CONJUGATING ABILITY AND INCOMPATIBILITY OF DEREPRESSED PLASMID pAP11-2

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In the course of a study of the pAP11 plasmid complex, discovered previously [3] in cells of the conditionally pathogenic strain of *Escherichia coli* AP43 (serogroup 020) plasmids pAP11-1 (Tc) and pAP11-2 (Col) were identified. The object of the present investigation was to study the genetic properties of F-like plasmid pAP11-2 in cells of serologically typed and untyped strains of *E. coli* (the plasmids and their genetic markers will be named in this paper in accordance with the recommendations of Novick et al. [9]).

EXPERIMENTAL METHOD

Standard strains of *E. coli* K-12, resistant to streptomycin (C600, AP106) or to nalidixic acid (AP115, AP132), and also serologically typed streptomycin-resistant strains of *E. coli* AP11 (serogroup 025) and AP13 (serogroup 0100) were used.

The senstivity of the bacteria to donor-specific phages and antibiotics, colicinogenicity, conjugation transmission of plasmids, and their ability to inhibit transfer (Tra) functions from one to another were determined by standard methods [4, 6]. Genetic marking of plasmid pAP11-2 was carried out by incorporating into its structure transposons Tn1 (resistance of the bacteria to ampicillin) and Tn9 (resistance to chloramphenicol) by the scheme developed previously [1, 5]. Compatibility (incompatibility) of the plasmids was studied by the usual method [7]. Strains of bacteria containing reference plasmids of different incompatibility groups were obtained from N. Datta (England), and plasmid R455 (Cm) was obtained from H. Tshäpe (East Germany).

EXPERIMENTAL RESULTS

After conjugation transmission of plasmid pAP11-2 (Col) from bacteria of strain AP43 into cells of strains of *E. coli* K-12 it was found to behave in these cells as derepressed for transfer functions. This plasmid was transmitted with high efficiency into cells of various plasmid-free (resistant) K-12 strains, rendering them sensitive to donor-specific phage MS2. For more detailed study of this plasmid its marked variants pAP11-2::Tn1 and pAP11-2::Tn9 were obtained.

Conjugation experiments with marked plasmids showed that the frequency of transmission into cells of serologically untyped strains (K-12) was about 1×10^{-1} to 4×10^{-1} (per cell of the donor strain), whereas they were transmitted into cells of typed strains AP11 (025) and AP13 (0100) with a frequency of about 4×10^{-4} to 4.5×10^{-4}. In all cases cells containing plasmid were sensitive to donor-specific phage, evidence of the effective (derepressed) synthesis of specific F like pili on their surface.

To investigate the character of genetic regulation of Tra functions of the test plasmid pAP11-2, the ability of its marked variance to inhibit transfer functions of reference derepressed plasmids F·lac and R386 in cells of strain AP132, and also to be inhibited by repressed plasmids R1, R100, R455, and pAP41::Tn1 were studied. For this purpose double plasmid transconjugants were obtained — cells of strain AP132 containing two plasmids (test and reference) at the same time. The results of the study of these transconjugants (Table 1) indicate that plasmid pAP11-2 inhibits the transfer functions of reference derepressed plasmid F·lac, reducing the frequency of its transmission from the double transconjugants (compared

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TABLE 1. Properties of Double Plasmid Transconjugants

