SHORT COMMUNICATION

Facile in vivo Transfer of Mutations Between the Bacillus subtilis Chromosome and a Plasmid Harbouring Homologous DNA

By K. F. CHAK,¹ H. DE LENCASTRE,² H-M. LIU† AND P. J. PIGGOT¹*  
¹Division of Microbiology, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.  
²Grupo de Genética Molecular, Instituto Gulbenkian de Ciência, Oeiras, Portugal  
(Received 26 July 1982)

Transformation of Bacillus subtilis strains carrying the point mutations spoIIA42 or spoIIA69 by a plasmid, pHM2, carrying the spoIIA⁺ allele gave occasional recombinants in which the plasmid had acquired the chromosomal mutation. Transformation of a spo⁺ strain by these derivative plasmids gave occasional recombinants in which the chromosome of the recipient had acquired the plasmid-borne spoIIA mutation. Both types of behaviour were recE⁺-dependent. Mutations spoIIA42 and spoIIA69 fell into a single complementation group.

INTRODUCTION

There are now several plasmid vectors with which it is possible to clone Bacillus subtilis genes and thence to study their expression in B. subtilis (Ehrlich et al., 1981; Kreft & Hughes, 1981). This clearly provides considerable impetus to B. subtilis research. Functional wild-type DNA has generally been cloned as it is readily identified using mutant hosts. Where a particular region is being studied in some detail and a number of chromosomal mutations have been characterized, it would be very useful to transfer these mutations to the cloned DNA. It would then be possible to perform complementation tests. Further, if the transferred mutations were nonsense mutations, it would help identify the translation product of the cloned DNA.

We have previously reported the isolation of a plasmid, pHM2, containing a functioning part of the spoIIA locus (Liu et al., 1982) from a clone bank of B. subtilis DNA inserted into pHV33 (Rapport et al., 1979). It was noted that a significant portion of the transformants of rec⁺ spoIIA⁻ strains by pHM2 were Spo⁺, and not Spo⁺ as expected. We show here that this results from the acquisition of the chromosomal mutation by the plasmid.

METHODS

Strains. The B. subtilis strains used were: BR151 (metB10 lys-3 trpC2); SL976 (spoIIA42 trpC2 metB10); SL986 (spoIIA42 metB10 recE4); SL989 (recE4 metB10 lys-3); SL1013 (spoIIA69 lys-3 metB10); SL1060 (spoIIA69 trpC2 recE4). All strains were in the genetic background of BR151. Strain SL989 was constructed by introducing the recE4 mutation by congression into BR151. The origin of other strains has been described previously (Liu et al., 1982). The Escherichia coli strain used, SL2022 (hsdR supE recA56), was a derivative of SL2009 (Liu et al., 1982) that had been cured of bacteriophage λ.

Transformation. Bacillus subtilis was transformed by the method of Bott & Wilson (1968). DNA was used at 1 μg ml⁻¹ for transformation. Chloramphenicol-resistant (Cm⁺) transformants were selected on nutrient agar containing 5 μg chloramphenicol ml⁻¹ for rec⁺ strains, and 3 μg chloramphenicol ml⁻¹ for recE4 strains. Transformants were scored for their sporulation phenotype (Spo⁺ or Spo⁻) by colony pigmentation (Iichinska.

† Present address: Institute of Epidemiology and Microbiology, Chinese Academy of Medical Sciences, Peking, China.
Short communication

1960; Schaeffer & Ionesco, 1960), and the scoring was confirmed by examination with a phase-contrast microscope. All other methods have been described previously (Liu et al., 1982).

RESULTS

Four mutant alleles of the spoIIA locus are complemented by pHM2 (Liu et al., 1982). Thus when recombination-deficient strains bearing any one of the mutations spo-42, spo-50, spo-69 or sas-I were transformed to Cm\(^R\) by pHM2, the transformants were Spo\(^+\). When isogenic Rec\(^+\) strains were used, up to 10% of the Cm\(^R\) transformants were Spo\(^-\) [Liu et al. (1982); Table 1, part (a)]. The Rec\(^+\)-dependence of the appearance of the Cm\(^R\) Spo\(^-\) transformants was consistent with some sort of recombination being involved in generating the phenotype. Several mechanisms might be considered: (a) interaction of the plasmid with the chromosome resulting in transfer of the chromosomal mutation to the plasmid, (b) insertional inactivation of a chromosomal spo gene, or (c) rearrangement or deletion of plasmid DNA without involvement of the chromosome. Tanaka (1979) has shown that plasmid rearrangements can occur independently of recE, so that (c) seems unlikely.

