 Auxo and *E. coli* P104. *Escherichia coli* D282 was used as an additional recipient in raffinose-utilization transmission experiments in which the donor possessed the K88 antigen. The mixed cultures were incubated, without shaking, at 37 °C for 24 h and then left at room temperature for another 24 h because it had been noted that some donors transmitted their plasmids at much higher frequencies at temperatures below 37 °C. They were then inoculated on to plates of the synthetic medium and of MacConkey agar base, both media containing the substrate under study and sodium nalidixate. The plates were incubated at 37 °C, the former for 48 h and the latter for 24 h. Any colonies that grew on the synthetic and any red-coloured ones that grew on the MacConkey plates were purified by plating and then inoculated on to the synthetic medium to confirm that they could utilize the particular substrate under test and that they were derived from the recipient strain. The fact that the K12 recipient strains were *spc^r* or *amp^r* in addition to *nal^r* and the availability of antisera against the other recipient strains facilitated their identification.

The rate of Sac transmission at different temperatures. Broth cultures of a strain whose sucrose-utilizing ability (Sac⁺) was transmissible and a *nal^r* prospective recipient strain were both grown at one or other of the temperatures to be studied and then inoculated in 1 ml amounts into the same 10 ml of broth pre-incubated at that temperature. The mixed culture was incubated at that temperature for 24 h. Then the number of prospective recipient organisms present was counted on ordinary MacConkey agar containing sodium nalidixate, and the number that had become Sac⁺ was counted on the synthetic medium containing sucrose and sodium nalidixate; if the donor strain possessed an antibiotic resistance determinant, counts were also made on ordinary MacConkey agar containing sodium nalidixate and the antibiotic to which the determinant conferred resistance.

The effect of passing Sac⁺ strains in broth at different temperatures. Sac⁺ strains were

passed daily in 10 ml broth maintained at 37 or 44 °C, the inoculum for each passage consisting of approximately 10^4 viable organisms. After each passage, the cultures were streaked on MacConkey agar base containing sucrose and the proportions of Sac⁺ and Sac⁻ colonies estimated.

The identification of strains possessing transfer factors only or genetic determinants only. This was achieved by mobilization tests (Anderson, 1965) employing the methods described by Smith & Gyles (1970) and Smith & Linggood (1970). The presence of a transfer factor in a strain was identified by its ability to mobilize a streptomycin/sulphonamide or a tetracycline (Tet) resistance determinant or a Sac determinant in a Tra⁻ strain of *E. coli* K12. Strains of *E. coli* K12 possessing F, I or other transfer factors were used to identify Tra⁻ strains possessing mobilizable determinants [I = I_{drd}(I); Meynell, 1968]. In attempts to mobilize determinants for substrate-utilizing ability, transfer factors linked to a Tet determinant were first introduced into the strains possessing these determinants and the resulting Tet⁺ forms of these strains then mated with an appropriate prospective recipient strain. The first part of this procedure was also employed when it was desired to introduce additional transfer factors into strains that already possessed one.

Elimination of plasmids from strains. This was attempted using acridine orange by the method of Hirota (1960), ethidium bromide by the method of Bouanchaud, Scavizzi & Chabbert (1968) and sodium lauryl sulphate by the method of Tomoeda, Inuzuka, Kubo & Nakamura (1968).

Fertility-inhibiting (fi) character of transfer factors. An F-linked Tet determinant was transmitted to an *E. coli* K12 strain possessing the transfer factor under examination. This Tet⁺ strain was then tested for visible lysis by the MS2 phage (Davis, Strauss & Sinsheimer, 1961). The result was confirmed by phage-testing an *E. coli* K12 Hfr strain to which the transfer factor with a determinant had been transmitted.

Hydrogen sulphide production. The method described by Cruikshank (1970) was employed.

Detection of strains possessing the K88 antigen. This was achieved by slide agglutination tests with appropriate antisera.

Haemolysin production. This was detected by spot-inoculating broth cultures on to plates of 'washed sheep blood' agar (Smith & Halls, 1967).

