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Division of Temperature-Sensitive *Streptococcus faecium* Mutants After Return to the Permissive Temperature

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The regrowth of 27 temperature-sensitive division mutants of *Streptococcus faecium* ATCC 9790 was examined after various periods of incubation at the nonpermissive temperature. Several of the mutants blocked at various stages of septum formation or of daughter-cell separation divided in a partially or completely synchronous way after a short incubation at the nonpermissive temperature. All four lytic mutants blocked early in the cell division cycle divided at a normal rate after a brief lag.

Rod-shaped, temperature-sensitive division mutants recovering from nonpermissive conditions usually exhibit an abrupt fragmentation of filaments subsequent to the temperature shift-down (1–3, 8–10). This response suggests that a potential for cell division is accumulated at the nonpermissive temperature. The division of filaments may be inhibited (1, 3, 8, 10) or not (2, 9, 10) by chloramphenicol, suggesting variability in a requirement for new protein synthesis. Regrowth of temperature-sensitive division mutants can be used to analyze the nature of the division block.

Streptococci differ from rod-shaped bacteria in that surface growth leads to formation of a pole. This pole has been considered the equivalent of a septum in rod-shaped, gram-positive bacteria (7). Consequently, the effects of release from inhibition of a division block can be examined without complications arising from rod or filament formation. Here we show the effects of preincubations at the nonpermissive temperature on division of four classes of temperature-sensitive growth and division mutants derived from *Streptococcus faecium* ATCC 9790 (see Table 1). Division ceased immediately after transfer from 30 to 42°C in class 1 and several minutes after the shift in class 2. Class 3 divided for 45 or more min after a temperature shift-up. Class 4 divided for at least 40 min and then lysed. The characterization of these mutants is given elsewhere (4).

MATERIALS AND METHODS

Bacterial strains. Temperature-sensitive cell growth mutants of *S. faecium* ATCC 9790 used in these experiments were classified according to a previous proposal (4) and are shown in Table 1.

Growth conditions. Bacterial strains were grown at 30°C in brain heart infusion broth (Difco Laboratories) containing 3% NaCl or in streptococcal broth containing 10 g of yeast extract, 10 g of tryptone, 20 g of glucose, 26.65 g of Na_2HPO_4, and 16.45 g of NaH_2PO_4 per liter (pH 6.8) for at least three doublings in cell number. Cultures were then shifted to 42°C for various times before transfer back to 30°C.

Cell numbers were evaluated with a Coulter Counter (model ZBI) equipped with a 30-μm orifice. Culture samples (0.5 ml) were diluted in 0.5 ml of ISOTON II (Coulter Scientific) containing 0.5% formaldehyde. Cell suspensions were fixed at room temperature for 20 min and then diluted 200-fold in ISOTON II, and cell numbers were counted.

TABLE 1. Temperature-sensitive cell division mutants of *S. faecium*

Strains	Class	Main characteristics of the class
NT2/10, SPT/2, SPT/119, NT1/49, NT2/57, SPT/104, NT2/105, NT1/76, NT2/110, NT2/73	1	Division stops immediately after temperature shift. Septa appear to be completed.
SPT/8, NT1/88, SPT/95, NT1/39, NT1/89, NT1/118, SPT/144, SPT/140, NT2/106, NT1/160	2	Division continues for several minutes after temperature shift. Chromosome replication appears to be completed.
NT1/131, NT2/1, NT2/113	3	Division continues for 45 or more min after temperature shift. Division block is before completion of chromosome replication.
NT2/7, SPT/63, NT1/45, SPT/91	4 (lytic)	Division continues for at least 60 min after temperature shift and then the culture lyses. Division block is before completion of chromosome replication.

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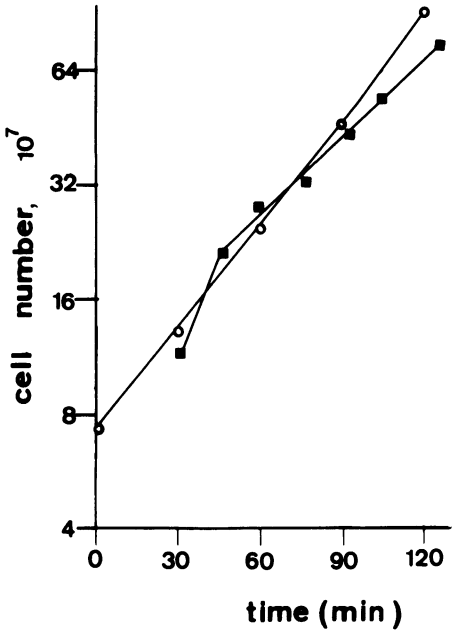

FIG. 1. Effect of temperature shift from 30 to 42°C for 30 min on subsequent increase in cell numbers of parental *S. faecium* ATCC 9790. An exponentially growing culture at 30°C was divided into two parts at time 0. One part was maintained at 30°C (○) and the other part was incubated at 42°C for 30 min and then transferred to 30°C (■). Samples were withdrawn from both cultures for cell number determination.

