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R Plasmids of the S Incompatibility Group Belong to the H2 Incompatibility Group

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Plasmids of the S and H2 incompatibility groups showed thermosensitive transfer and a bacteriophage inhibition phenotype and were incompatible with one another.

We have determined that the plasmids which had earlier been classified as belonging to the S incompatibility group are closely related to and incompatible with H2 plasmids. Previous studies (1, 9, 11) have demonstrated that both H and S plasmids have a thermosensitive mechanism of transfer. In this communication, we present evidence that the S plasmids belong to the H2 subgroup of the H incompatibility group and that the S plasmids share with known H2 plasmids the properties of mutual entry exclusion and bacteriophage inhibition.

S plasmids were described in 1975 after their isolation from *Serratia marcescens* (6), and their temperature-sensitive transfer step was shown to depend on the incubation temperature of the donor (9). Plasmids of the H1 and H2 incompatibility subgroups (earlier designated as H_1 and H_2 subgroups; see reference 1) have been isolated from strains of *Salmonella* found throughout the world (1, 10), and one H2 plasmid has been isolated from *Shigella* (13). In a previous study, representative plasmids belonging to 14 incompatibility groups (A, C, FI, FII, FIV, Iα, J, M, N, O, P, T, W, and X) were tested for their ability to inhibit the development of λ, T1, T4, T5, and T7 phages in R^+ strains of *Escherichia coli* (13). None of the plasmids from these 14 incompatibility groups inhibits the same phages as do plasmids of the H2 group. Therefore, a useful characteristic for identifying H2 plasmids is their ability to inhibit the development of bacteriophages λ, T1, T5, and T7 but not T4 (12, 13). The mechanism of phage inhibition is unknown at present, but deoxyribonucleic restriction without plasmid-mediated modification may be involved (16). Plasmids of the H1 subgroup do not interfere with phage development (12).

Phage inhibition experiments. The plasmids used in this study are shown in Table 1. Plasmids of the H, S, and L incompatibility groups were tested for their ability to reduce the number of plaques and the plaque size of phages λ, T1, T5, and T7. Titrations were performed concurrently with R^+ and R^- cells, using the *E. coli* C derivative RG176 (4). The plasmid of the L incompatibility group, R831, which had been isolated from *S. marcescens* and shown not to have a thermosensitive mechanism of transfer (6), did not inhibit the development of any of the phages. In contrast, both plasmids of the S group reduced the efficiency of plating and plaque size of all four phages (Table 2).

Entry exclusion between S and H plasmids. The *E. coli* K-12 strain RG192. a rifampin-resistant derivative of CSH73 (8), was used for the entry exclusion and incompatibility experiments. The S plasmids were transferred from RG176 to RG192 and to strain RG192 derivatives that contained various H plasmids, as shown in Table 3, during a 3-h mating period at 26°C in Penassay broth (Difco antibiotic medium no. 3). Transconjugant clones were selected by using 100 μg of rifampin per ml and one of the following antibiotics: kanamycin (8 μg/ml); tetracycline (8 μg/ml); ampicillin (24 μg/ml); chloramphenicol (16 μg/ml); or streptomycin (10 μg/ml). The ability of H plasmids to exclude the entry of S plasmids is shown in Table 3. The entry exclusion (Eex) index was approximately 10 when an S plasmid was transferred to the *E. coli* K-12 strain containing an H1 plasmid, but was 100 to 1,000 between and S and an H2 plasmid. Eex indexes of about 10 were obtained previously for matings between strains containing H1 and H2 plasmids, whereas Eex indexes of 100 to 1,000 were obtained for matings between H1 and H1 or between H2 and H2 plasmid-containing strains (13, 14). Similar results were obtained when plasmid transfer was conducted in the opposite direction, that is, when an H1 or H2 plasmid was introduced into RG176 carrying either of

TABLE 1. *Plasmids used in this study*

Plasmid designation	Incompatibility group	Resistance pattern[a]	Species, place, and date or origin	Source or reference
pRG1251	H1	Ap Cm Sm Sp Su Tc	*Salmonella typhi*, Thailand, 1972	
pRG1241	H1	Cm Sm Sp Su Tc	*S. typhi*, Mexico, 1972	12
pRG1241-1[b]	H1	Cm Sm Sp Su	*S. typhi*, Mexico, 1972	pRG1241
pAS251-2	H2	Cm Km Sm Tc	*S. typhimurium*, Canada, 1974	12
pAS251-2-4[c]	H2	Cm	*S. typhimurium*, Canada, 1974	12
pSD296	H2	Sm Tc	*S. typhimurium*, Canada, 1974	Taylor, Shermer, and Grant (submitted)
pSD114	H2	Cm Km Sm Tc	*S. anatum*, Canada, 1974	12
R477	S	Cm Km Sm Sp Su Tc Hg	*Serratia marcescens*, U.S.A., 1969	6, 7
R477-1[d]	S	Sm Sp Su Tc Hg	*S. marcescens*, U.S.A., 1969	N. Datta
R478	S	Cm Km Tc Hg	*S. marcescens*, U.S.A., 1969	6, 7
R831	L	Km Sm	*S. marcescens*, U.S.A., 1969	6, 7

[a] Resistances are designated as follows: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamides; Hg, mercuric ions; Tc, tetracycline.
[b] Tc-sensitive segregant of pRG1241, obtained by mutagenesis with ethyl methane sulfonate.
[c] Spontaneous loss of Km and Sm resistance from pAS251-2T$^-$.
[d] Spontaneous loss of Cm Km from R477.