Detection of production of heat-labile enterotoxin. This was achieved by the rabbit ligated intestine test employing the method of Smith & Linggood (1971).

The survival of Sac⁺ and Sac⁻ organisms of Salm. typhimurium in the alimentary tract of chicks. Approximately 3×10^8 viable organisms of a nal^r strain of *Salm. typhimurium* of phage type 29 and a similar number of organisms of this strain in which a Sac determinant and its transfer factor had been established were mixed and given immediately by mouth to three-day-old chicks. At intervals, cloacal swabs were rubbed gently over the surface of plates of brilliant green agar (Oxoid, CM263) containing 0.5 % sucrose, 20 µg sodium nalidixate and 1 µg novobiocin/ml; few of the organisms normally found in faeces grow on this medium and the colonies of those that do can easily be differentiated by their morphology from those of the *Salm. typhimurium* strain. The plates were incubated at 37 °C for 24 h and the ratio of Sac⁺ (colourless) colonies and Sac⁻ (red) colonies of the *Salm. typhimurium* strain that grew on them estimated. Apart from one group whose diet was supplemented with 1 % sucrose, all the chicks were fed on a commercial diet free from antibiotics.

The survival of Raf⁺ and Raf⁻ organisms of E. coli in the alimentary tract of chickens. The technique was similar to that employed in the previous study except that the chicks were given equal numbers of organisms of a nal^r spe^r mutant of a Raf⁻ O9:K9 strain of *E. coli* and of organisms of this strain into which a Raf determinant and its transfer factor had been

Table 1. *The ability of 320 strains of E. coli to utilize different substrates and to transmit this property*

Substrate	No. of strains that utilized the substrate	No. of strains that transmitted this property
Sucrose	152	3
Raffinose	163	27
Lactose	320	0
Dulcitol	99	0
Salicin	12	0

established. Chick faeces were cultivated on MacConkey agar base containing 0.5% raffinose and 20 μ g each of sodium nalidixate and spectinomycin and 1 μ g of novobiocin/ml.

Bacterial counts. These were performed by the method of Miles & Misra (1938).

RESULTS

The incidence in enterobacteria of ability to utilize different substrates and to transmit this property

The results of examining 320 apparently epidemiologically unrelated strains of *E. coli* for ability to ferment different substrates and to transmit this property are summarized in Table 1. Of these strains, 85 had been isolated from humans, 34 from calves, five from lambs, 127 from pigs, 67 from chickens and two from dogs. About half of them had been isolated from the faeces of healthy subjects; the remainder had been associated with diarrhoea or bacteraemia. None of the strains was found to transmit their ability to utilize lactose, dulcitol or salicin but three (2%) of the 152 strains that utilized sucrose and 27 (17%) of the 163 that utilized raffinose did. The transfer factor responsible for transmission of sucrose utilization in one of the three strains, H155, and transfer factors F and I were used in attempts to mobilize the genetic determinants for sucrose utilization in 50 of the *E. coli* strains; the attempts were unsuccessful.

Apart from the four strains of *Salm. thompson* received from Professor Kasitiya, which our examinations suggested were derived from the same clone and therefore will be referred to subsequently as the Sac⁺ *Salm. thompson* strain, none of 100 *Salmonella* strains (representing a variety of serotypes), 15 *Shigella flexneri* strains and six *Sh. sonnei* strains utilized sucrose. Nor did any of these strains utilize lactose, raffinose or salicin; 88 of the *Salmonella* strains utilized dulcitol but none of them transmitted this character.

The nature of the transmissible sucrose-utilizing ability in the E. coli and Salm. thompson strains

Of the three *E. coli* strains whose sucrose-utilizing ability was transmissible, two, H155 and H156, had been isolated from the faeces of humans and one, F62, had been isolated from the heart of a chicken suffering from bacteraemia.

