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SHORT COMMUNICATIONS

Efficiency of Spontaneous Transformation of *Bacillus subtilis* as Dependent on the Presence of Conjugative Plasmid p19 in the Donor Strain

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Transformation by DNA present in a growing culture is called spontaneous, or natural, transformation. DNA may be released as a result of cell lysis or some other processes impairing the cell envelope; it may occur in the culture liquid in a free state or be adsorbed on the cell surface [1, 2]. Spontaneous transformation has been described for a number of bacteria, including *Bacillus subtilis*. The conditions favoring cell contact (e.g., adsorption of bacteria on suspended solid particles) promotes spontaneous transformation [3, 4].

It is known that conjugation begins with the contact of donor and recipient cells, and this contact can last for a rather long time. The agglutination of cells is due to changes in their surfaces. In donor cells, these changes are determined by the gene products of conjugative plasmids (thus, in *Escherichia coli*, the formation of sex pili is determined by the genes of the F plasmid *tra* operon). Aggregates of donor and recipient cells may include a large number of cells and may be visible to the naked eye as flocks in the mixture of donor and recipient cultures (e.g., in enterococci or *Bacillus thuringiensis*) [5, 6].

In our laboratory, we have been studying conjugation in *B. subtilis*; the conjugative plasmid in these studies is a large plasmid, p19, harbored by the soil strain *B. subtilis* 19 [7]. According to our data, the transfer frequency of plasmid p19 is very high (almost 100% of the recipient strains acquire it [7]). Prolonged contacts of donor and recipient cells are likely to occur in this conjugal mixture. Therefore, it was of interest to investigate the efficiency of the transfer of chromosomal genes occurring in it via spontaneous transformation.

In the experiments on spontaneous transformation, the prototrophic strains *B. subtilis* 19 (p19 pUB110) and *B. subtilis* 19 (pUB110) were used as DNA donors. The latter strain lacks the large conjugative plasmid p19; however, both strains harbor the small plasmid pUB110 containing a gene determining resistance to kanamycin. This plasmid can be mobilized, i.e., it can be transferred via conjugation at the expense of the large plasmid. Auxotrophic mutants of *B. subtilis* 19 were used as the recipients; these mutants lacked the large plasmid and required histidine, leucine, or threonine or had two mutations simultaneously, requiring histidine and cysteine. In addition, all of these strains had chromosomal mutations determining resistance to 5 μg/ml of chloramphenicol and 15 μg/ml of streptomycin. These auxotrophic strains were obtained in our laboratory by nitrosoguanidine treatment.

Cultures of recipient and donor strains grown overnight in liquid LB medium were tenfold diluted with Spizizen medium and grown on a shaker at 37°C for 3 h; the medium in which the auxotrophic recipients were grown contained all the amino acids that they required in a concentration of 50 μg/ml. The grown donor and recipient cultures were mixed in equal volumes and incubated for two more hours. In the experiments involving DNase treatment, a solution of DNase (Calbiochem) in 0.05 M $MgCl_2$ was added, to a final concentration of 500 μg/ml, to the donor and recipient cultures prior to their mixing. After 20-min incubation with DNase, the cultures were mixed and incubated as described above. Then, dilutions of the bacterial mixture were plated onto minimal Spizizen agar supplemented with 5 μg/ml of chloramphenicol and 15 μg/ml of streptomycin. Donor cells could not grow in this medium because they were sensitive to both antibiotics, whereas recipient cells could not grow due to amino acid deficiency. Only recipient cells that had acquired from the donor strain one of the wild prototrophy-determining alleles (e.g., *his*) could yield colonies on this medium. The auxotrophic mutants that we employed virtually did not revert to prototrophy (the reversion frequency was less than 10^{-7}). If the transfer of plasmid pUB110 was to be monitored, the mixture of donor and recipient strains was plated onto Spizizen agar supplemented with the corresponding amino acid, kanamycin (50 μg/ml), chloramphenicol, and streptomycin. On

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Table 1. Transfer of chromosomal genes determining prototrophy and of the plasmid kanamycin-resistance gene in experiments with donor strains possessing and lacking the conjugative plasmid p19

Transferred allele	Donor strains	
	B.subtilis 19 (p19pUB110)	*B.subtilis* 19 (pUB110)
	Number of bacteria with the allele acquired in 0.1 ml of the cell mixture	
His$^+$	2×10^5	1.6×10^3
Kan^R*	2.4×10^5	6.0
Leu$^+$	0.7×10^5	3.3×10^2
Thr$^+$	2.7×10^4	3.4×10^3

* Data on the number of kanamycin-resistant colonies were obtained in experiments with the recipient strain *B. subtilis* 19 (p19pUB110)*his*.

