TRANSFORMABILITY OF DNA POLYMERASE-DEFICIENT MUTANTS OF BACILLUS SUBTILIS

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Summary. The effect of a deficiency in DNA polymerase on recombination in Bacillus subtilis has been studied. It is concluded that the major DNA polymerase of B. subtilis is not required for recombination, and that the recombination deficiency of a previously described DNA polymerase-deficient mutant is actually due to a rec mutation. Genetic crosses imply that this recombination deficiency is not recA or recB.

Recent models for mechanisms of genetic recombination in bacteria require a number of enzymatic activities, among them polymerization of deoxyribonucleic acid (DNA). It is possible that certain classes of recombination-deficient mutants are deficient in a particular DNA polymerase.

The polA (DNA polymerase I-deficient) mutation in Escherichia coli does not markedly reduce the frequency of recombination during conjugation (1). However, Gass, et al., (2) have described a methyl-methane sulfonate (MMS)-sensitive mutant of Bacillus subtilis, JB1-49(59), which has a greatly reduced level of DNA polymerase and is also recombination-deficient (Rec^-) (3,4). We have examined the possible role of this DNA polymerase in recombination, and we have concluded that the polymerase deficiency does not reduce recombination as measured by transformation. The polA mutant described (2) apparently carries an additional mutation in some recombination function (see also Ref. 5). The defective function appears not to be recA or recB (6).

MATERIALS AND METHODS

The polymerase-deficient strains tested were JB1-49(59), a highly mutagenized strain (3), and a derivative by transformation, HA160, which presumably differs from the Pol^+ strain HA101 only in the polymerase mutation (7). These

Table I

Bacterial Strains

Designation	Genotype	Source	
168 Trp$^-$	trpC2	D. S. Nasser	(8)
HA101	his$B^{a,b}$ leu, metB^{a}	B. S. Strauss	(2)
JB1-49(59)	cys, his$A^{a,b}$ trpC, polA, rec-3	B. S. Strauss	(2, 3, 4)
HA160	his$B^{a,b}$ leu, metB^{a}, PolA	B. S. Strauss	JB1-49(59) -x HA101 (5)
BR58	pyrA1, trpC2, ilvA2	F. Young	
SB70	hisB2, tyrA1	E. Nester	(9)
GSY1025	trpC2, metB4, recA1	J. Hoch	(10)
GSY1028	trpC2, metB4, recB2	J. Hoch	(10)

[a] unpublished observations
[b] B. S. Strauss, personal communication

and other strains used are described further in Table 1.[1] DNA was extracted by the Marmur procedure (11), omitting treatment with ribonuclease and precipitation with isopropanol. Competent cells were prepared as described previously (C.T. Hadden and D. Billen, J. Bacteriol., in press). Competent cells were exposed to a saturating amount of DNA for 30 minutes, and the exposure was terminated with pancreatic deoxyribonuclease (10 µg/ml for 5 min.). Transformants for nutritional markers were enumerated on the media described previously (12). MMS-resistant colonies were selected by plating cells on nutrient agar (Difco) which had been made 2 mM in MMS (Eastman Kodak Co.) immediately prior to pouring the plates.

RESULTS

The effect of the Pol$^-$ mutation on transformation and linkage in *B. subtilis* HA160 was tested by a cross in which the donor DNA carried the *trpC2* mutation, normally linked to *hisB* (9). Transformants were plated on agar supple-

[1] Abbreviations: Trp, typtophan; His, histidine; Leu, leucine; Met, methionine; Cys, cysteine; Ura, uracil; Tyr, tyrosine; CFU, colony-forming units; Tf, transformants.

mented with Leu, Met, and Trp, to determine the total number of His^+ transformants, or Leu and Met, to select recombinant (His^+ Trp^+) progeny. The tests of JB1-49(59) were carried out similarly, with the appropriate changes in supplementation to select for Trp^+ (total) or Trp^+ Tyr^+ (recombinant) transformants.