RESULTS

A temperature shift from 30 to 42°C for 30 min had no significant effects on subsequent cell division at 30°C of the parental strain (Fig. 1). When a class 4 mutant was shifted to 42°C for 15 min and then regrown at 30°C, cell division continued at a normal rate (Fig. 2). However, after 30 min at 42°C, at 30°C a brief 15-min lag was followed by a resumption of cell division at a preshift rate. When class 1, 2, or 3 mutants were shifted to 42°C for various periods of time and then shifted back to 30°C, the resulting divisions were highly dependent on duration of the incubation at 42°C (Fig. 3). After 45 to 60 min at 42°C (Fig. 3), cells from these mutant classes failed to divide further when shifted back to 30°C. Prolonged incubation of such cultures for up to 24 h in liquid media at 30°C did not give any increase in cell number (data not shown). On the other hand, when the same cells were kept at 42°C for 15 to 30 min, divisions occurred upon return to 30°C.

Four representative regrowth division patterns are shown in Fig. 3. In the first (Fig. 3A), the cells doubled in a synchronous way for two cycles. The same doublings were seen if cells were preincubated at 42°C for 25 or 30 min. One mutant showed this pattern. The mutants shown in Fig. 3B, C, and D gave multiple or incomplete steps when regrown at 30°C after 15 min at 42°C. The divisions were delayed for variable lengths of time when the preincubation at 42°C was for 15 to 30 min. Eight mutants behaved as shown in Fig. 3B, nine as in Fig. 3C, and five as in Fig. 3D.

In several experiments containing representatives of all mutant classes, the addition of chloramphenicol (20 μg/ml) during the recovery completely prevented subsequent divisions (data not shown). These results are consistent with the view that the cell divisions observed are not due to the separation of cells previously arrested in the chloramphenicol-insensitive D2 phase of the division cycle.

DISCUSSION

Our data differ in several respects from those reported previously for rod-shaped organisms (1–3, 8–10). First is the inability of class 1, 2, and 3 mutants to recover after incubation at 42°C for periods of over 30 to 45 min (Fig. 3). Cells exposed to the temperature block remain viable and when returned to 30°C are capable of colony formation on solid media (5).

Second is the stepwise nature of the regrowth after a short incubation at 42°C (Fig. 3). In some instances (Fig. 3A and B), the steps were synchronous or partially synchronous. In all class 1, 2, or 3 mutants studied, the first detectable biochemical change after a shift to 42°C was inhibition of protein synthesis occurring between 0 and 30 min at 42°C, whereas DNA synthesis continued for 0.20 to 1.40 doublings (manuscript in preparation). Consequently, the division synchrony observed at 30°C must be due to blockage or breakdown of an essential division element(s) during incubation at the nonpermissive temperature, followed by periodic synthesis at the permissive temperature. From the lack of regrowth in the presence of chloramphenicol, at least one of the elements may be a protein. The periodicity varied among mutants and also between experiments using the same mutant (Fig. 3A and B versus C and D). It is interesting to observe that class 4 mutants, blocked early in the division cycle, did not exhibit division synchrony when regrown at 30°C. On the other hand, no correlation could be found between regrowth pattern and timing of the cell division block in class 1, 2, or 3 mutants. These results suggest that in *S. faecium* there are unique features about the terminal

