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A new mutator strain of *Bacillus subtilis*

Jean-François Viret and Juan Carlos Alonso
Max-Planck-Institut für molekulare Genetik, Ihnestrasse 73, D-1000 Berlin 33, Federal Republic of Germany

Summary. *Bacillus subtilis* strain SB1207, widely used in our laboratory, was found to be highly temperature-sensitive and to exhibit a strong SOS-independent mutator phenotype at elevated temperatures. Both chromosomal and plasmid-borne genes were affected by the mutator. Lethality and mutator phenotype could not be attributed to a replication shut off or to thymine starvation. Due to the high frequency of base misincorporation, the mutator phenotype probably results from an editing defect rather than from a post-replication defect (mismatch repair).

Key words: *mut*-(Ts) – Error-prone repair – Proof-reading – Replication

Spontaneous mutations occur in *Escherichia coli* at a frequency of 10^{-10} to 10^{-9} per base pair per cell generation (Cox 1976). The study of mutator strains which have an increased rate of spontaneous and/or induced mutagenesis (Cox 1976; Loeb and Kunkel 1982; Sargentini and Smith 1985) has led to important advances in the understanding of the mechanisms ensuring the fidelity of DNA replication. In *Bacillus subtilis*, few strains with such a phenotype have been reported; those mutants known map in 3 different loci: (i) *polC* coding for DNA polymerase III core enzyme (*mut-1, dnaF*133, *polC*25) (Gross et al. 1968; Gass and Cozzarelli 1973; Love et al. 1976); (ii) *dna*-8132 the function of which is still unknown (Sadaie and Narui 1976) and (iii) *polA* coding for DNA polymerase I (*polA*59) which has little mutator activity by itself but acts synergistically with *dna*-8132 (Sadaie and Narui 1976). Other properties of these mutants have been reviewed by Mazza and Galizzi (1978). In addition to deficiencies in the proteins involved in DNA synthesis, strong mutator phenotypes can result from imbalances in the pools of deoxyribonucleotides (Kunz 1982). A well known example is thymine starvation which can lead to high rates of mutagenesis in both *E. coli* and *B. subtilis* (Bresler et al. 1973).

Offprint requests to: J.C. Alonso

Materials and methods

Bacterial strains and plasmids. Bacterial strains used are listed in Table 1. The *recE*4 and *mut*-1207(Ts) mutations were transferred from the original backgrounds by congression. The *rec*F15 mutation Harford (1974) has been placed into SB1207 by gene conversion (Iglesias and Trautner 1983) using precF15 plasmid (Alonso et al. 1987). *thrA*5 and *metB*5 mutations are suppressor-susceptible (Georgopoulos 1969); nevertheless, CU1050 is phenotypically auxotroph for both amino acids due to the presence of the *hom*-1050 mutation which affects the common biosynthetic pathway to threonine and methionine. The presence of *hom*-1050 was demonstrated both phenotypically and genetically.

Table 1. Bacterial Strains

Strain	Relevant genotype	Origin and comments
CU1050	*sup*-3 *leu*-1050 *thrA*5 *metB*5 *hom*-1050 *att*SPβ	Zahler et al. (1977). The *leu* marker was originally designated *leuA*8 (Zahler, personal communication)
SB1207	as CU1050, *mut*-1207(Ts)	Laboratory collection[a]
BG98	as SB1207, *recE*4	This work
BG80	as SB1207, *recF*15	This work
YB886	*metB*10 *trpC*2 *amyE xin*-1 *att*SPβ	Yasbin et al. (1980)
BG111	as YB886, *mut*-1207(Ts) (*trpC*$^+$)	This work
BD337	*trpC*2 *thrA*5 *mut*-1	D. Dubnau; Gross et al. (1968)
VUB240	*hisA*1 *thrA*5 *purA*16 *dna*-8132	Laboratory collection; Hara and Yoshikawa (1973)

[a] Due to its unexpected phenotype, CU1050 was renamed SB1207 by I. Stroynowski. The origin of *mut*-1207(Ts) is unknown