TABLE 2. *Bacteriophage inhibition by H and S plasmids in Escherichia coli*[a]

Plasmid designation	Incompatibility group	Relative EOP (R^+/R^-)[b]			
		λ	T1	T5	T7
pRG1251	H1	1	1	1	1
pRG1241	H1	1	1	1	1
pAS251-2	H2	10^{-4}	10^{-1}	$<10^{-8}$	10^{-1}
pSD114	H2	10^{-4}	10^{-1}	$<10^{-8}$	10^{-1}
R477-1	S	10^{-1}	0.5	0.5	10^{-1}
R478	S	10^{-1}	0.5	0.5	10^{-1}

[a] *E. coli* C, RG176, was used in all experiments.
[b] EOP, Efficiency of plating. Phage λcI and T5 plaques have a pin-point morphology on the hosts harboring S or H2 plasmids. Phage T1 and T7 plaques are reduced to 1 to 2 mm on the hosts harboring S or H2 plasmids.

the S plasmids; Eex indexes of 10 were obtained between H1 and S and 100 to 1,000 between H2 and S.

Incompatibility between S and H plasmids. Transconjugant clones were selected by using the method and bacterial strains described above. Selection was made for the recipient strain by using 100 μg of rifampin per ml and for the incoming plasmid by using the antibiotic concentrations as given above.

The incompatibility status of the S plasmid with an H1 or H2 plasmid was determined by using a modification of the colony test (15), which has been described elsewhere (13). Five transconjugant clones were diluted in 0.05 M sodium phosphate (pH 7.2) and plated on MacConkey agar without antibiotics. After 16 h of incubation at 37°C, 100 colonies were toothpicked to MacConkey agar, reincubated for 16 h, and replica plated to MacConkey agar containing one of the following antibiotics: kanamycin (8 μg/ml); ampicillin (24 μg/ml); tetracycline (8 μg/ml); chloramphenicol (16 μg/ml); or streptomycin (10 μg/ml).

Incompatibility of each of the S plasmids was observed with both H1 and H2 plasmids. The results of colony tests with R478 are shown in Table 4. The results for R477-1 were very similar. In all the colony tests, the H1 or H2 plasmid was displaced by the entry of the S plasmid. Likewise, when an H2 plasmid was introduced into RG176 carrying either of the S plasmids, the S plasmid was displaced in all trans-

TABLE 3. *Entry exclusion between plasmids of the H and S incompatibility groups*

Incoming plasmid[a]	Resident plasmid[b]	Marker selected[c]	Transfer frequency[d]	Eex index[e]
S(R478)	H1(pRG1251)	Km	2×10^{-4}	10
S(R477-1)	H1(pRG1241-1)	Tc	5×10^{-4}	10
S(R478)	H2(pSD296)	Cm	1×10^{-6}	≥100
S(R477-1)	H2(pAS251-2-4)	Tc	1×10^{-5}	≥100

[a] Host was *E. coli* C, RG176.
[b] Host was *E. coli* K-12, RG192.
[c] Abbreviations as in Table 1, footnote *a*.
[d] Determined from a 3-h mating at 26°C in Penassay broth, measured as transconjugants per donor.
[e] Relative frequency of R-plasmid transfer (S plasmid) by conjugation with a strain of *E. coli* K-12 without and with a second plasmid (H plasmid).

TABLE 4. *Colony test for incompatibility between plasmids of the H1, H2, and S incompatibility groups*[a]

Expt no.	Plasmid		Marker selected[b]	Transconjugant clone	% Daughter colonies containing:		
	Incoming	Resident			Incoming plasmid only	Resident plasmid only	Both
1[c]	H1(pRG1251)	S(R478)	Ap	1	100	0	0
				2	94	6	0
				3	52	32	16
				4	40	32	28
				5	60	38	2
2[c]	H2(pSD296)	S(R478)	Sm	All 5	100	0	0
3[d]	S(R478)	H1(pRG1251)	Km	All 5	100	0	0
4[d]	S(R478)	H2(pSD296)	Cm	All 5	100	0	0

[a] In each experiment, five transconjugant clones were picked, diluted in 0.05 M sodium phosphate, and grown under nonselective conditions as described in the text. From each clone, 100 daughter colonies were picked and tested for the loss of R478 (chloramphenicol or kanamycin resistance), pRG1251 (ampicillin resistance), or pSD296 (streptomycin resistance).
[b] Abbreviations as in Table 1, footnote *a*.
[c] Host was RG176.
[d] Host was RG192.

conjugants tested. When an H1 plasmid is introduced, it is less likely to displace the resident S plasmid, and some transconjugant clones contain both plasmids. In a previous study (14), we have shown that this type of weak incompatibility exists between plasmids of the H1 and H2 incompatibility groups and that retention of the H2 plasmid is usually favored over retention of the H1 plasmid. Both S plasmids are compatible with F*lac*, whereas H1 plasmids are incompatible with F (10). Thus it appears that the plasmids isolated from *S. marcescens* and placed in the S incompatibility group by Hedges et al. (6) belong to the same incompatibility group as H2 plasmids, which have often been found in species of *Salmonella*.

The results described here conflict with those of Hedges et al. (6), who placed the plasmids R478 and R477-1 in the S incompatibility group after testing them against representative plasmids of 23 incompatibility groups, including H1 but not H2 (2, 3, 5). In our study, the S plasmids were incompatible with both H1 and H2 plasmids. We suggest that the weak incompatibility that has been observed between H1 and H2 plasmids (14) may have been responsible for the discrepancy.

The two S plasmids tested here exhibit properties of bacteriophage inhibition, entry exclusion, and incompatibility characteristic of plasmids of the H2 subgroup. Both S and H plasmids exhibit thermosensitive transfer (1-3). We therefore recommend that henceforward plasmids previously classified as S be regarded as members of the H2 incompatibility group.

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