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Compatibility of pTM89, a New F-Like R Factor, and of Derivative Plasmids

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pTM89, an *fi*$^+$ R factor that controls the production of repressed F-type pili, is incompatible with plasmids belonging to the FII and P groups. The results of P1 transduction show that all of the resistance markers of pTM89 are part of a single replicon, which also includes RTF. When the compatibility of different derivative plasmids was investigated, it was found that they fall into two classes. Those of the first class have lost the compatibility of pTM89 for the P group but are still incompatible with FII group, whereas those of the second class are compatible with plasmids of both groups. Plasmids of the latter class that are also compatible with each other and, therefore, apparently lack any determinant for compatibility are genetically stable and self-transmissible. It appears, therefore, that compatibility between plasmids cannot be explained by the hypothesis of competition for a maintenance site.

Compatibility has been largely used for the classification of plasmids since a compatibility group is, by definition, a set of plasmids that cannot stably coexist in pairs inside a cell (7). It has been proposed that incompatibility between plasmids is the result of a competition for a single maintenance site in the bacterial cell (20). In this respect, a compatibility group should be a fairly homogeneous set of elements, but the aberrant behavior of plasmids that are incompatible with those of two different groups has been described for plasmids found in nature (8, 13, 24) or constructed in a laboratory (5). On the other hand, F derivatives defective for incompatibility function have been reported (12, 22, 26).

In the present paper we describe the behavior of an R factor, termed pTM89, that is incompatible with plasmids of two widely separated groups, and of some derivative plasmids that seem to lack the determinants for compatibility.

MATERIALS AND METHODS

Bacteria and plasmids. The bacteria and plasmids used are listed in Table 1.

Media. Nutrient broth and agar, minimal broth and agar, and soft agar were prepared by the method of Clowes and Hayes (3).

Recognition of the type of pili and interaction of plasmids with an F factor. Pili types and plasmid-F factor interaction were determined as described by Datta et al. (10).

Transduction. Transduction with P1 bacteriophage was carried out as described by Lennox (21).

Transfer of R factors. Donors and recipient were overnight, standing broth cultures containing about 10^9 bacteria per ml. Donor and recipient were mixed in a 1:1 ratio and diluted 1:50 with fresh broth. Mixtures were incubated overnight at 37 C, and 0.05-ml volumes of appropriate dilutions were plated on selective media suitable for counting the number of colony-forming units of donor, recipient, and recombinant. Recombinants were selected for chromosomal characters of the recipient and for one resistance marker of the incoming plasmid.

Compatibility of R factors. Recipient clones, carrying two plasmids, derived from crosses in which both donor and recipient were carrying plasmids, were purified on nonselective medium and tested by replica plating for the presence of each plasmid to give a measure of compatibility. When there was evidence of the presence of the antibiotic resistance determinants from the two factors in the same cell, the pairs were used as the donor in conjugation or transduction tests, with appropriate recipients, to show whether the two factors coexisted as separate entities or whether a recombinant plasmid was formed.

RESULTS

The general characteristics of pTM89, an R factor present in a strain of *Escherichia coli* isolated in Trieste, Italy, are given in Table 1, where a number of reference plasmids received mainly from N. Datta are also listed. It has been determined, by using the male-specific phage MS2 (11), that pTM89 controls the production of F-type pili with the character of repressed F-like R factors.

All of the resistance markers of pTM89 are transduced by phage P1 as a single unit, with

TABLE 1. *Bacteria and plasmids used*

Bacterium/plasmid	Compatibility group	Chromosomal characters/ genetic markers[a]	Reference
E. coli K-12 J53-1 F⁻		*lac*⁺ *pro*⁻ *met*⁻ *nal*^{R}	3
E. coli K-12 J62-1 F⁻		*lac*⁻ *pro*⁻ *his*⁻ *trp*⁻ *nal*^{R}	3
E. coli Hfr C		*met*⁻	3
Plasmid			
R386	FI	T *fi*⁺	16
R1	FII	A S C K Su *fi*⁺	16
R136	FII	T *fi*⁺	16
ColB-K98	FIII	Colicin B production, *fi*⁺	16
R124	FIV	S T *fi*⁺	16
F*olac*	FV	Lactose utilization, *fi*⁺	6
Hly-P212	FVI	Hemolysin production, *fi*⁺	23
R64	Iα	S T *fi*⁻	17
R483	Iβ	S Tp *fi*⁻	17
JR66a	Iω	S K *fi*⁺	17
RA1	A-C	T Su *fi*⁻	14
R57b	A-C	A K Su Gk *fi*⁻	14
R46	N	A S T Su *fi*⁻	7
RP4	P	A T K *fi*⁻	10
RP4a	P	A K *fi*⁻	This paper
S-a	W	S K C Su *fi*⁻	15
R391	J	K *fi*⁻	4
R726	H	S T C Su *fi*⁻	14
R69(IP)	M	A T K *fi*⁻	2
R387	K	S C *fi*⁻	15
R^{16}	0	A S T Su *fi*⁻	19
R6K	X	A S *fi*⁻	18
R401	T	A S *fi*⁻	4
pTM89		S C A K T Su *fi*⁺	This paper
pTM71	FII	S C K Su *fi*⁺	Samer et al., in press

[a] Abbreviations denote resistance to: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Gk, gentamycin and kanamycin; Su, sulfonamide; and Tp, trimethoprim.

occasional deletion of the T marker (Table 2). The drug-resistant transductants were able to transfer their drug resistance markers by conjugation. It is evident, therefore, that all of the resistance markers of pTM89 are part of a single replicon that includes also RTF.

