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

N. A. Ivanov, E. G. Danilova, N. A. Medvedkova, and A. P. Pekhov

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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.

Department of Microbiology, Khabarovsk Medical Institute. Department of Biology and General Genetics, P. Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 78-80, August, 1983. Original article submitted October 1, 1982.

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TABLE 1. Frequency of Transformation of *B. subtilis* by Unpurified Staphylococcal Lysates

Donor strain	Markers of plasmids	Selective agent	Frequency of transformation
Staph. aureus No. 1 163	Penicillinase + resistance to to cadmium	Penicillin, 0.5 U/ml	$1{,}68 \cdot 10^{-10}$
	The same	Cadmium, 10^{-4} M	$2{,}25 \cdot 10^{-10}$
	Resistance to chloramphenicol	Chloramphenicol, 15 μg/ml	$2{,}62 \cdot 10^{-10}$
Staph. epidermidis No. 1 17 nonbacteriocinogenic, resistant to cadmium	Resistance to cadmium	Cadmium, 10^{-4} M	$12{,}39 \cdot 10^{-10}$
Staph. epidermidis No. 1 17 wild type	Bacteriocinogenicity + resistance to cadmium	Cadmium, 10^{-4} M	$20{,}6 \cdot 10^{-10}$

TABLE 2. Spontaneous Loss of Plasmids by Staphylococci and by Transformed Variants of *B. subtilis*

Origin of plasmid and phenotype	*B. subtilis*		Staphylococci		*P*
	total number of colonies studied	% of spontaneous loss	total number of colonies studied	% of spontaneous loss	
Penicillinase plasmid of strain *Staph. aureus* No. 1 163	550	0,54±0,3	552	2,72±0,69	<0,01
Plasmid of resistance to chloramphenicol of strain Staph. aureus No. 1 163	549	51,37±2,13	552	14,3±1,82	<0,001
Plasmid of resistance to cadmium of nonbacteriocinogenic variant of strain Staph. epidermidis No. 1 17	534	10,67±1,34	574	1,22±0,46	<0,001
Plasmid complex of resistance to cadmium and bacteriocinogenicity of wild-type strain Staph. epidermidis No. 1 17	476	3,78±0,87	1000	0±0,63	<0,001

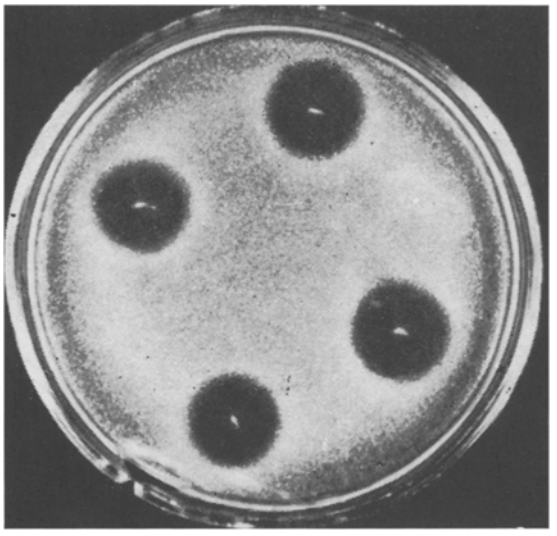

Fig. 1. Formation of zones of inhibition of growth of indicator strain by strain *Staph. epidermidis* No. 117.

Sensitivity to antibiotics and to ions of metal was determined by the usual method [2]. Penicillinase activity was determined by an iodometric method [4]. Spontaneous loss of plasmid was determined by seeding the original strain on laminated NA followed by reseeding of the colonies on dishes with a selective agent and counting the number of colonies which had lost the relevant feature.

EXPERIMENTAL RESULTS

The results of experiments on transformation of *B. subtilis* by unpurified staphylococcal lysates are given in Table 1.