Table 2. Effect of treatment with DNase on the transfer of chromosomal genes determining prototrophy and of the plasmid kanamycin-resistance gene*

Transferred allele	Number of bacteria with the allele acquired in 0.1 ml of the cell mixture	
	with DNase treatment	without DNase treatment
His$^+$	2	8×10^2
Cys$^+$	1	2.4×10^2
Kan^R	7.2×10^2	3.8×10^3

* Donor strain: *B. subtilis* 19 (p19pUB110); recipient strain: *B. subtilis* 19 *his*, *cis*, *str*^{r}, *cm*^{r}.

this medium, only recipient cells that had acquired plasmid pUB110 could grow.

Typical results are presented in Table 1. If a strain harboring plasmid p19 was used as the donor, the number of spontaneous transformants acquiring the wild *his*, *leu*, and *thr* alleles was large (2×10^5, 0.7×10^5, and 2.7×10^4, respectively, in 0.1 ml of the cell suspension). The transformation frequency with respect to these markers was $2–3 \times 10^{-3}$ as calculated per recipient cell. If the strain lacking the conjugative plasmid was used as the donor, the above values decreased 10- to 100-fold for different markers.

We also considered it interesting to determine under the same conditions the efficiency of the mobilization of plasmid pUB110. The mobilization efficiency was nearly the same in all of the strains (in 0.1 ml of the donor and recipient strain mixture, $2–2.4 \times 10^5$ kanamycin-resistant cells appeared, which corresponded to 0.2–0.3 % of recipient cells). If the donor strain lacked the large plasmid, the number of kanamycin-resistant colonies decreased abruptly and was as low as 6–20 colonies per dish inoculated with 0.1 ml of the donor and recipient cell mixture. Apparently, these colonies were produced by both spontaneous transformants and spontaneous kanamycin-resistant mutants.

In these experiments, in cases where donor cells contained the conjugative plasmid, the prototrophic colonies could be yielded not only by transformants, but also by transconjugants that had acquired the prototrophic alleles. To check the latter possibility, we performed experiments that employed pretreatment of partner cells with DNase (see above). A strain carrying two mutations that determined requirements for histidine and cysteine was used as the recipient (although spontaneous transformation occurred in this strain at a lower rate than in the aforementioned strains that carried single auxotrophic mutations). The treatment with DNase virtually completely abolished the emergence of prototrophic colonies after plating of the strain mixture (Table 2). However, the emergence of kanamycin-resistant colonies was affected by DNase treatment to a lesser extent: in the experiment reported in Table 2, their number decreased about fivefold. In some analogous experiments (data not shown), DNase treatment decreased the number of kanamycin-resistant colonies no more than twofold.

Thus, the presence in the donor cells of *Bacillus subtilis* 19 of the large plasmid p19 not only promoted the conjugal transfer of plasmids but also stimulated the transfer of chromosomal DNA by transformation. The transformational transfer of the small plasmid pUB110 was also stimulated, albeit much more weakly. The transfer of small plasmids by spontaneous transformation has already been described earlier [8], but those studies were performed with a laboratory strain *B. subtilis* 168, which exhibits a higher competence than the soil strain *B. subtilis* 19 used in the present study.

It deserves attention that the initial stages of conjugation—a process that in nature primarily results in the transfer of plasmids [9, 10]—also promote gene transfer via transformation in *B. subtilis*. A natural question to ask is whether this species is capable of the conjugal transfer of chromosomal genes, characteristic of many other bacteria. Under conditions of the experiments performed in the present work, the frequency this process, if not zero, was low enough for it to be masked by gene transfer via spontaneous transformation. However, in our laboratory, experiments aimed at clarifying this point are currently in progress.

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