When pTM89 or pTM89-1 and pTM89-2, two spontaneous segregants used, when required, to avoid overlapping of resistance, were tested with plasmids of different groups, they proved to be compatible with R386, ColB-K98, R124, F*olac*, Hly-P212, R64, R483, JR66a, R57b, RA1, R46, R391, R726, R69(IP), R387, R^{16}, R6K, R401, and S-a (representative of groups FI, FIII, FIV, FV, FVI, Iα, Iβ, Iω, A-C, N, J, H, M, K, O, X, T, and W). Evidence of incompatibility, on the contrary, was obtained in the tests with R136 and R1 (group FII) and with RP4 (group P) (Table 3). RP4a, a derivative of RP4 obtained by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which lacks the tetracycline resistance marker, was used in some of the tests to avoid overlapping of resistances.

TABLE 2. *Drug-resistant transductants obtained in transduction of pTM89 factor by phage P1*

Selected marker	No. of colonies	No. of transductants with unselected markers:			
		A	C	T	S
A	166	166	166	104	166
S	126	126	126	80	126
T	206	206	206	206	206
C	41	41	41	34	41

It is evident that pTM89 and RP4 could not stably coexist in pairs, the resident plasmid being most frequently eliminated. In some cases, however, the two plasmids do coexist in the same cell with the elimination from pTM89 of the T marker or sometimes of the S and T markers. In fact, pTM89-1, which spontane-

TABLE 3. *Compatibility of pTM89 with R factors of groups FII and P*

Plasmid in the donor	Plasmid in the recipient	Frequency of transfer	Analysis of recombinants			
			No. of tested clones	Plasmid in the subclones		
				Both	Incoming	Resident
pTM89	RP4a	4×10^{-1}	8	144[a]	259	0
RP4a	pTM89	3×10^{-2}	5	371[b]	0	0
pTM89-1	RP4a	5×10^{-2}	7	418[c]	0	0
pTM89-1	RP4	1×10^{-1}	2	101[c]	0	0
RP4	pTM89-1	5×10^{-2}	2	80[c]	0	0
pTM89	R1	$<10^{-6}$		—[d]	—	—
R1	pTM89	3×10^{-5}	5	0	512	14
pTM89-1	R136	4×10^{-1}	4	6[e]	59	11
R136	pTM89-1	1×10^{-3}	3	28[e]	262	0

[a] 130 out of 144 subclones lost the T marker of pTM89.
[b] All of the subclones lost the T marker of pTM89.
[c] Independent transmission of the two factors from the pair by conjugation.
[d] No recombinants formed.
[e] Unstable doubles segregating cells that have lost either factor.

ously lost the T marker, was compatible with RP4. On the other hand, pTM89 and the T⁻ segregant pTM89-1 were both found to be incompatible with R1 and R136 (group FII).

One explanation for these results is that pTM89 has two incompatibility loci, one of which is linked to T and is frequently lost from the plasmid upon introduction of RP4.

A number of derivative plasmids have been obtained in the course of the experiments. The history and characters of these plasmids are as follows: pTM89-1 (markers: S C A Su fi^+), about 10% of the clones carrying pTM89 and of of conjugants and transductants derived therefrom; pTM89-2 (markers: A C fi^+), 1 of 73 replica-plated colonies from the cross J62-1 (pTM89) × J53-1; pTM90 (markers: A C fi^+), 2 of 10 scored clones from the pair J62-1 (pTM89-1) (RP4a), separated from RP4a by P1 transduction; pTM91 (markers: C fi^+), 5 of 45P1 transductants from the pair J62-1 (pTM89-2) (RP4a); pTM92 (markers: A fi^+), from the pair J62-1(pTM89-1) (pTM71) separated from pTM71 by P1 transduction. pTM71 is another F-like R factor isolated in Trieste and also belongs to the FII group (L. Samer, C. Monti-Bragadin, and N. Babudri, Ann. Sclavo, in press).

All of these plasmids are stable genetically, self-transmissible, and control the production of F pili with the character of repressed F-like R factors.

The results of compatibility tests of these plasmids are summarized in Table 4. It is evident that pTM89-2 and pTM90 are incompatible with R136 and in the crosses give the same kind of results as pTM89 and pTM89-1 when these latter plasmids are crossed with R factors of the FII group, namely, the preferential elimination of the resident plasmid.

pTM91 and pTM92, on the contrary, are compatible with R136, with each other, and with plasmids representative of all the F-like groups.