In preliminary mating studies, *E. coli* H155 transmitted its sucrose-utilizing determinant (Sac) to *E. coli* K12 more efficiently at 28 °C than at 37 °C. Seven of 80 isolates of *E. coli* K12 that had not acquired the Sac determinant of H155 in these matings were found to have acquired its transfer factors (Tra). Of the seven isolates, two had acquired an fi⁺ transfer factor subsequently designated TS (thermo-sensitive), and three an fi⁻ transfer factor subsequently designated TR (thermo-resistant); the remaining two had acquired both

Table 2. The transfer frequency of the sucrose-utilizing determinant (Sac) of *E. coli* H155 and a tetracycline-resistance determinant (Tet) from one strain of *E. coli* K12 to another by the transfer factors of H155 and by transfer factor F

Transfer factors possessed by donor strain of <i>E. coli</i> K12	Proportion* of <i>E. coli</i> K12 recipient organisms acquiring determinant at:									
	22 °C		28 °C		33 °C		37 °C		41 °C	
	Sac	Tet	Sac	Tet	Sac	Tet	Sac	Tet	Sac	Tet
TS	10 ⁻¹	10 ⁰	10 ⁰	10 ⁰	10 ⁻²	10 ⁻²	10 ⁻⁴	10 ⁻⁴	10 ⁻⁵	10 ⁻⁵
TR	10 ⁻⁵	10 ⁻³	10 ⁻⁴	10 ⁻²	10 ⁻⁴	10 ⁻¹	10 ⁻⁴	10 ⁻¹	10 ⁻⁴	10 ⁻²
TS and TR	10 ⁻¹	10 ⁰	10 ⁰	10 ⁰	10 ⁻²	10 ⁻¹	10 ⁻⁴	10 ⁻¹	10 ⁻⁴	10 ⁻¹
F	10 ⁻⁵	10 ⁻³	10 ⁻⁴	10 ⁻¹	10 ⁻⁴	10 ⁰	10 ⁻³	10 ⁰	10 ⁻⁴	10 ⁰

* To the nearest power of 10.

transfer factors. TS and TR and transfer factor F were then used to mobilize a tetracycline-resistance determinant (Tet) in an *E. coli* K12 Tet⁺Tra⁻ strain and to transfer it to an *E. coli* K12 Sac⁺Tra⁻ strain. The latter had been obtained by passaging at 44 °C an *E. coli* K12 strain that had acquired the Sac determinant and TS transfer factor of H155 during mating with that strain (see below). As a result of these mobilization experiments, four different Sac⁺Tet⁺ strains of *E. coli* K12 were obtained, one possessing transfer factor F, one transfer factor TS, one transfer factor TR and one both TS and TR. The effect of incubation temperature on the transmission of Sac and Tet from these four strains to an *E. coli* K12 strain is shown in Table 2. Transfer factor TS transmitted both determinants much more efficiently at the lower incubation temperatures and TR and F transmitted them more efficiently at the higher temperatures. Tet, and to a lesser extent Sac, was transmitted by the strain possessing TS and TR at a high rate at all the incubation temperatures studied.

The Sac⁺ *Salm. thompson* strain possessed only one transfer factor, an fi⁻ one. This transfer factor was closely associated with the Sac determinant and with a Tet determinant which the strain also possessed; the association between the Sac and Tet determinants was less close. For example, all isolates of *E. coli* K12 selected from matings with the *Salm. thompson* strain as Sac⁺ or Tet⁺ were all Tra⁺ and all isolates selected as Sac⁻Tet⁻ were all Tra⁻, but about 10% of Sac⁺ isolates were Tet⁻ and 0.1% of Tet⁺ isolates were Sac⁻. The transfer factor of the *Salm. thompson* strain was more thermo-sensitive than the TS transfer factor of *E. coli* H155 in that it transferred the Sac and Tet determinants very poorly both at 28 and 37 °C. For example, in matings with the *Salm. thompson* strain, the approximate proportions of *E. coli* K12 organisms that acquired these determinants at 37 and at 28 °C were 1 in 10⁶ and 1 in 10⁵, respectively, whereas the proportion that acquired them at 22 °C was 1 in 10².