Table 2 shows that the polymerase-deficient strain HA160 transforms with as high an efficiency as the isogenic HA101. Furthermore, the polymerase deficiency has no effect on linkage between the hisB and trpC markers. Similar results were

Table II

Test Cross	Colonies/ml X 10^{-5} BR58→ HA101	BR58→ HA160	SB70→ 168 Trp^-	SB70→ JB1-49(59)
Total CFU/ml	680	745	570	1040
Total Tf/ml	16.05	12.37	5.02	0.014
Recombinants/ml	6.3	3.70	2.82	0.007
% Transformation	2.4	1.7	0.88	0.0029
Recombinant/total Tf	0.39	0.30	0.56	0.52

Transformations were carried out as described in the text. Both recombinant type and total transformants were selected directly, on the appropriately supplemented agar.

reported by Laipis and Ganesan (5) with polA5, an independantly isolated DNA polymerase mutation. As a confirmation of the linkage data, the trpC markers of a number of His^+ transformants growing on agar supplemented with Trp were tested by replica-plating. The ratios of recombinant to total transformants determined in this way were identical to the results in Table 2.

In contrast, the polymerase-deficient strain JB1-49(59) was about 300-fold less transformable than strain 168 Trp^- (Table 2), although the extent of linkage observed between trpC and tyrA is unchanged in JB1-49(59) (Table 2). These linkage data were also confirmed by replica-plating. Since this strain is the

donor of the mutant polymerase gene in HA160, it seems clear that the polymerase mutation in itself cannot be responsible for low transformability in JB1-49(59).

Dubnau, et al. (4) have described JB1-49(59) as bearing the mutation rec-3. They further state (4) that rec-3 behaves identically to rec-2 (recB2) (6) in transformation and transduction. To determine whether rec-3 is actually an allele of recB, we performed a transformation cross in which competent cells of recA and recB mutants were exposed to DNA from JB1-49(59) and subsequently tested for resistance to MMS, since Rec$^-$ cells are sensitive to MMS. Thus, if JB1-49(59) is recB, some recA recipients should be transformed to MMS resistance, but recB recipients should not. Competent cultures of GSY1025 and GSY1028 were exposed to a saturating concentration of DNA for 60 min., treated with deoxyribonuclease, and incubated an additional 2 hrs to allow expression of the newly transformed genes. They were then plated on nutrient agar or nutrient agar containing 2mM MMS. Table 3 shows that both recA and recB recipients are transformed to MMS-resistance by DNA from JB1-49(59), while only recB recipients are transformed by recA DNA. Therefore it is likely that the rec-3 mutation is an allele not of recB, but of a separate gene, which should be designated recD.

DISCUSSION

At this time there seems to be no indication that the major DNA polymerase of B. subtilis plays an essential role in recombination, either in the frequency of recombination or (as indicated by the linkage studies) in the length of the integrated segment. This appears to be the case also with E. coli (1). Furthermore, the recD mutation also has no effect on linkage. That is, any recombination event which is completed will be either normal or lethal.

It seems most likely that the simultaneous loss by JB1-49(59) of DNA polymerase and of ability to recombine was a fortuitous result of mutagenization of the parental strain (3). Derivatives of JB1-49(59) have been isolated which are Pol$^-$ Rec$^+$ (HA160) or Pol$^+$ Rec$^-$ (BD193[4, 13]), so there can be little question that the two deficiencies in JB1-49(59) represent lesions in separate genes. The recombination deficiency of JB1-49(59), described as rec-3 (4) or mms (3), has been

Table III

Transformation of Rec$^-$ strains to MMS-resistance

by DNA from Rec$^-$ mutants

DNA	Recipient Type	CFU/ml -MMS ($x10^{-8}$)	+MMS ($x10^{-3}$)	Fraction MMS-Resistant ($x10^{5}$)
No DNA	recA	2.5	.005	.002
	recB	1.5	.005	.003
GSY1025	recA	7.9	.05	.006
(recA)	recB	2.3	3.6	1.56
JB1-49(59)	recA	6.9	21.2	3.06
(rec-3)	recB	1.3	4.2	3.34

Transformations were carried out as described in the text. RecA and recB recipients were GSY1025 and GSY1028, respectively. The frequencies of Met$^+$ transformants were: JB1-49(59) → recA, 3.23×10^{-5}; JB1-49(59) → recB, 1.14×10^{-5}.

assigned to recB (14, 15). The basis for this assignment is unclear, and the data in Table 3 imply that rec-3 is probably not recA or recB, but rather should be designated rec D3

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Addendum: A new Rec⁻ mutation, recC4, has been described by R.P. Sinha and V. N. Iyer (J. Mol. Biol. 72, 711 [1972]). Unlike rec-3 (5), this mutation does not confer sensitivity to MMS, so rec-3 cannot belong to recC.