Moreover, pTM91, which is derived from a combination with RP4a, has been tested with the latter plasmid and found compatible with it.

DISCUSSION

pTM89, which controls the resistance to streptomycin, chloramphenicol, ampicillin, and tetracycline, is an R factor remarkable in several aspects. That it exists as a single replicon is evident from the results of P1 transduction (25). However, it is incompatible with plasmids of two widely unrelated groups of plasmids, FII and P. An analogous behavior has been described for R62, a hybrid R factor that is incompatible with plasmids of groups I and N (13). It seems, therefore, that the occurrence of hybrid plasmids in nature is not unusual.

In crosses between pTM89 and incompatible plasmids, loss of unselected antibiotic resistance markers was frequently observed. In most cases, the hybrids so formed were stable and the modified plasmids coexisted in pairs as separate entities.

This fact is reminiscent of the interaction of

group H resistance factors with the F factor, as described by Smith et al. (24).

Anderson (1) also gave evidence that a state of structural instability may exist when incompatible plasmids are present in the same cell, and result in liberation of regions of the affected plasmids.

Less frequently spontaneous segregants of pTM89 were also formed.

All of the derivative plasmids remained self-transmissible and P1 transducible.

The ultimate results of crosses between incompatible plasmids are highly unpredictable, even for factors belonging to a single compatibility group. Elimination of either plasmid, loss of unselected markers, and formation of a joint plasmid are the possible outcomes. It has been reported that the direction of the cross and the individuality of the two crossed plasmids are important variables that affect the result (9, 17). The same holds true for crosses described in the present paper. Loss of the T marker and sometimes of the S marker was the result of introducing RP4 into a strain carrying pTM89, whereas the elimination of the resident plasmid was the most frequent event in the opposite cross. Loss of S and C markers from pTM89-1 was observed when a strain carrying this factor was crossed with pTM71, an R factor of group FII isolated in Trieste, but never when pTM89-1 was crossed with R136 (16), which also belongs to group FII.

Two derivative plasmids, pTM91 and pTM92, the former controlling resistance to chloramphenicol and the latter to ampicillin, proved to be compatible with each other and with all of the F-like plasmids we tested. F-like plasmids have been divided into four compatibility groups by Hedges and Datta (16). A fifth group was subsequently added by Datta (6) and a sixth was added by Monti-Bragadin et al. (23). Had pTM91 and pTM92 been isolated from field material, they would have been taken as prototypes of two additional groups of F-like plasmids. The same conclusion could have been drawn even if they had been produced in the laboratory; however, in this case, there is at least an alternative explanation, namely, they were derived by a process that eliminated the region responsible for their incompatibility. It is not possible to decide whether pTM91 and pTM92 derive from pTM89 by the deletion of some genetic material or are the result of a recombination of the two plasmids present in the pair. However, they definitely lack the compatibility of both plasmids used in the crosses from which they derived.

The reasons why plasmids are incompatible are obscure (7, 13), but whatever explanation is accepted for this phenomenon it should be born in mind that plasmids may exist, as pTM91 and pTM92, which are genetically stable and self-transmissible without possessing any compatibility function. It might be that similar plasmids exist in nature, as for example R124, which is the only representative of its compatibility group (16); i.e., it is incompatible with no plasmid.

F factors defective for incompatibility functions (*inc*$^-$) have been described (12, 22, 26) as being integrated in the host chromosome.

TABLE 4. *Compatibility of derivative plasmids*

Plasmid in the donor	Plasmid in the recipient	Frequency of transfer	Analysis of recombinants			
			No. of tested clones	Plasmid in the subclones		
				Both	Incoming	Resident
pTM89-2	R136	2×10^{-3}	5	0	137	1
R136	pTM89-2	1×10^{-1}	6	34[a]	157	12
pTM90	R136	1×10^{-2}	6	0	133	0
R136	pTM90	1×10^{-1}	6	0	70	0
pTM91	R136	1×10^{-3}	3	129[b]	0	0
R136	pTM91	1×10^{-1}	3	76[b]	0	0
pTM92	R136	3×10^{-4}	12	472[b]	0	0
R136	pTM92	2×10^{-2}	12	394[b]	0	0
pTM91	pTM92	2×10^{-7}	3	93[b]	0	0
pTM92	pTM91	1×10^{-5}	3	70[b]	0	0
pTM91	RP4a	1×10^{-4}	3	97[b]	0	0
RP4a	pTM91	1×10^{-1}	2	55[b]	0	0

[a] Unstable doubles; upon subcultivation they show symmetrical loss of one of the two factors.
[b] Stable pairs; the two factors are transmitted independently by conjugation.

There is evidence that the *inc* mutation prevents their autonomous replication or is not expressed when the episome is replicated autonomously (12). pTM91 and pTM92 are, therefore, different from Inc$^-$ F factors since they are genetically stable, autonomously replicating elements with a normal ability for self-transmission.

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