Introduced plasmid	Resident plasmid	Frequency of transmission of introduced plasmid	Properties of transconjugants: sensitivity to phage MS2	frequency of transmission of plasmids into C600 cells: introduced	frequency of transmission of plasmids into C600 cells: resident	index of decrease in frequency of transmission of plasmids: introduced	index of decrease in frequency of transmission of plasmids: resident
pAP11-2::Tn1	F'lac	$9 \cdot 10^{-3}$	+	$2 \cdot 10^{-1}$—$5,8 \cdot 10^{-1}$	$2 \cdot 10^{-3}$—$7 \cdot 10^{-3}$		27—95
pAP11-2::Tn1	R386	$1,5 \cdot 10^{-1}$	++	$1 \cdot 10^{-2}$—$1,3 \cdot 10^{-2}$	$2 \cdot 10^{-3}$—$3,4 \cdot 10^{-3}$	23—30	0,9—1,6
R1	pAP11-2::Tn1	$5 \cdot 10^{-5}$	—	$1,2 \cdot 10^{-3}$—$1,7 \cdot 10^{-3}$	$1,6 \cdot 10^{-2}$		21
R100	pAP11-2::Tn1	$0,4 \cdot 10^{-1}$	—	$1 \cdot 10^{-3}$—$3,5 \cdot 10^{-4}$	$1 \cdot 10^{-2}$—$1,5 \cdot 10^{-2}$		20—30
R100	pAP11-2::Tn9	$7 \cdot 10^{-4}$	—	$2,4 \cdot 10^{-3}$—$2,5 \cdot 10^{-3}$	$1,9 \cdot 10^{-3}$—$2,6 \cdot 10^{-3}$		115—158
R445	pAP11-2::Tn1	$1,8 \cdot 10^{-4}$	—	$1,1 \cdot 10^{-2}$—$1,1 \cdot 10^{-4}$	$1,2 \cdot 10^{-3}$—$4,8 \cdot 10^{-3}$		27—108
pAP41::Tn1	pAP11-2::Tn9	$2,8 \cdot 10^{-3}$	—	$4,1 \cdot 10^{-3}$—$5,1 \cdot 10^{-3}$	$5,3 \cdot 10^{-3}$—$5,6 \cdot 10^{-3}$		57—61
pAP11-2::Tn1	pAP53::Tn9	$4,3 \cdot 10^{-1}$	—	$5,0 \cdot 10^{-3}$—$6,5 \cdot 10^{-3}$	$4,8 \cdot 10^{-3}$—$6,5 \cdot 10^{-3}$	140—200	180—200
pAP53::Tn9	pAP11-2::Tn1	$2,3 \cdot 10^{-1}$	—	$5,4 \cdot 10^{-3}$—$7,0 \cdot 10^{-3}$	$5,3 \cdot 10^{-3}$—$8,0 \cdot 10^{-3}$	140—180	120—180
—	pAP11-2::Tn1		++		$3,0 \cdot 10^{-1}$—$3,4 \cdot 10^{-1}$		
—	pAP11-2::Tn9		++		$3,2 \cdot 10^{-1}$		
—	F'lac		+		$1,9 \cdot 10^{-1}$		
—	R386		++		$3,2 \cdot 10^{-3}$		
—	pAP53::Tn9		+		$9,8 \cdot 10^{-1}$		

with cells carrying only one plasmid F·lac). Meanwhile it was unable to inhibit the transfer functions of derepressed plasmid R386. In accordance with existing views [8] these data suggest that plasmid pAP11-2 contains a regulator gene (genes) responsible for synthesis of an inhibitory protein (inhibitor), which is active against the control systems of structural Tra-genes of plasmid F·lac, but not of plasmid R386.

Meanwhile reference repressed plasmids R1 and R100, which have a control system of the fin OP type [8] inhibit plasmid pAP11-2 (Table 1). A similar effect also is observed in the case of plasmid R455 and pAP41::Tn1. These results can probably be explained by the presence of a mutation in the system of genetic control of Tra functions of plasmid pAP11-2, making this system insensitive to its own inhibitory protein. Meanwhile this system remains sensitive to inhibitors of certain other plasmids (including those of the fin OP type).

To continue the study of the characteristics of the control system of plasmid pAP11-2 a comparative study was made of this and of derepressed plasmid pAP53, which we previously classed in a new control test system [2]. It will be clear from Table 1 that double transconjugants containing the two above-mentioned plasmids simultaneously are characterized by resistance to phage MS2 and by a reduced frequency of transmission of each of the plasmids, i.e., these plasmids mutually inhibit their own Tra functions irrespective of the order of their introduction into the recipient cells and of the marker used (Tn1 or Tn9). This state of affairs, and also the ability of plasmid pAP41::Tn1 to inhibit Tra functions of plasmid pAP53 [2] as well as plasmid pAP11-2 (Table 1), are evidence of the structural and functional similarity of the genetic control systems of plasmids pAP11-2 and pAP53.