Spo\(^-\) Cm\(^R\) transformants from strains SL976 and SL1013 were chosen for further study. These strains contain mutations spo-42 and spo-69 respectively, which are point mutations (Yudkin & Turley, 1981) mapping at opposite ends of the group of mutations complemented by pHM2. Two plasmid preparations, pPP1 and pPP2, were made from separate Cm\(^R\) Spo\(^-\) clones from a SL1013 \(\times\) pHM2 cross, and two, pPP3 and pPP4, made from separate clones from a SL976 \(\times\) pHM2 cross. The mobility of these plasmids was indistinguishable from that of pHM2 on electrophoresis in 0·8% (w/v) agarose gels; the HindIII and EcoRI restriction patterns were indistinguishable from the corresponding patterns for pHM2. Thus there was no gross physical difference between these plasmids and pHM2. The plasmids were derived from a chimeric plasmid, pHV33, that can replicate in E. coli as well as B. subtilis (Ehrlich et al., 1981). They transformed E. coli SL2022 to ampicillin-resistance, and all plasmids used subsequently were prepared from E. coli.

Table 1. Transformation of B. subtilis strains by plasmid preparations

(a) Selection for Cm\(^R\), using covalently closed circular plasmid DNA as donor. Results are given as the number of transformed colonies counted (obtained from 0·4 ml culture, except SL1013 which was obtained from 0·2 ml culture) with the percentage of Spo\(^+\) colonies shown in parentheses.

(b) Selection for Spo\(^+\), using linear plasmid DNA as donor. Plasmid DNA was converted to linear form by restriction endonuclease digestion at the single PstI site (Liu et al., 1982). Results are given as the number of transformant colonies counted (from 0·2 ml culture).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>pHM2</th>
<th>pPP1</th>
<th>pPP2</th>
<th>pPP3</th>
<th>pPP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL976</td>
<td>spoI42A42 rec(^+)</td>
<td>3480 (94·4)</td>
<td>2319 (2·8)</td>
<td>1599 (2·2)</td>
<td>2852 (0)</td>
<td>4196 (0)</td>
</tr>
<tr>
<td>SL986</td>
<td>spoI42A42 recE4</td>
<td>403 (100)</td>
<td>788 (0)</td>
<td>582 (0)</td>
<td>558 (0)</td>
<td>808 (0)</td>
</tr>
<tr>
<td>SL1013</td>
<td>spoI469 rec(^+)</td>
<td>1074 (95·5)</td>
<td>2052 (0)</td>
<td>2360 (0)</td>
<td>1326 (3·3)</td>
<td>2156 (3·4)</td>
</tr>
<tr>
<td>SL1060</td>
<td>spoI469 recE4</td>
<td>1274 (100)</td>
<td>406 (0)</td>
<td>630 (0)</td>
<td>437 (0)</td>
<td>603 (0)</td>
</tr>
<tr>
<td>BR151</td>
<td>spo(^+) rec(^+)</td>
<td>9480 (100(*)</td>
<td>4882 (87·2)</td>
<td>2758 (89·1)</td>
<td>3168 (74·9)</td>
<td>611 (81·3)</td>
</tr>
<tr>
<td>SL989</td>
<td>spo(^+) recE4</td>
<td>1258 (100)</td>
<td>670 (100)</td>
<td>508 (100)</td>
<td>414 (100)</td>
<td>ND</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Donor</th>
<th>Relevant genotype</th>
<th>pHM2</th>
<th>pPP1</th>
<th>pPP2</th>
<th>pPP3</th>
<th>pPP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL976</td>
<td>None</td>
<td>20, 13+</td>
<td>5040</td>
<td>4220</td>
<td>3710</td>
<td>25</td>
<td>12‡</td>
</tr>
<tr>
<td>SL1013</td>
<td>None</td>
<td>0, 0†</td>
<td>908</td>
<td>0</td>
<td>0</td>
<td>320</td>
<td>328</td>
</tr>
</tbody>
</table>

ND, Not determined.

