Microbiology, Vol. 71, No. 4, 2002, pp. 488–490. Translated from Mikrobiologiya, Vol. 71, No. 4, 2002, pp. 567–569.

SHORT COMMUNICATIONS

The Kanamycin-Induced Elimination of the Large Conjugative Plasmid p19 from the *Bacillus subtilis* Strain 19 Harboring Also the Small Plasmid pUB110 of Kanamycin Resistance

O. V. Lotareva, V. Z. Nezametdinova, E. A. Fedorina, and A. A. Prozorov

Vavilov Institute of General Genetics, Russian Academy of Sciences, ul. Gubkina 3, Moscow, 117809 Russia

Received December 12, 2001

Like many other bacteria, bacilli harbor a variety of plasmids differing in size and type of replication: small (6–10 kbp) plasmids of sigma replication and large (more than 30 kbp) plasmids of theta replication. Some plasmids play a significant part in bacterial metabolism, while others are not important for bacteria (these are the so-called cryptic plasmids, typical of many soil strains of *Bacillus subtilis* [1, 2]). One of the functions of large bacillar plasmids is the conjugative transfer (mobilization) of small plasmids from one cell to another. Earlier, we described the mobilization of the small plasmid pUB110 in the soil strain *B. subtilis* 19 carrying also the large (95 kbp) plasmid p19, but provided no evidence that it is the plasmid p19 that mobilizes the plasmid pUB110 [3, 4]. This paper presents such evidence and describes some characteristics of plasmids in this strain.

Experiments were carried out with the *B. subtilis* strain 19 (p19 pV pUB110) constructed earlier [6]. The strain bears the large plasmid p19, the small cryptic plasmid pV, and the plasmid pUB110 responsible for the strain resistance to 50 μg/ml kanamycin. The laboratory strain *B. subtilis* trpC2 thr5 cm^r [4] served as the recipient. Conjugal transfer experiments were conducted using a liquid medium [4]. Plasmid DNA was isolated by the Birnboim and Doly procedure [6] and analyzed by electrophoresis in 1% agarose (Serva) with a Tris–borate buffer.

Earlier estimations showed that the mean frequency of conjugation (expressed as a percentage of transformed recipient cells) was about 1% and substantially varied from experiment to experiment [5]. To understand the reason for such a variation, cells from the colonies of the recipient strain grown in the presence of 50 μg/ml kanamycin (to prevent the growth of cells with the spontaneously eliminating plasmid pUB110) were tested for the ability to transfer this plasmid. About 40% of the 30 colonies tested contained cells incapable of conjugal transfer. The other cells exhibited the original level of the mean conjugation frequency (about 1%), but their progeny (at least in two cell generations) was again heterogeneous with respect to the conjugal transfer ability. At the same time, the progeny of clones incapable of the conjugal transfer of the small plasmid did not contain cells with such a capability.

To test the assumption that the heterogeneity of the donor strain is related to the elimination of the large conjugative plasmid, plasmid DNA isolated from each type of cells (i.e., capable and incapable of the conjugal transfer of plasmid pUB110) was analyzed by electrophoresis. The results were ambiguous because of the presence of other plasmids in the donor strain and because of the low number of copies of the large plasmid. To overcome this difficulty, the DNA isolated from the donor strain was digested by restriction endonuclease *Bam*HI and again analyzed by electrophoresis. (This approach is based on the knowledge that, unlike the plasmids pUB110 and pV, the plasmid p19 lacks the recognition sites of *Bam*HI.) The electrophoresis showed that only the digested plasmid DNA of bacterial cells capable of conjugal transfer contained a band corresponding to the plasmid p19 (Fig. 1). These data unequivocally indicate that the donor strain grown in the presence of kanamycin contains a number of cells lacking the plasmid p19 and, correspondingly, incapable of the conjugal mobilization of the plasmid pUB110.