Both *E. coli* H155 and the Sac⁺ *Salm. thompson* strain were lysogenic but not for *E. coli* K12 strains. Furthermore, their saccharose-utilizing ability could not be transmitted by membrane filtrates (average pore diameter 450 mμ) of broth cultures. It was transmitted during mixed culture at 22 or 28 °C to three strains of *Salm. typhimurium* belonging to different phage types, to one strain each of *Salm. typhi*, *Salm. cholerae-suis*, *Salm. dublin*, *Salm. gallinarum* and *Sh. flexneri*, to one of two strains of *Sh. sonnei*, to three strains of *E. coli* isolated from the faeces of healthy human beings, and to two strains of *E. coli* enteropathogenic for animals. Transmission was not achieved to one strain each of *Proteus mirabilis*, *P. rettgeri* and *Pseudomonas aeruginosa*.

The Sac plasmid was not eliminated from *E. coli* H155 or from the *Salm. thompson* strain

Table 3. *The effect of passaging Sac⁺ strains on their sucrose-utilizing ability*The Sac⁺ strains were passaged daily in broth at 44 °C.

Strain	No. of Sac ⁻ colonies amongst 200 colonies on day:						
	1	2	3	4	5	6	7
<i>E. coli</i> H155	0	0	0	0	0	0	0
<i>Salm. typhimurium</i> possessing the Sac determinant of <i>E. coli</i> H155*	0	0	0	0	0	0	0
<i>E. coli</i> K12 possessing the Sac determinant and the TS Tra of <i>E. coli</i> H155	0	0	0	0	0	0	0
<i>Salm. thompson</i>	0	6	70	150	170	170	180
<i>E. coli</i> K12 possessing the Sac and Tet determinants and Tra of <i>Salm. thompson</i>	0	0	5	30	100	140	160

* Not known whether the TR transfer factor (Tra) of H155 was present in addition to the TS one.

by treatment with acridine orange, ethidium bromide or sodium lauryl sulphate. However, after four passages in broth containing sodium lauryl sulphate, only Sac⁻ organisms were found in cultures of one of three strains of *E. coli* K12 in which the Sac plasmid of H155 had been established; all the organisms isolated from cultures of the other two K12 strains similarly treated were still Sac⁺.

No Sac⁻ organisms were found after seven daily passages in broth at 37 °C of *E. coli* H155, the Sac⁺ *Salm. thompson* strain and three strains in which their Sac determinants had been established. However, after passage at 44 °C they were found in cultures of the *Salm. thompson* strain and an *E. coli* K12 strain possessing its Sac determinant, their numbers increasing with each passage until eventually they were more common than Sac⁺ ones (Table 3); all the Sac⁻ isolates tested were also Tet⁻. No Sac⁻ organisms were found after seven passages at 44 °C in cultures of H155 or in cultures of one strain each of *Salm. typhimurium* and *E. coli* K12 in which the Sac determinant of H155 had been established. All of 20 isolates from the seventh passage of the H155 culture had lost their TS transfer factor and so had 9 of 20 isolates from the passaged Sac⁺ K12 culture; isolates from the passaged Sac⁺ *Salm. typhimurium* culture were not examined for transfer factor activity.

Unlike *E. coli* H155 and the *Salm. thompson* strain, the other two strains that transmitted their ability to utilize sucrose, *E. coli* F62 and H156, did so at very low rates. The proportion of *E. coli* K12 organisms that acquired the Sac determinants of these two strains during mixed culture was never higher than 1 in 10⁷; sometimes no Sac⁺ *E. coli* K12 organisms were isolated. Transfer was never observed at 22 or 28 °C, only at 37 °C. Both strains possessed antibiotic-resistance determinants, those of H156 being transferred at a very high rate to *E. coli* K12 and those of F62 at a moderate rate; their transfer factors were fi⁺. The establishment of transfer factors F or I in an *E. coli* K12 strain in which the Sac determinant of F62 had previously been established increased the transfer rate of that determinant to the extent that approximately 1 in 10⁴ organisms of an *E. coli* K12 recipient strain acquired it during mixed culture with these F⁺ or I⁺ donor strains. The corresponding figure for a similar experiment involving F and the Sac determinant of *E. coli* H156 was 1 in 10⁶. Apart from transferring (on one occasion only) the Sac determinant of *E. coli* F62 to an *E. coli* strain isolated from a human and the Sac determinant of *E. coli* H156 to a *Salm. typhimurium* strain, all attempts to transfer these determinants to the same range of recipient strains that

Table 4. *The classification of 320 strains of E. coli according to source, ability to utilize raffinose and ability to transmit this property*

All the strains associated with diarrhoea were enteropathogenic.

