GENETIC TRANSFER, INHERITANCE, AND PHENOTYPIC EXPRESSION OF PLASMID RP4::Mu cts GENES IN *Bacillus cereus*

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Genetic exchange in intact cells of naturally nontransformable species of bacilli was limited until recently to transduction [12]. Since then the phenomenon of plasmid transfer during combined culture of bacilli, known as transception [10], has been described and is considered to be similar to transfer of conjugative plasmids in Gram-negative bacteria [4, 7]. Unlike conjugation, however, transception in bacilli is due to predominantly cryptic plasmids, which have no selective markers, making the use of this genetic exchange more difficult [6]. A parallel search for marked plasmids of heterologous origin, with a broad spectrum of hosts, capable of genetic transfer in bacilli, is therefore being conducted [3]. In our view, the hybrid plasmid RP4::Mu cts of *E. coli* [9] is promising. Like plasmid RP4, it has a wide range of hosts, it controls resistance to antibiotics, and contains a genome of a mutant phage-transposon, capable of insertion into chromosomes of different species of bacteria, and inducing various chromosomal aberrations and endowing the host cell with the feature of temperature sensitivity. A phenomonologic description of the ability of *B. cereus*, during combined culture with *E. coli* cells containing plasmid RP4::Mu cts, to inherit kanamycin resistance and temperature sensitivity, has been published [5, 11]. The aim of the present investigation was to study physical and functional behavior of plasmid RP4::Mu cts 62 during intergeneric transfer from *E. coli* into *B. cereus* and its subsequent intraspecific transfer in *B. cereus*.

EXPERIMENTAL METHOD

E. coli GA 570 leu pro his thr thi str recA, lysogenic for Mu c^+ and containing plasmid RP4::Mu cts 62 with Ap^R Tc^R Km^R markers, was used as the primary donor. The recipients were phototrophic strains of *B. cereus* obtained from Goebel [8]: GP7 in intergeneric transfer and DSM 318 Rif^R in intraspecific transfer. The donor for intraspecific transfer. The donor for intraspecific transfer was transcipient *B. cereus* 682, isolated during this investigation, and a derivative of strain GP7 with plasmid RP4::Mu cts 62, transmitted from *E. coli* GA 570. Standard methods of culture of bacteria in liquid LB medium and agarized BHI medium, and selection of antibiotic-resistant and temperature-sensitive (T^S) clones were used [2]. To transfer the plasmid, cultures of donors' and recipients' cells were grown separately until the logarithmic phase, mixed in a ratio of 1:1 by volume, incubated in LB medium for 24-72 h at 32°C, and seeded on dishes with kanamycin (100 μg/ml) for selection of clones of transcipients. Counterselection of *E. coli* donors' cells was undertaken with *B. cereus* 682 deficient in amino acids, by the addition of rifampicin (100 μg/ml) to the medium. The selected clones of transcipients were subjected to fivefold "purification" under selective conditions and their temperature sensitivity was analyzed as nonselective marker. Electrophoresis of DNA and localization of plasmid RP4::Mu cts 62 in plasmid and chromosomal DNA fractions of transcipient cells by the blot hybridization method, were carried out as described by Maniatis et al. [1].

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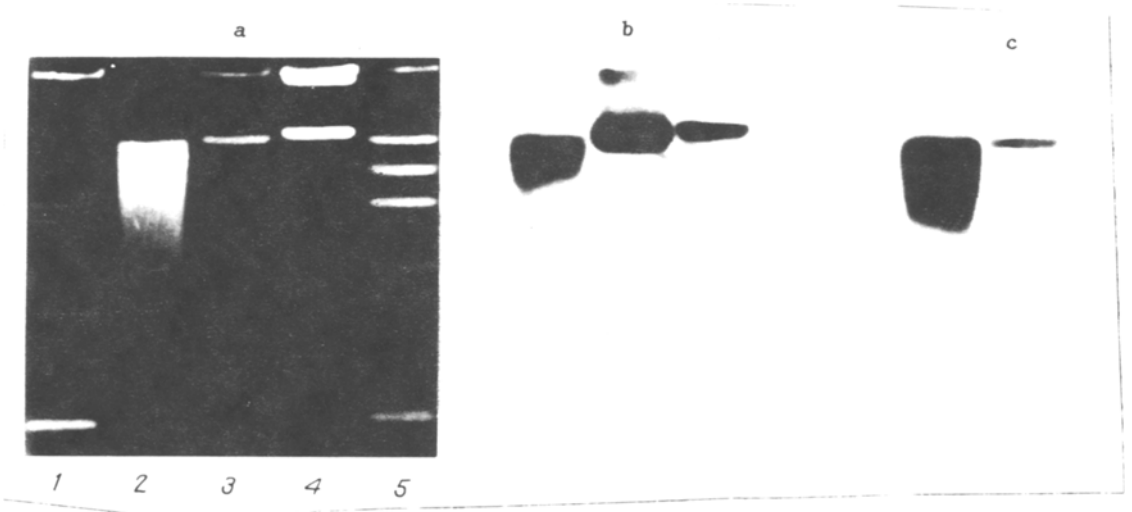

Fig. 1. Electrophoresis (a) and blot-hybridization autoradiography of fraction of plasmid DNA of *B. cereus* 682 transcipient with probes of DNA of phage Mu cts 62 (b) and ^{32}P-labeled plasmid RP4 (c). Location of probes on b and c identical with a: 1) total plasmid DNA of recipient, 2) DNA of phage Mu cts 52, 3) DNA of plasmid RP4::Mu cts 62, 4) DNA of plasmid RP4, 5) Hind III-fragments of DNA of phage λ.