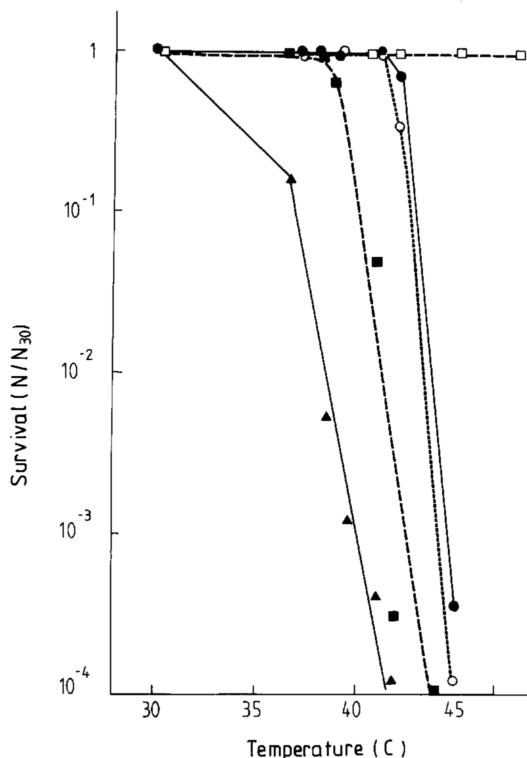

Fig. 1. Plating efficiencies (normalized to 30° C) of SB1207 and derivatives at different temperatures. Unless specified otherwise cells exponentially growing at 30° C in TY medium were plated. (●) SB1207; (○) SB1207 (stationary phase cells); (▲) BG111; (■) BG98; (□) CU1050

Media. TY broth medium (Biswal et al. 1967) was used for growth of liquid and solid cultures and Spizizen's minimal medium (Spizizen 1958) for the determination of new auxotrophs.

Transformation. Competent cells were prepared and transformed as described by Alonso and Trautner (1985).

Physical and chemical treatments. The dose of the DNA damaging agent required to reduce the survival of cells to 10% of the untreated ones was basically determined from inactivations curves as reported by Friedman and Yasbin (1983).

DNA determination. DNA synthesis was monitored using the diphenylamine assay of total DNA (Burton 1956).

Mutagenesis tests. The mutation frequency was determined either directly or indirectly by plating exponentially growing cells (grown at 30° C) at different temperatures. For direct selection, the frequency of streptomycin-resistant mutants was estimated by plating onto plates containing streptomycin at the indicated concentration. For indirect selection, the production of new auxotrophs or reversion of the *recF*15 mutation was measured. Colonies grown on TY plates at different temperatures were either replica plated onto amino acid supplemented minimal plates or onto TY plates containing 300 μg/ml MMS. The latter concentration is about 4-fold over the minimal inhibitory concentration for the *recF*15 background.

Results and discussion

In this communication, we report the identification of a new temperature-sensitive mutator strain of *B. subtilis*, SB1207, derived from the SPβ-free strain CU1050 of Zahler et al. (1977) (Table 1). Both strains plate at 30° C with equal efficiencies; however, SB1207 contains at least one additional mutation that leads to a 10^4-fold reduction of the plating efficiency at 45° C whether growing or stationary phase cells are plated (Fig. 1). The mutation conferring temperature-sensitivity was transferred by congression to strain YB886, which is cured of SPβ and non-inducible for PBSX prophage, at a frequency compatible with the transfer of one (or closely linked) mutations. In the resulting strain BG111, the lethal phenotype is partially expressed at 37° C and totally expressed at 42° C, whereas strain BG98, a *recE*4 derivative of SB1207, presents an intermediate phenotype (Fig. 1).

The colonies of SB1207, BG111 and BG98 surviving incubation at non-permissive temperature are highly heterogeneous in size and morphology indicating a possible mutator phenotype (see below). To test if these strains were impaired in DNA repair, we determined their sensitivy to DNA-damaging agents. SB1207 was only 2- to 3-fold more sensitive to UV light, methyl methanesulfonate (MMS) and mitomycin C (MC) when plated 42° C rather than at 30° C. As expected from the presence of the *recE*4 mutation, strain BG98 was 30- to 50-fold more sensitive than SB1207 to any DNA-damaging agent independent of the temperature. In addition, SB1207 and BG111 are transformed for chromosomal markers with a wild-type efficiency at both 30° C and 42° or 37° C respectively. A strong impairment in recombination or DNA repair is thus unlikely.