Source of strains		No. examined	No. that utilized raffinose	No. that transmitted this property
Host	Clinical condition			
Humans	Neonatal diarrhoea	25	13	0
	Bacteraemia	19	5	0
	Normal*	41	12	0
Calves	Neonatal diarrhoea	5	0	0
	Bacteraemia	2	1	0
	Normal*	27	21	0
Pigs	Neonatal and post-weaning diarrhoea	51	31	21
	Normal*	72	22	0
Chickens	Bacteraemia	50	44	5
	Normal*	17	8	1
Others	Various	7	6	0

* From faeces of healthy individuals.

had been used in corresponding studies with *E. coli* H155 and the Sac⁺ *Salm. thompson* strain were unsuccessful.

Escherichia coli K12 strains in which the Sac determinants of *E. coli* H155, H156, F62 and the Sac⁺ *Salm. thompson* strain had been established, were grown in peptone water containing 24 different 'sugars' used to identify bacteria. Apart from fermenting sucrose, they had neither acquired nor lost the ability to ferment any of the other substrates except that they now fermented raffinose. Because only about 1 in 10⁶ organisms of cultures of these strains grew into colonies on the synthetic medium containing raffinose as sole energy source, this event was considered mutational in origin.

The nature of the transmissible raffinose-utilizing ability in the E. coli strains

The 320 strains of *E. coli* referred to in Table 1 were classified according to their source, their ability to utilize raffinose and their ability to transmit this property (Table 4). A higher proportion of the strains from pigs affected with *E. coli* diarrhoea than of the strains from other sources transmitted the ability to utilize raffinose. Because of this, an additional 47 apparently epidemiologically-unrelated *E. coli* strains enteropathogenic for pigs were examined and the results for all 98 classified serologically (Table 5) with particular regard to possession of the K88 antigen, known to be controlled by a transmissible plasmid in some porcine enteropathogenic strains (Ørskov & Ørskov, 1966). Cultures of *E. coli* K12 or D282 that acquired raffinose-utilizing ability (Raf⁺) from these enteropathogenic strains were examined, one per donor, for K88 antigen and for two other characters also known to be transmissible in some porcine enteropathogenic strains, haemolysin production (Smith & Halls, 1967) and enterotoxin production (Smith & Halls, 1968). Irrespective of their other antigens, all 41 enteropathogenic strains possessing the K88 antigen utilized raffinose and 37 of them transmitted this property to the *E. coli* K12 or D282 recipient strains. Furthermore, all 37 Raf⁺ cultures of *E. coli* K12 or D282 obtained from these matings also acquired the determinants for K88 production from the donor strains. Fourteen of them also acquired the determinants for enterotoxin production (Ent) but only one had also acquired the

Table 5. *The ability of different serotypes of E. coli enteropathogenic for pigs to utilize raffinose, to transmit this property and to co-transmit other properties*

The K antigens in quotation marks are not Internationally-recognized ones. All the K88⁺ strains were enterotoxigenic and haemolytic; of the K88⁻ strains, all except 11 were enterotoxigenic and all except five were haemolytic.

