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SHORT COMMUNICATIONS

The Conjugal Transfer of Plasmid pUB110 in *Bacillus subtilis* in Soils of Different Natural Landscapes

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The wide distribution of the horizontal transfer of bacterial genetic material (plasmids and chromosomal genes) is confirmed by direct and indirect evidence, which includes the detection of identical chromosomal genes and cosmopolitan plasmids in evolutionarily diverse bacteria; experimental data on transformation, transduction, and conjugation in model microcosms; and the observation of horizontal gene transfer in natural soil and aquatic ecosystems [1–3]. It should be noted that most of the relevant investigations were performed on enterobacteria, whereas bacilli are much less studied in this respect [4].

For several years, our laboratory has been concerned with the study of conjugation between various strains of *Bacillus subtilis* and other bacilli with the involvement of the large plasmid p19, which was first detected in a *B. subtilis* strain isolated from a Belarussian forest soil. This plasmid is capable of self-transfer on solid and in liquid media and the mobilization of small plasmids (pUB110 in particular) at a rate of about 1% per recipient cell [5]. The study of the mobilization of pUB110 in model microcosms with sterile and nonsterile soils showed that such mobilization may occur at 30 and 22–23°C, being 50 times less frequent in the microcosm with nonsterile soil [6].

The aim of this work was to study the possibility of the conjugal mobilization of plasmid pUB110 in soils of various natural landscapes in the Moscow region (meadow, spruce forest, and garden). The experiments, which were carried out in July and August 2003, revealed the transfer of pUB110 in a meadow soil. The next spring (specifically, in the last decade of March 2004), spores of transconjugants were still detected in the meadow soil.

These experiments were performed by using *B. subtilis* 19(p19 pUB110) as the donor strain and *B. subtilis* 19 lacking the large plasmid p19 [7] as the recipient strain. The donor strain was resistant to 50 μg/ml kanamycin due to the presence of plasmid pUB110, which was preliminarily introduced into the regenerating bacillar protoplasts [5]. The recipient strain was resistant to 15 μg/ml streptomycin and 5 μg/ml chloramphenicol. The gene for chloramphenicol acetyltransferase, which is responsible for chloramphenicol resistance, was transferred to the chromosome of strain 19 from the *Staphylococcus aureus* plasmid pC194 incorporated into the *B. subtilis* chromosome. *B. subtilis* cells resistant to chloramphenicol were plated onto agar media supplemented with a lethal dose of streptomycin (15 μg/ml). The plates were tested for spontaneous streptomycin-resistant mutants. The transconjugants that acquired plasmid pUB110 could grow on LB agar (Sigma) with kanamycin, streptomycin, and chloramphenicol (50, 15, and 5 μg/ml, respectively), whereas the growth of recipient cells on such agar was suppressed by kanamycin and the growth of donor cells was suppressed by streptomycin and chloramphenicol. Experiments were carried out with spruce forest, meadow, and garden soils. The garden is located near the Tarasovskaya station of the Yaroslavl railroad (25 km north of Moscow).

Donor and recipient bacterial cells grown separately on LB agar were washed off from the plates with sterile tap water and inoculated into soils. For this purpose, a portion of soil (about 500 g) excavated from a certain place was mixed with a suspension of the donor and recipient cells and the inoculated soil was placed back. In the course of the experiment, this soil was sampled at regular intervals at a depth of 3 cm. Simultaneously with soil sampling, soil temperature at this depth was measured. The experiment was conducted in July and the first half of August 2003. In the first half of July, the daytime temperatures of the meadow and garden soils were between 22 and 29°C. The temperature of the soil under spruce trees was 2–3°C lower. At night, the temperature of the soils decreased slower than that of the air. By the end of July, the daytime soil temperature slightly decreased.

The soil samples (2 g) were suspended in physiological saline solution (8 ml) in a Shuttel for 20 min and then in a Vortex for 1 min. Large soil particles were allowed to settle, and the supernatant was diluted and plated onto LB agar with kanamycin to determine the total number of viable vegetative cells and spores of the recipient strain and onto LB agar with chloramphenicol and streptomycin to determine the total number of cells and spores of the donor strain. To assay only spores, before plating, the supernatants of the soil suspensions were incubated at 80°C for 20 min to kill vegetative cells [6]. The results were expressed in colony-forming units (CFU). To count transconjugants with plasmid pUB110, LB agar was supplemented with all three antibiotics. To suppress the growth of molds, LB agar was supplemented with 100 μg/ml nystatin.