EXPERIMENTAL RESULTS

In experiments with intergeneric crossing of *E. coli* GA 570 with *B. cereus* GP7 by seeding on dishes under selective conditions, after 2-3 days of incubation single colonies were found with the morphological and cultural properties of *B. cereus*. Combined inheritance of Km^R T^S markers, determined by plasmid RP4::Mu cts 62, by the isolated clones indicates that they can be regarded as transcipients of *B. cereus* with the plasmid transmitted to them from *E. coli*. Due to the presence of a resident plasmid pBC16 in cells of the recipient strain GP7, determining its resistance to tetracycline [8], the Tc^R marker of plasmid RP4::Mu cts 62 cannot be identified in clones of the transcipients, and the high natural resistance of the species *B. cereus* to ampicillin precludes detection of another plasmid marker, namely Ap^R, in the transcipients. The frequency of formation of transcipients of primary colonies of *B. cereus* transcipients, calculated per *E. coli* donor cell is 10^{-9}. In control experiments with separate culture of donor's and recipient's strains, growth of colonies was not found. The population of primary colonies of *B. cereus* transipients was found to be unstable during "purification" and subsequent subcultures. More than 10% of the clones completely lost their viability, and among the survivors intensive and spontaneous segregation of clones lacking one or both Km^R T^S markers of the plasmid was observed. In the absence of selective pressure, a single subculture in liquid LB medium led to simultaneous loss of both plasmid markers in more than 90% of transcipient clones. Nevertheless, by successive and prolonged subcultures under selective conditions with continuous clonal selection, clones of transcipients stable with respect to Km^R T^S markers could be isolated. One of them, identified as *B. cereus* 682 and stable under nonselective conditions, was used in the subsequent experiments.

To determine the localization of plasmid RP4::Mu cts 62 in cells of the *B. cereus* 682 transcipient, the fraction of plasmid DNA was isolated and subjected to electrophoretic fractionation in agarose gel. Besides plasmid bands, corresponding to the two resident plasmids pBC16 and pBC15 of the recipient strain GP7, with mol. wt. of 2.8 and 23 megadaltons [8], no other extrachromosomal structures were found in gels after electrophoresis (Fig. 1a). The possibility remained of integration of plasmid RP4::Mu cts 62 or its derivative with the chromosome of *B. cereus*. To shed light on this problem, fractions of plasmid and chromosomal DNA of the transcipient cells were studied in the blot-hybridization test with DNA of phage Mu cts 62 and plasmid RP4 as probes, labeled with ^{32}P in the nick-translation reaction. It will be clear in Fig. 2b, c that the two zones did not hybridize with the plasmid DNA of the transcipient, but effectively bound with homologous DNA in control tests. Crossed hybridization of DNA of phage Mu cts and plasmid RP4 in the control can be explained on the grounds that the phage was obtained by induction from cells of an *E. coli* strain in which its genome was incorporated into plasmid RP4, and for that reason its DNA contained short fragments of the DNA of this plasmid.

Fig. 2. Electrophoresis and blot-hybridization autoradiographs of chromosomal DNA of *B. cereus* 682 transcipient with probes of DNA of phage Mu cts 62 (b) and plasmid RP4 (c), labeled with ^{32}P. Location of probes on b and c identical with a: 1) unrestricted DNA, 2) DNA restricted by Pst 1.

The probes did not interact with DNA of plasmid-free strains of *E. coli*, tested as the negative control, confirming their specificity and that they can be used in hybridization of DNA of *B. cereus*. Unlike the plasmid fraction, the chromosomal DNA of the transcipient bound specifically with both probes. Binding took place both with native DNA and with its high-molecular-weight fragments obtained by degradation by restriction endonuclease Pst 1, although with a weaker autoradiographic signal (Fig. 2). This was evidently due to the "gene dose" involved in hybridization. It can be concluded from the results as a whole that plasmid RP4::Mu cts 62 can be inserted into the chromosome of *B. cereus*, probably in the form of a single, possibly deleted structure, and can express some of its genes in the bacilli. The clone of transcipient studied produced no infectious Mu cts 62 phage particles either spontaneously or under conditions of thermoinduction.

In the state preceding stabilization of inheritance of plasmid markers, the *B. cereus* 682 transcipient was tested for donor ability to transmit the Km^R T^S Tc^R markers (the plasmid carries the tet gene) into cells of recipient strain *B. cereus* DSM 318 Rif^R Km^S-Tc^S-T^R. In seedings on dishes from mixed cultures under selective conditions, delayed growth of colonies with Rif^R Km^R Tc^R T^S markers was observed. The fact that they belonged to strain DSM 318 was confirmed by identification by electrophoresis of one resident cryptic plasmic pBC6, with molecular weight of 8 megadaltons [8], belonging to the recipient. The frequency of transmission of plasmid markers Km^R Tc^R T^S in intraspecific crossing of *B. cereus* was 10^{-6}-10^{-7}, calculated per colony-forming unit of the donor (the bacilli were connected into short chains). This is at least two orders of magnitude higher than the frequency of formation of transcipients in intergeneric transfer of RP4::Mu cts 62. In control experiments with separate culture of the two strains, and also with mixed growth of the colonies under conditions similar to the experimental conditions was not observed.

The results are evidence of the possibility of transfer of markers of plasmid RP4::Mu cts 62 in strains of *B. cereus* and the genetic exchange system described can be used in addition to natural transception in bacilli.

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