Serotype	No. of strains examined	No. that utilized raffinose	No. that transmitted this property	No. that co-transmitted* production of		
				K88 antigen	Enterotoxin	Haemolysin
Possessing K88 antigen						
O8:K87,88ab	9	9	9	9	4 (9)	0
O8:K87,88ac	4	4	4	4	1 (4)	1 (2)
O8:K?,88ac	2	2	2	2	2 (2)	0
O45:K'E65',88ac	4	4	3	3	1 (3)	0
O64:K'V142',88ac	1	1	1	1	0	0
O138:K81,88ac	1	1	0	0	0	0
O141:K85ab,88ab	7	7	5	5	2 (4)	0
O147:K89,88ac	4	4	4	4	1 (4)	0
O149:K91,88ac	7	7	7	7	3 (4)	0
O?:K?,88ac	2	2	2	2	0	0
Not possessing K88 antigen						
O9:K9	2	0	—	—	—	—
O45:K'E65'	2	1	0	—	—	—
O64:K?	1	1	0	—	—	—
O101:K?	2	0	—	—	—	—
O138:K81	9	9	0	—	—	—
O139:K82	11	3	0	—	—	—
O141:K85ab → ac	12	0	—	—	—	—
O141:K85ac	18	0	—	—	—	—

* This means that the particular isolate of the *E. coli* K12 or D282 recipient strain selected as Raf⁺ had also acquired the K88, enterotoxin or haemolysin determinant from the donor; testing ten Raf⁺ isolates from the same mating, instead of one, increased the detection rate of donors of enterotoxin or haemolysin production; the final number detected is given in parentheses.

determinants for haemolysin production (Hly). When ten Raf⁺ recipient cultures from each of the 37 matings with the Raf donor strains were examined, instead of one, the number of matings in which Ent transmission was detected increased from 14 to 30 and the number in which Hly transmission was detected increased from 1 to 2; for convenience, the ten colonies in each batch were incubated together in broth before testing for enterotoxin production. Of the 37 Raf donor strains, 14 were resistant to tetracyclines, 14 to streptomycin, 8 to chloramphenicol and 5 to neomycin. Antibiotic sensitivity tests on the 37 Raf⁺ *E. coli* K12 or D282 recipient cultures revealed that 6 of the donor strains had co-transmitted their tetracycline resistance with raffinose-utilizing ability, 3 their streptomycin resistance, 8 their chloramphenicol resistance and 4 their neomycin resistance.

In contrast to the K88⁺ strains, only 14 of the 57 enteropathogenic strains that did not produce the K88 antigen utilized raffinose and none of them transmitted this property. Attempts to mobilize the genes for raffinose utilization in these 14 strains by transfer factors F and I were unsuccessful. Nine of the Raf⁻K88⁻ enteropathogenic strains were mated with a Raf⁺K88⁺ enteropathogenic strain, P233, of antigenic structure O8:K87,88ac. All nine acquired the Raf determinant of P233, the Raf⁺ isolates tested being K88⁺ also.

To study the relationship between the K88 and Raf determinants more closely, four high-transmitting K88⁺ enteropathogenic strains were mated with one or other of five prospective recipient strains. No recipient organisms that were Raf⁺K88⁻ or Raf⁻K88⁺

were isolated from any of these matings – all of 115 Raf⁺ isolates were K88⁺ and all of 160 Raf⁻ isolates were K88⁻. This close relationship, however, was not always noted. For example, three O141:K85ab,88ab strains that were not tested for raffinose utilization until they had been maintained on Dorset egg medium at 5 °C for about two years yielded Raf⁺ and Raf⁻ colonies, all of which were K88⁺. Nor was a close relationship noted when cultures of eight of the K88⁺ enteropathogenic strains were treated with sodium lauryl sulphate or acridine orange. K88⁻ organisms were isolated from all eight as a result, those from seven of them being K88⁻Raf⁺; no Raf⁻ organisms at all were isolated from these seven treated strains. Four of seven K88⁻Raf⁺ cultures were still able to transmit their Raf determinants, but three were no longer able to; of two such cultures selected from one treated strain, one could still transmit its Raf determinant and the other could not.