* A single Spo\(^-\) transformant was obtained, the lesion was not complemented by pHM2 and is thought to be a spontaneous mutation.
† Duplicate determinations.
‡ Covalently closed circular plasmid used.
Recombination of plasmid mutations with chromosomal mutations

Plasmids pPP1 and pPP2 gave Spo+ recombinants with SL976 (Table 1) but not with SL1013 (from which they were derived). Plasmids pPP3 and pPP4 gave Spo+ recombinants with SL1013 but not with SL976 (from which they were derived); the appearance of the recombinants was rec+-dependent (Table 1). The ability of pPP1 and pPP2 to correct only spo-42, and of pPP3 and pPP4 to correct only spo-69, was also seen when linear plasmid DNA was used as donor and selection was for Spo+ (this DNA gave no CmR transformants). It seems reasonable to conclude that pPP1 and pPP2 harbour the spo-69 mutation, whilst pPP3 and pPP4 contain the spo-42 mutation. However, from these experiments we cannot rule out the possibility that the plasmids contain small deletions covering the mutations in question.

Induction of mutations in the chromosome by plasmids

When plasmids pPP1 to pPP4 were used to transform strain BR151 to CmR a significant proportion of the transformants were Spo−. This appearance was recE+-dependent as no Spo− transformants were obtained in comparable crosses with SL989 (Table 1). It seemed possible that mutations had been transferred from the plasmids to the chromosome. To test this, two Spo− CmR transformants from each cross were isolated. Growth for approximately 10 generations in the absence of chloramphenicol yielded Spo− CmS bacteria which were presumed to have lost the original plasmid. The mutants were confirmed to be SpoII by microscopic examination, and the mutations were shown to be 55−70% co-transformed with lys-3, which is characteristic of spoIIA mutations. A Spo− strain derived from pPP1 gave Spo+ recombinants with SL976 DNA but not with SL1013 DNA, whilst a strain derived from pPP3 gave Spo+ recombinants with SL1013 and not with SL976 DNA (data not shown). It seems reasonable to conclude that the mutations are indeed spoIIA mutations derived from the donor plasmid. The mutations reverted at a frequency of about $10^{-7}$−$10^{-8}$ which is similar to that of the original mutations spo-42 and spo-69 (Yudkin & Turley, 1981), and would not be exhibited by small deletions (see above). From this it is concluded that pPP1 and pPP2 carry the mutation spoIIA69 and pPP3 and pPP4 carry spoIIA42.

Complementation test

Plasmids pPP1 to pPP4 gave no Spo+ transformants when the recE4 strains SL986 and SL1060 were used as recipients (Table 1). This indicates that spo-42 and spo-69 fall into a single complementation group. Although plasmid copy number is not known, it seems unlikely to have influenced the result as pHM2 renders SL986 and SL1060 Spo+, whilst pPP1 to pPP4 do not affect the sporulation of SL989.

DISCUSSION

The present work establishes an easy method for transferring point mutations from the chromosome on to a plasmid harbouring the wild-type allele. The method has been used to demonstrate that mutations spo-42 and spo-69 fall into a single complementation group. The work also demonstrates that mutations can be transferred from a plasmid on to the chromosome of a recipient strain without loss of any other recipient genetic markers. This should be of use when it is desired to investigate the phenotypes resulting from genetically-engineered plasmid-borne mutations. It may also be of use in transferring mutations from one strain to another. It is particularly easy to follow the process with spo mutations where the sporulation phenotype is indicated by colony pigmentation (Ilichinska, 1960; Schaeffer & Ionesco, 1960). However, the frequency of mutant transfer is such that the method should also be applicable where less direct screening methods are used. Moreover it may be possible to increase this frequency by using plasmid monomer preparations that have been freed from oligomers (Iglesias et al., 1981). These authors have shown that deletions and insertions in plasmid-borne chromosomal DNA can be corrected by interaction with the B. subtilis chromosome during transformation. Our results extend their observations to point mutations, to the ability to change wild-type to mutant, and to alterations to either chromosome or plasmid. Iglesias et al. (1981) considered that correction
could occur either by mismatch repair or by recombination. A more detailed analysis of the system described here, using several very closely linked spo mutations may help to distinguish between these possibilities.

REFERENCES