Experiments were then performed to quantify rates of mutagenesis after plating at different temperatures. Table 2 reveals that at the non-permissive temperature up to 40%–50% of the surviving cells of SB1207 and BG111 represent clones with new auxotrophies. This high rate of mutagenesis is also observed in BG98 and is thus independent of the *recE* gene product (de Vos et al. 1983). By analogy with the *E. coli* system (Sargentini and Smith 1985), the independence from an active *recE* gene product, i.e. from error-prone repair, can be considered an essential condition for the definition of a mutator phenotype. Consequently,

Table 2. Mutator phenotype of SB1207 and related strains

Strain	Percentage of colonies with new auxotrophies					
	30° C	38° C	39° C	41° C	42° C	45 °
SB1207	<0.3[a] (290)	n.d.	n.d.	n.d.	<1[a] (96)	41 (250)
BG111[b]	<0.4[a] (243)	<0.4[a] (255)	20 (137)	31 (105)	39 (113)	48 (133)
BG98 *recE*4	<0.5[a] (197)	n.d.	n.d.	n.d.	n.d.	39 (246)

n.d. not determined

The frequency of new auxotrophs after plating on TY was scored at different temperatures. The numbers of colonies tested are given in parenthesis

[a] No colony with new auxotrophies was scored

[b] Frequencies are the mean of two experiments

Table 3. Comparison of *B. subtilis* mutator strains

Strain	Plates incubated at	Frequency of Streptomycin-resistant mutants		
		Streptomycin concentration (μg/ml)		Relative efficiency of plating on TY plates
		50	100	
SB1207[a]	30° C	1.8×10^{-6}	1.2×10^{-6}	1
	42° C	2.2×10^{-5}	1.4×10^{-5}	5.7×10^{-1}
	45° C	1.9×10^{-2}	1.0×10^{-2}	1.8×10^{-4}
BG111[a]	30° C	7.0×10^{-6}	5.8×10^{-6}	1
	37° C	9.5×10^{-5}	5.0×10^{-5}	3.8×10^{-2}
	45° C	5.2×10^{-2}	1.0×10^{-2}	3.5×10^{-5}
BG98 *recE*4	30° C	5.9×10^{-6}	3.4×10^{-6}	1
	39° C	1.4×10^{-5}	8.6×10^{-6}	6.4×10^{-1}
	45° C	1.2×10^{-1}	6.1×10^{-2}	4.6×10^{-5}
BD337[a] (*mut*-1)	30° C	3.0×10^{-3}	2.7×10^{-3}	1
	45° C	1.2×10^{-2}	5.9×10^{-3}	4.7×10^{-1}
VUB244 (*dna*-8132)	30° C	1.1×10^{-5}	3.7×10^{-6}	1
	45° C	$<3.5 \times 10^{-3}$ [b]	$<3.5 \times 10^{-3}$ [b]	6.8×10^{-6}
YB886[a]	30° C	5.8×10^{-6}	2.9×10^{-6}	1
	45° C	6.8×10^{-6}	1.4×10^{-6}	1

[a] Frequencies are the mean of two experiments
[b] No streptomycin-resistant colony was scored

the temperature-sensitive mutation was designated *mut*-1207(Ts) and the corresponding phenotype termed Tsm (for *t*emperature-*s*ensitive *m*utator). As observed for temperature-sensitivity, the Tsm phenotype is expressed at a significantly lower temperature in strain BG111.

The mutator phenotype of SB1207 and its derivatives was further characterized and compared to two other reported mutator strains of *B. subtilis*. In this case we measured the rate of production of streptomycin-resistant (Sm^r) mutations which are known to lie within the *rpsL* gene (Piggot and Hoch 1985). Results presented in Table 3 confirm the temperature dependence of the Tsm phenotype. At 45° C, the frequency of Sm^r mutants in both *recE*$^+$ and *recE*4 backgrounds is a high as 1%–10% whereas at 30° C it is comparable to the wild-type. The efficiency of mutagenesis observed for the Tsm phenotype is at least of the same order of magnitude as for the *mut*-1 phenotype; the latter mutation however does not drastically reduce cell viability.