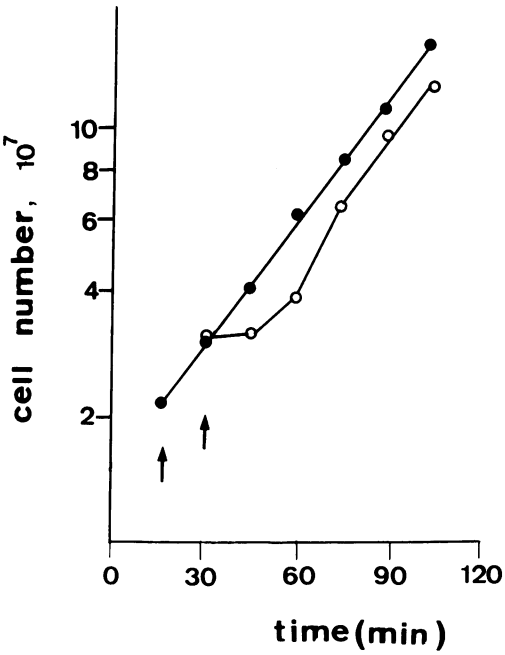

FIG. 2. Effect of temperature shift-up for 15 or 30 min on subsequent cell division at 30°C of mutant NT1/131 (class 3). An exponentially growing culture of the NT1/131 strain at 30°C was incubated at 42°C. At 15 (●) and 30 (○) min, samples were transferred to 30°C (see arrows). Samples were withdrawn from both cultures for cell number determination.

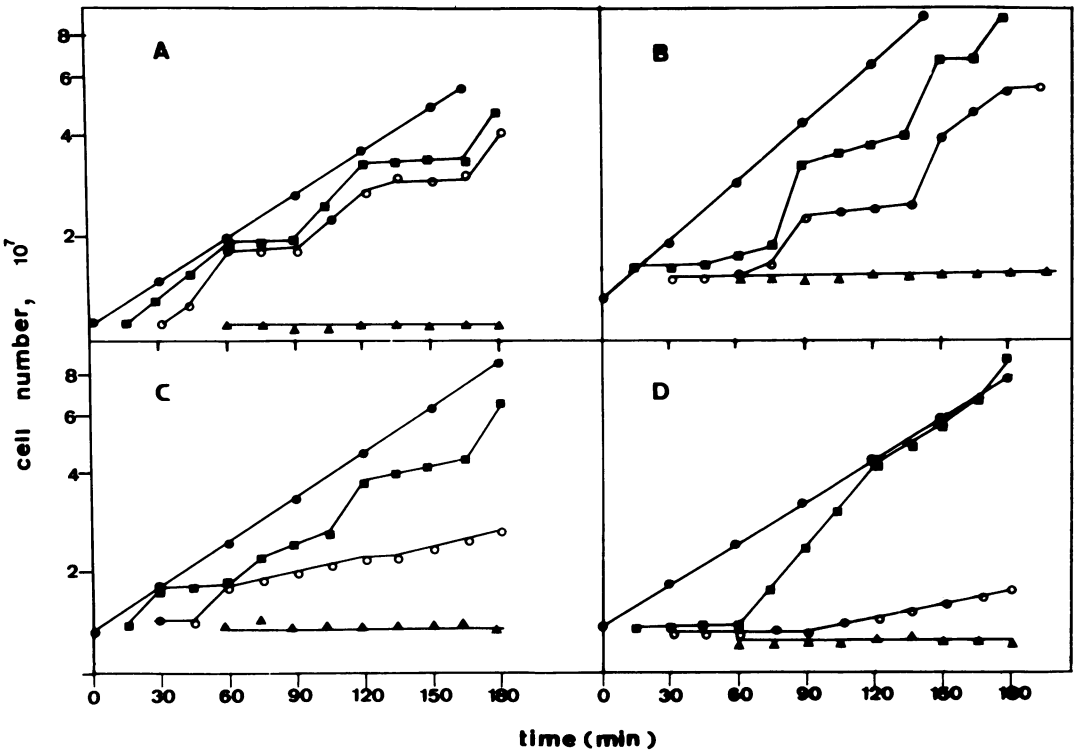

FIG. 3. Representative regrowth division patterns of four thermosensitive class 1 or class 2 mutants after incubation at 42°C for various times. (A) Strain NT2/10 (class 1); (B) SPT/8 (class 2); (C) NT1/88 (class 2); (D) SPT/2 (class 1). Exponentially growing cultures at 30°C were divided at time 0 into two parts. One part was maintained at 30°C as a control (●). The other part was incubated at 42°C. At 15 (■), 30 (○), and 60 (▲) min after the temperature shift-up, samples were transferred back to 30°C. Samples were withdrawn from the cultures for cell number determination.

stages of division. Possibly these features are due to the intimate relationship between surface growth, pole formation, and division (7). It has been shown previously (6) that in *S. faecium* protein synthesis is required for 18 to 20 min after completion of chromosome replication.

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