All 43 strains referred to in Tables 4 and 5 that transmitted raffinose-utilizing ability did so much more efficiently at 37 °C than at 28 or 22 °C. Strains that acquired the Raf determinant did not, as a result, acquire the ability to utilize sucrose or any of the other substrates tested. Some strains were much better donors than others. For example, in matings between 27 donor strains and *E. coli* K12, the proportion of K12 organisms that acquired the Raf determinant varied from 1 in 2 to 1 in 10⁷ (median, 1 in 500). None of the 43 Raf donor strains produced hydrogen sulphide so it was impossible to obtain additional information on the significance of the high incidence of transmissible raffinose-utilizing ability amongst *E. coli* strains that transmit the ability to produce hydrogen sulphide (Ørskov & Ørskov, 1973). The results of fi tests indicated that 12 of 16 enteropathogenic Raf⁺ strains and four of six non-enteropathogenic ones possessed fi⁻ transfer factors, the other six possessing fi⁺ ones.

Raffinose-utilizing ability was transmitted from an enteropathogenic and a non-enteropathogenic Raf⁺ strain of *E. coli* to the same *E. coli*, *Salmonella* and *Shigella* strains into which sucrose-utilizing ability had been transmitted from *E. coli* H155 and from the Sac⁺ *Salm. thompson* strain; the strains that had acquired the Raf determinant from the enteropathogenic strain also acquired its K88 determinant. As in the case of the Sac⁺ strains, the Raf⁺ strains failed to transmit to one strain each of *Pr. mirabilis*, *Pr. rettgeri* and *Pseudomonas aeruginosa*.

The survival of Sac⁺ and Sac⁻ organisms of Salm. typhimurium in the alimentary tract of chicks

The proportion of Sac⁺ and Sac⁻ *Salm. typhimurium* organisms in the faeces of nine chicks that had been given equal numbers of each orally was assessed on eleven occasions during the next 18 days. The Sac⁺ strain had been obtained by establishing the Sac determinant and TS transfer factor of *E. coli* H155 in the Sac⁻ strain. Of the 99 faecal specimens examined, 55 yielded colonies of the *Salm. typhimurium* strain. The proportion of the colonies obtained from each of these 55 specimens that were Sac⁺ varied from 0 to 75% (median 10%); in only three specimens did the Sac⁺ organisms outnumber the Sac⁻ ones. Usually more Sac⁻ than Sac⁺ organisms were found in the contents of the crop, small intestine and caeca of the chicks when they were killed at the end of the experiment.

When the experiment was repeated, but employing food containing 1% sucrose, the results were the same. In this experiment, ten uninoculated chicks were kept in contact with the ten infected ones. They subsequently became infected, the numbers of the Sac⁻ *Salm. typhimurium* organisms isolated from their faeces, crop, small intestine and caeca greatly outnumbering the Sac⁺ ones.

To ensure that the results of these infection experiments were not a reflection of loss of the

Sac determinant by Sac⁺ organisms, they were repeated, the chicks being infected with Sac⁺ *Salm. typhimurium* organisms only. Sac⁻ *Salm. typhimurium* organisms were never isolated from the faeces of these chicks.

*The survival of Raf⁺ and Raf⁻ organisms of an O9:K9 strain of E. coli
in the alimentary tract of chicks*

The proportion of Raf⁺ and Raf⁻ organisms of an O9:K9 strain of *E. coli* in the faeces of ten chicks that had been given equal numbers of both organisms orally was assessed on seven occasions during the next 16 days. The Raf⁺ strain had been obtained by establishing the Raf determinant and transfer factor of a non-enteropathogenic *E. coli* strain in the Raf⁻ strain. During the first seven days after infection, Raf⁺ O9:K9 organisms were found rather more commonly in the faeces of most of the chicks than were the Raf⁻ ones. At the subsequent examinations, however, the reverse was true – at the two final examinations all ten specimens yielded more Raf⁻ than Raf⁺ O9:K9 organisms.