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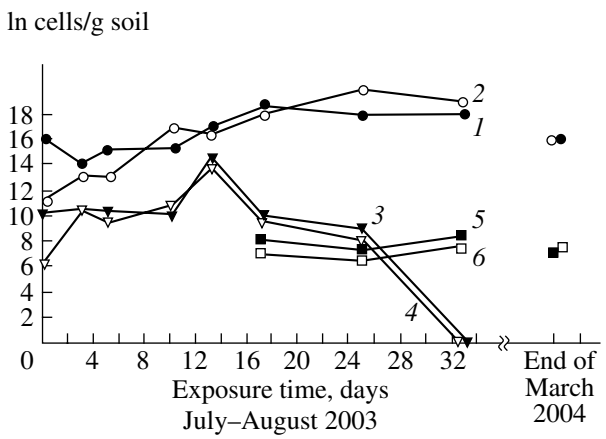

The dynamics of the number of donor, recipient, and transconjugant *B. subtilis* cells in the meadow soil (ln/g soil): (*1*) CFU of the recipient strain; (*2*) spores of the recipient strain; (*3*) CFU of the donor strain; (*4*) spores of the donor strain; (*5*) CFU of transconjugants; (*6*) spores of transconjugants.

At the zero point of the experiment (actually, after several hours of incubation of the inoculated soil), colony-forming units in the soil were mainly represented by vegetative cells (about 100%), the number of donor cells being considerably smaller than that of recipient cells. This could be due to a decrease in the copy number of plasmid pUB110 in the donor cells, which undergo nutritional starvation in the soil. As a result, these cells may become less resistant to kanamycin. In the course of the experiment, virtually all vegetative cells transformed into spores (figure). The number of CFU of the donor strain remained at a constant level for 10 days. By the 13th day, this number increased by two orders, then showing a gradual decline, so that the donor bacteria could not be detected in the soil by the 33rd day of the experiment. The dynamics of the number of recipient cells in the soil was characterized by a 5-fold decrease by the 3rd day, a 20-fold increase (with respect to the original level) by the 17th day, and a steady level during the rest of the experiment. It should be noted that the results presented in the figure refer to the meadow soil. The experiments with the forest and garden soils gave similar results (data not shown).

Transconjugants (both vegetative cells and spores) were only detected in the meadow soil by the 17th day of the experiment. The conjugation rate was approximately 2.9×10^{-5} per viable recipient cell and remained unchanged until the end of the summer. In the next spring, specifically, at the end of March 2004, the CFU values of the recipient strain and transconjugants were found to be the same as they were in the middle of August 2003 (figure). All the CFU were represented by spores.

Thus, we revealed the conjugal transfer of the small plasmid pUB110 in the natural meadow soil but not in the spruce forest and garden soils, although the dynamics of the viable donor and recipient bacterial cells in the soils of all three natural landscapes were similar. The meadow soil under study was clayish and had pH 6.0, i.e., slightly higher than that of the other soils studied (pH 5.5). The possibility cannot be excluded that clay in the meadow soil promotes conjugation, as was shown earlier for another type of horizontal gene transfer (the spontaneous transformation of *B. subtilis*) in experiments with the clay mineral montmorillonite [8]. In central Russia, the average temperature of the upper soil horizons in summer is sufficient for conjugation. The same inference (in particular, that a temperature of 22–23°C is sufficient for the conjugal transfer of plasmid pUB110) was made earlier on the basis of the results of laboratory experiments with the use of nutrient media and model microcosms [5, 6].

The conjugal transfer of plasmids in *B. subtilis* and related bacilli is likely to be widespread in nature, as is evident from the presence of *mob* genes (i.e., genes that are necessary for the conjugal mobilization of small plasmids) in some cryptic plasmids borne by soil *B. subtilis* strains [9].

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