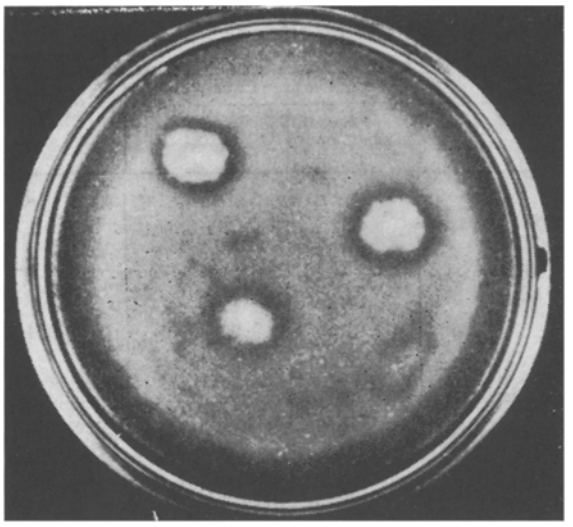

Fig. 2. Formation of zones of inhibition of growth by clones of *B. subtilis* transformed by bacteriocinogenic plasmid.

Table 1 shows that transformation by unpurified lysate of strain *Staph. aureus* No. 1 163 took place with equal frequency during selection of transformants by resistance to cadmium and by resistance to penicillin. All transformants resistant to cadmium produced penicillinase, but transformants resistant to penicillinase also produced penicillinase and were resistant to cadmium ions. This suggests that resistance to cadmium and penicillinase activity are controlled by one plasmid, in agreement with data in the literature [8, 9].

Strain *Staph. aureus* No. 1 163 also possessed resistance to chloramphenicol, evidently connected with the presence of the plasmid. The frequency of transformation during selection for chloramphenicol was close to the frequency during selection for resistance to penicillin and cadmium ions, but not all transformants produced penicillinase and not all were sensitive to cadmium ions. This is evidence that these types of resistance are controlled by different plasmids.

Unpurified lysate from a variant of strain *Staph. epidermidis* No. 1 17 which had acquired resistance to cadmium ions during growth in mixed culture with the wild-type strain was able to transform the *B. subtilis* strain with higher frequency than lysate from strain *Staph. epidermidis* No. 1 17, possessing bacteriocinogenic properties and resistance to cadmium ions, also took place with a higher frequency. Since bacteriocinogenicity is not a selective marker, in this series of experiments selection was carried out for resistance to cadmium ions. Altogether 241 transformants resistant to cadmium ions were tested for their ability to produce bacteriocin. This property was possessed by 8.71 ± 1.82% of clones studied. Bacteriocinogenicity and resistance to cadmium ions in strain *Staph. epidermidis* No. 1 17 are thus controlled by different plasmids, which can form associations with each other and can be transmitted as such by tranformation of *B. subtilis*.

On the whole the experiments showed that unpurified lysates of strains of staphylococci containing plasmids can transform *B. subtilis*. The frequency of transformation coincides with that obtained in similar experiments [6], but is lower than that in experiments with purified plasmid DNA [7].

The next step was to study behavior of staphylococcal plasmids in *B. subtilis*. The penicillinase plasmid of strain *Staph. aureus* No. 1 163 induced resistance in its host to 5 U/ml of the antibiotic. A similar level of resistance was observed also in *B. subtilis* (25 cultures selected for resistance to penicillin and cadmium ions were investigated). The plasmid of resistance to chloramphenicol behaved somewhat differently, for it induced resistance to 250 μg/ml in the staphylococcus and from 100 to 25 μg/ml of the antibiotic in 25 transformants of *B. subtilis* studied.

This property 24 h later was weaker in clones of transformants of strain *Staph. epidermidis* No. 1 17 which had acquired the ability to produce bacteriocin than in the parental strain. Growth inhibition zones formed by strain *Staph. epidermidis* No. 1 17 and by the transformed strain of *B. subtilis* can be compared in Figs. 1 and 2. It must be pointed out

that during culture for 48 h the size of the zones formed by the transformants was close to the size of zones formed by staphylococci.

Table 2 gives data on spontaneous loss of plasmids by strains of staphylococci and transformants of *B. subtilis*. It will be clear from Table 2 that in all cases but one clones of *B. subtilis* lost plasmids significantly more often than the parental strains.

The results thus confirm that unpurified lysates of staphylococci containing plasmids can transform a strain of *B. subtilis*, but replication and expression of the plasmids in the new host follow a less satisfactory course than in the parental strains.

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