In the final experiments plasmid pAP11-2 (its marked variants) was investigated in compatibility tests with reference plasmids of nine known incompatibility groups of F-like plasmids. The test plasmid pAP11-2::Tn1 proved to compatible in 100% of cases with reference plasmids of groups FIII, FV, FVI, FVII, FVIII, and FIX and with reference plasmid of the FII group in 80-100% of cases. Incompatibility of this plasmid simultaneously with reference plasmids of groups FI and FIV was discovered (the indices of compatibility were 0-5 and 0-17% respectively). Similar results also were obtained in experiments with plasmid pAP11-2::Tn9. Control experiments showed that plasmids pAP11-2::Tn1 and pAP11-2::Tn9 are also incompatible with each other (index of compatibilty 0-5%).

The results are evidence that the Tra functions of plasmids pAP11-2 and pAP53 have common features of their control systems, although these plasmids are different in origin and behavior in tests of compatibility (incompatibility) with plasmids from different incompatibility F-groups.

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TRANSFORMATION OF *Bacillus subtilis* BY UNPURIFIED LYSATES CONTAINING STAPHYLOCOCCAL PLASMID DNA

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Plasmids of some bacteria are known to be able to replicate in cells of other bacteria, sometimes even if taxonomically very far removed from one another [1, 3]. For instance, *Bacillus subtilis* can be transformed by staphylococcal plasmid DNA [7]. It has recently been shown [6] that not only purified plasmid DNA, but also unpurified lysates of staphylococcal cultures possess transforming activity.

The object of this investigation was to study transformation of *B. subtilis* by plasmid DNA contained in unpurified lysates of staphylococci and to study the expression of staphylococcal plasmids in a new host.

EXPERIMENTAL METHOD

Strains *B. subtilis* No. 168 and *Staphylococcus aureus* No. 1 163, possessing a plasmid complex including plasmids of resistance to chloramphenicol, and the penicillinase plasmid controlling simultaneously resistance to cadmium ions, and *Staph. epidermidis* No. 117, possessing resistance to cadmium ions and ability to produce bacteriocin and its nonbacteriocinogenic variant, to which resistance to cadmium ions was transmitted from the wild-type strain in mixed culture.

Unpurified lysates were obtained by the method described previously [6]. A competent culture of *B. subtilis* No. 168 was obtained and the transformation procedure carried out by the method in [5]. Strain *B. subtilis* No. 168 was grown on slopes of nutrient agar (NA) with the addition of citrated donors' blood for 16 h at 37°C. Washings were prepared from this culture in nutrient broth (N), containing 10^8 cells according to an optical turbidity standard; 2.5 ml of washings was tranferred to a test tube and cultured for 37°C with shaking for 4 h. The growing cultures was centrifuged at 3500 rpm for 5 min. The residue was diluted with 1 ml minimal medium of the following composition: ammonium sulfate 0.2%, K_2HPO_4 1.4%, KH_2PO_4 0.6%, sodium citrate ($\cdot 7H_2O$) 0.02%, glucose 0.5%. The resulting suspension of culture was treated with 0.1 ml lysate of a staphylococcal culture. The mixture was kept for 30 min at 37°C, after which 1 ml of NB was added to it and the samples were incubated at 37°C for 2 h. Samples 0.1 ml in volume were seeded on dishes containing one of the following agents for selection: penicillin 0.5 U/ml, chloramphenicol 15 μg/ml, cadmium nitrate ($\cdot 3H_2O$) 10^{-4} M. The seeded dishes were kept at 37°C for 48 h. Dishes seeded with DNA and dishes of the same medium seeded with the original strain without addition of DNA served as the control. The number of transformants was counted relative to the total number of cells of the recipient strain.

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