It has been shown in *E. coli* that *recF* mutants have a reduced capacity in UV mutagenesis (Clark et al. 1982). We have evidences showing that the *B. subtilis recF* gene and its *E. coli* counterpart have analogous functions (our unpublished results). To further determine if the expression of SOS functions is involved, we studied Tsm mutagenesis in a *recF*15 background (strain BG80) by measuring reversion of *recF*15 (see Materials and methods). When present only on the chromosome, reversion of the *recF*15 mutation occurred at a frequency of about 3.0×10^{-4} at 45° C but was undetectable at 30° C. If in addition the *recF*15 mutation was present on a plasmid such as precF15, based on the multicopy pC194 replicon, the frequency of reversion observed for BG80 cells was about 20 times higher. This is in agreement with the increased number of targets for mutagenesis expected from a plasmid copy number of about 15 (Alonso and Trautner 1985).

Mutagenesis in the *recF*15 genetic background further supports its independence from error-prone repair. In addition, reversion of the precF15 plasmid reveals that the Tsm phenotype works in trans, as expected for a replicon like pC194 which only encodes a protein involved in the initiation of replication (J.C.A. and R. Tailor, submitted) and otherwise relies on host functions for DNA replication.

Strong mutator phenotypes can also be observed when *B. subtilis dna*(Ts) mutants are plated under non-permissive conditions (A. Demierre and D. Karamata, personal communication). However in SB1207 and BG111, DNA synthesis is not affected by a shift up to the non-permissive temperature (data not shown), showing that the Tsm phenotype does not result from a strong deficiency in DNA replication. About 30 min after the shift, partial lysis of the cell population occurs followed by resumption of growth during which observations in the phase contrast microscope reveal swollen cells and highly distorted chains suggesting perturbations of cell wall metabolism. Similar perturbations have been reported for *dna*(Ts) mutants (Viret et al. 1985). The same is observed in strain BG111, ruling out the involvement of prophage PBSX induction in the observed lysis. Due to the observed pattern of growth after shift up, suggesting a possible leakiness of *mut*-1207(Ts), no experiments were performed to check the Tsm phenotype in liquid cultures. However, temperature shift experiments under conditions where translation was temporarily blocked (data not shown) may suggest a defect at the gene product level.

Attempts to map the *mut*-1207(Ts) mutation by PBS1 transduction using a kit of *B. subtilis* reference strains (Dedonder et al. 1977) were not successful due to the high reversion frequency of some of the available markers. However, our data indicate that *mut*-1207(Ts) probably lies between *purB*33 and *metC*3, *leuA*8 and *thrA*5 or *hisA*1 and *purA*16 without detectable linkage to any of these markers. No locus concerned with DNA metabolism has been reported to date in these regions (Piggot and Hoch 1985). The ab-

sence of linkage with *leuA* excludes the possibility that *mut*-1207(Ts) maps in the *polA* locus (see Piggot and Hoch 1985). In addition, no linkage was found with *mut*-1 and *dnaF133* belonging to *polC* locus (see above) or with *dna*-8132 by calculating recombination indices after transformation (Karamata and Gross 1970).

From our data, we can rule out the involvement of SOS functions e.g. of error-prone repair in the expression of the Tsm phenotype. Most likely, the *mut*-1207(Ts) mutation affects the activity of the DNA replication machinery in such a way that replication can still proceed but with an extremely poor fidelity. The high frequency at which mutations take place allows us to also exclude a defect in mismatch repair. If a strong imbalance in the pools of DNA precursors cannot be ruled out, both the fact that the Tsm phenotype is unaffected when thymine or thymidine are added to the plates and the absence of linkage of *mut*-1207(Ts) with *thyA* or *thyB* loci (data not shown) make it unlikely that it results from thymine starvation. Moreover, transfection of SB1207 competent cells with SPP1 DNA gives the same yield of phages at 30° C and 42° C suggesting that, at the latter temperature at least (see Fig. 1), the pools of precursors are nearly normal. Thus to account for the Tsm phenotype, the most plausible target could be a defect in proof-reading activity.

The Tsm phenotype we report here constitutes a powerful tool for the mutagenesis of given genes present both on the chromosome or on plasmids, even if its molecular basis is still unclear. Indeed, two questions remain open: (i) the avenue by which mutations arise and (ii) the type of mutations produced; these might be quite specific since no reversion of the *leu*-1050 and *metB*10 markers was observed.

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