The loss of the plasmid pUB110 correlated with specific changes in the morphology of the colonies of *B. subtilis* strain 19 (p19, pV, pUB110) grown in the presence of 50 μg/ml kanamycin. As soon as after 1 day of incubation, in addition to the colonies of the normal

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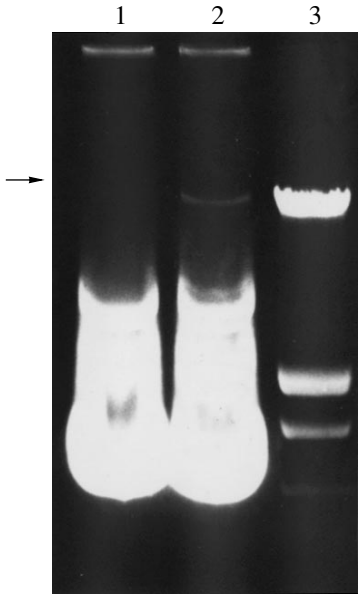

Fig. 1. Electrophoresis of the plasmid DNA isolated from two *B. subtilis* strains and digested with the restriction endonuclease *Bam*HI. Lanes: (1) *B. subtilis* strain incapable of conjugation; (2) *B. subtilis* strain capable of conjugation; and (3) phage λ DNA digested with *Eco*RI and *Hin*dIII. The arrow shows the position of the DNA fragment that corresponds to plasmid p19.

size, one could observe large colonies (Fig. 2). After 2 days of incubation, the number of such colonies increased. In the absence of kanamycin, large colonies did not appear. The appearance of large colonies was stimulated by increasing concentrations of kanamycin (up to 150 μg/ml), so that only large colonies grew at this concentration of the antibiotic. Cells from these "super-kanamycin-resistant" colonies were incapable of conjugation (all 15 tested colonies of this type showed the absence of conjugal transfer). At the same time, none of the 17 colonies grown in the absence of kanamycin lost the ability for conjugal transfer.

The small cryptic plasmid pV, 8.1–8.3 kbp in size [5], could also eliminated from the *B. subtilis* strain 19 at a high frequency (among the 10 colonies tested, 4 colonies showed the absence of this plasmid). This plasmid was not obviously related to conjugation, since it was present in cells both capable and incapable of mobilizing the plasmid pUB110.

Thus, the *B. subtilis* strain 19 with the plasmid pUB110 was unstable and easily lost the large conjugative plasmid p19. Extreme resistance to kanamycin may be associated with some structural alterations of the plasmid pUB110 or with variations in the number of its copies (it should, however, be noted that we failed to reveal any changes in the electrophoretic pattern of the plasmid DNA isolated from cells with different susceptibility to kanamycin). It is also unclear why extreme resistance to kanamycin and the loss of the large plasmid p19 should be related and why the growth of cells even at a moderate kanamycin concentration is not compatible with the simultaneous occurrence of the plasmids pUB110 and p19 in these cells. We may suggest that the plasmid p19 and the specific form of the plasmid pUB110 that determines extreme kanamycin resistance are incompatible. This may be associated with the malfunction of the mechanism responsible for the equal partition of the large plasmid copies between daughter cells. It should be noted in this regard that none of the 30 tested colonies of the *B. subtilis* strain 19 lacking plasmid pUB110 exhibited the spontaneous elimination of the large plasmid p19.

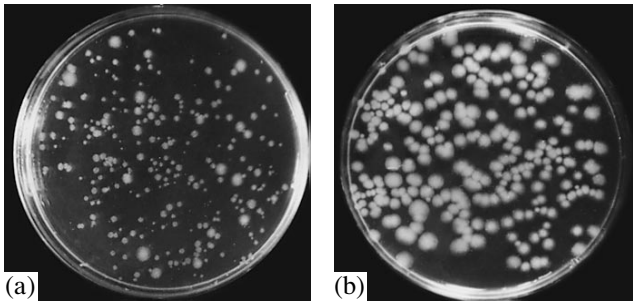

Fig. 2. Colonies of the *B. subtilis* strain 19 (p19 pV pUB110) grown (a) in the presence of 50 μg/ml kanamycin and (b) in its absence.

The high-frequency spontaneous elimination of large and small plasmids was also described for other bacillar species, including the *B. thuringiensis* strain capable of conjugation [7]. In the case of this species, the appearance of plasmidless clones was related to the altered replication of plasmids at an elevated temperature.

The fact that the loss of the large plasmid and the loss of the conjugal transfer capability of the *B. subtilis* strain 19 are correlated unambiguously indicates that the plasmid p19 possesses mobilizing activity.

We are grateful to A.V. Grigor'eva from the Russian Chemical and Technical University for her assistance in the experiments and P.S. Vasil'eva for her technical assistance.

This work was supported by grant no. 01-04-49497 from the Russian Foundation for Basic Research.

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