DISCUSSION

There seems little doubt that sucrose-utilizing ability in the *Salm. thompson* strain and in *E. coli* H155 is controlled by transmissible plasmids. The significantly increased transfer rate that resulted from the establishment therein of transfer factors F or I suggested that this might also be the case in *E. coli* F62 and H156, strains which previously had transferred their ability to utilize sucrose at very low frequencies. Both the *Salm. thompson* strain and *E. coli* H155 possessed transfer factors belonging to the thermo-sensitive (TS) class, first described by Terawaki, Takayasu & Akiba (1967) in a strain of *Proteus vulgaris*. The transfer factor of the *Salm. thompson* strain was identified as TS because not only was its Sac determinant transmitted much more efficiently at low temperatures but it was eliminated by incubation at 44 °C. The identification of the TS transfer factor of *E. coli* H155 presented a more difficult problem in that, although its Sac determinant was transmitted much more efficiently at temperatures below 37 °C, it was not eliminated by incubation at 44 °C. However, the Sac determinant in this strain was not linked to the transfer factor and therefore was not a reliable indicator of the presence of the transfer factor; this was demonstrated when all 20 isolates from a culture of *E. coli* H155 that had been passaged at 44 °C were found to have lost their TS transfer factor but to be still Sac⁺. The elucidation of the transfer factor status of *E. coli* H155 was further complicated in that it possessed, in addition to the TS transfer factor, a transfer factor (TR) of the more usual type, i.e. one that transfers most efficiently at 37 °C. The TR transfer factor could transmit the Sac determinant, albeit poorly, and so could transfer factors F and I, indicating the absence of an exclusive relationship between Sac determinants and TS transfer factors. The TS transfer factors of the Sac⁺ *Salm. thompson* strain and of *E. coli* H155 were obviously different from each other, in that they reacted differently to the fi test and transferred Sac most efficiently at different temperatures, the *Salm. thompson* transfer factor at 22 °C and the *E. coli* H155 transfer factor at 28 °C. Le Minor *et al.* (1973) provided no information on the effect of temperature on the frequency with which three *Salmonella* strains they examined transmitted their Sac determinant, so it remains to be discovered whether TS transfer factors are commonly associated with Sac determinants in nature. The fact that the Sac determinants of the *Salm. thompson* strain and of *E. coli* H155 were transmitted at such low frequencies at 37 °C, and the fact that the Sac determinant of H155 did not confer survival value on *Salm. typhimurium* in the alimentary tract of chicks, make it unlikely that these Sac determinants could spread to other entero-

bacteria in the alimentary tract of homothermic animals. Such spread would be more likely to occur in poikilothermic animals or in sewage. Both strains may well have acquired their determinants in either of these two environments.

The apparent lack of contribution of transmissible raffinose-utilizing ability to the survival of *E. coli* in the alimentary tract of chicks also raises the question of the high incidence of strains found to possess this character. However, it was principally a feature of the porcine enteropathogenic strains that possessed the K88 antigen and in these strains the Raf and K88 determinants, although not linked, were closely associated with each other. Although the association between these two determinants and the enterotoxin determinant (Ent) was not so obvious, there was a tendency for all three determinants to be transmitted together. It is difficult to understand how Ent can confer a survival value to organisms possessing it. This is not the case with K88 because it enables *E. coli* organisms possessing it to proliferate in the anterior small intestine of pigs (Smith & Linggood, 1971; Jones & Rutter, 1972), a region which normally contains relatively few bacteria. It may be that Raf and Ent owe their present ecological status, at least in part, to their association with the K88 determinant rather than to any specific contribution to survival they themselves make. It is significant in this respect that naturally-occurring K88⁻ porcine enteropathogenic strains possess a transmissible Ent determinant different from that possessed by naturally-occurring K88⁺ strains (Smith & Gyles, 1970) and that none of the K88⁻ enteropathogenic strains found to utilize raffinose in the present study could transmit that property although transmissible Raf determinants could be established in them.

The examination of recipient organisms that had received the Raf determinant for other transmissible characters possessed by the donor strains, e.g. K88, Ent and resistance determinants, provided an excellent example of how transmissible characters for which good selection methods exist, e.g. Raf, can be utilized to detect transmission of characters for which only poor selection methods exist, e.g. K88 and Ent. These studies also warn against ascribing properties to a particular transmissible determinant when they may, in reality, be those of a different but closely associated determinant.

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