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SHORT PAPER

Surface exclusion by ColV–K94

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SUMMARY

Measurement of ColE1 transfer by ColV2, in *E. coli* K12, showed that ColV2 specifies a surface exclusion system. This system is different from those specified by F*lac*, and by ColVB*trp* and R1-19. Like these other two systems, the ColV2 surface exclusion system is subject to inhibition by the appropriate transfer inhibitor, and is not expressed in stationary phase cells.

It has previously been shown that the two F-like plasmids ColVB*trp* and R1-19 both determine a surface exclusion system different from that specified by F itself (Alfaro & Willetts, 1972). Although cells carrying ColV-K94 (ColV2) showed no surface exclusion in matings with cells carrying F*lac*, ColVB*trp* or R1-19, the possibility remained that ColV2 specified a third, unrelated, surface exclusion system.

Measurement of ColE1 transfer by ColV2 has now shown that this is the case, and that the ColV2 surface exclusion system, like those of F and of R1-19, is inhibited by the appropriate transfer inhibitor and by growth of cells to stationary phase. Attempts to extend this technique to R100-1 failed, since ColE1 was unexpectedly transferred very poorly by this sex factor.

MATERIALS AND METHODS

Bacterial strains. Donor strains, carrying ColE1 together with F*lac*, ColV2 or R100-1, were derivatives of JC6256 (Str^S; Alfaro & Willetts, 1972). Recipient strains were derivatives of ED664 ($ColE1^R$ Lac^- Str^R; Alfaro & Willetts, 1972) for matings with donor strains carrying F*lac* or R100-1, and of ED3801 (a $ColV2^R$ $ColE1^R$ derivative of the Str^R strain JC3272; Alfaro & Willetts, 1972) for matings with the donor strain carrying ColV2.

Media. These have been described previously (Willetts & Finnegan, 1970).

Mating conditions. One ml each of exponentially growing broth cultures of donor and recipient strains, at about 2×10^8 cells per ml, were mixed and incubated at 37 °C for 30 min. All cultures were tested to ensure that $\geqslant 98\%$ of the cells carried the expected plasmid(s).

Flac and R100-1 transfer were determined by measuring the formation of $Lac^+[Str^R]$ and $Tet^R[Str^R]$ progeny of ED664, respectively. ColE1 and ColV2 transfer were measured as described previously (Alfaro & Willetts, 1972). The indicator strain for ColE1 was ED57 (a $ColV2^R$ derivative of JC3272) and for ColV2 was ED664 (which is $ColE1^R$).

Table 1. *ColE1 transfer by three sex factors*

Sex factor in donor	Sex factor transfer	ColE1 transfer
F*lac*	38	39
ColV2	70	44
R100–1	62	2×10^{-2}

The recipient strain was ED664 or ED3801, as appropriate. Transfer is expressed as the number of progeny per 100 donor cells.

RESULTS AND DISCUSSION

The absence of phenotypically distinguishable derivatives of ColV2 previously prevented testing for the presence of a surface exclusion system (Alfaro & Willetts, 1972). However, ColV2 is known to promote efficient transfer of the non-transmissible plasmid ColE1 (Kahn & Helinski, 1964; Table 1), and the frequency of ColE1 transfer should therefore serve as a measure of the frequency of mating between donor cells carrying ColE1 and ColV2, and recipient cells with or without ColV2. Kahn & Helinski (1964) originally used this technique to demonstrate the absence of surface exclusion in matings between donor cells carrying ColV2 and ColE1, and recipient cells carrying F.

The validity of the technique was confirmed in experiments measuring surface exclusion by F*lac* (Table 2). Transfer of ColE1 from donor cells carrying F*lac* and ColE1 was reduced 400-fold by the presence of F*lac* in the recipient cells, and this reduction was abolished either by using stationary phase recipient cultures, or by the simultaneous presence in the recipient strain of the *fin*$^+$ plasmid R100 (the *fin*$^+$ product is one component of the F transfer inhibitor; Finnegan & Willetts, 1972). These results are in close agreement with, and therefore reflect, the known properties of the F*lac* surface exclusion system (Lederberg, Cavalli, & Lederberg, 1952; Willetts & Finnegan, 1970).

Analogous experiments using a donor strain carrying ColV2 and ColE1 showed that ColE1 transfer was reduced 540-fold when the recipient strain carried ColV2 (Table 2.) ColV2 must therefore specify a surface exclusion system. As in the case of F*lac*, surface exclusion by ColV2 was abolished when stationary phase recipient cultures were used, or by the simultaneous presence in the recipient cells of R100. R100 has been shown to inhibit transfer of ColV2, as well as F*lac* (Finnegan & Willetts, 1972).

Despite the close relationship between F*lac* and ColV2, exemplified by their incompatibility (Kahn, & Helinski, 1964; MacFarren & Clowes, 1967) and the similarity of their transfer systems (Alfaro & Willetts, 1972), their surface exclusion systems are different. This is shown by the high levels of transfer of ColE1 by F*lac* to cells carrying ColV2, and by ColV2 to cells carrying F*lac* (Table 2). These results confirm those of previous experiments showing the absence of surface exclusion between cells carrying an F factor and cells carrying ColV2 (Kahn & Helinski, 1964; MacFarren & Clowes, 1967; Alfaro & Willetts, 1972). Furthermore, the surface exclusion system of ColV2 is different from that specified by ColVB*trp* and R1-19 (Alfaro & Willetts, 1972). Three separate surface exclusion systems can therefore be distinguished.

R100-1 transferred ColE1 very poorly (Table 1), preventing investigation of its surface exclusion system by this technique. The low level of transfer was unexpected since all components of the F*lac* transfer system except the *traI* and *traJ* products can be supplied by R100-1 (Willetts, 1971). The *traI* product is not required for ColE1 transfer (Alfaro & Willetts, 1972), and if the *traJ* product serves to induce other transfer genes, rather than form, with their products, a complex directly required for transfer (Willetts, 1971; Finnegan and Willetts, *manuscript in preparation*), it should not be required either. How-

Table 2. *Surface exclusion as determined by ColE1 transfer*

F*lac*, ColE1 donor		ColV2, ColE1 donor	
Plasmid(s) in recipient	Surface exclusion index	Plasmid(s) in recipient	Surface exclusion index
None	1	None	1
F*lac*	400	ColV2	540
R100	2	R100	1
F*lac*, R100	7	ColV2, R100	6
F*lac**	1	ColV2*	15
ColV2	4	F*lac*	6

The surface exclusion index is the ratio of the frequencies of ColE1 transfer to plasmid-free, and to plasmid-containing, strains. A high value therefore indicates a high level of surface exclusion, and corresponds to a low level of transfer.

* These were stationary phase cultures (shaken overnight), diluted to 2×10^8 cells/ml immediately prior to mating. They were compared to similar cultures of plasmid-free strains.

ever, the recent discovery by Grindley *et al.* (1973) of surface exclusion between R100-1 and R136*drp*2 supports the idea that R100-1 specifies a fourth surface exclusion system (Alfaro & Willetts, 1972).

Note added in proof. We have recently found that R538*drd* specifies the same surface exclusion system as ColV2. Also, that R100–1 is surface excluded by R136*fin*$^-$ (85-fold reduction in transfer frequency), but not by R136 itself.

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