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Streptococcus faecium Mutants That Are Temperature Sensitive for Cell Growth and Show Alterations in Penicillin-Binding Proteins

PIETRO CANEPARI,[1]* MARIA DEL MAR LLEÒ,[1] ROBERTA FONTANA,[1] AND GIUSEPPE SATTA[2]

Istituto di Microbiologia dell'Università di Verona, Verona,[1] and Istituto di Microbiologia dell'Università di Siena, Siena,[2] Italy

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The penicillin-binding proteins (PBPs) of 209 cell division (or growth) temperature-sensitive mutants of *Streptococcus faecium* were analyzed in this study. A total of nine strains showed either constitutive or temperature-sensitive conditional damage in the PBPs. Analysis of these nine strains yielded the following results: one carried a PBP 1 constitutively showing a lower molecular weight; one constitutively lacked PBP 2; two lacked PBP 3 at 42°C, but not at 30°C; one was normal at 30°C but at 42°C lacked PBP 3 and overproduced PBP 5; two were normal at 42°C and lacked PBP 5 at 30°C; one constitutively lacked PBP 5; and one carried a PBP 6 constitutively split in two bands. The mutant lacking PBP 3 and overproducing PBP 5 continued to grow at 42°C for 150 min and then lysed. Revertants selected for growth capability at 42°C from the mutants altered in PBPs 5 and 6 maintained the same PBP alterations, while those isolated from the strains with altered PBP 1 or lacking PBP 2 or PBP 3 showed a normal PBP pattern. Penicillin-resistant derivatives were isolated at 30°C from the mutants lacking PBP 2 and from that lacking PBP 3. All these derivatives continued to show the same PBP damage as the parents, but overproduced PBP 5 and grew at 42°C. These findings indicate that high-molecular-weight, but not low-molecular-weight, PBPs are essential for cell growth in *S. faecium*. This is in complete agreement with previous findings obtained with a different experimental system. On the basis of both previous and present data it is suggested that PBPs 1, 2, and 3 appear necessary for cell growth at optimal temperature (and at maximal rate), but not for cell growth at a submaximal one (or at a reduced rate), and an overproduced PBP 5 is capable of taking over the function of PBPs 1, 2, and 3.

In earlier studies we suggested that penicillin-binding proteins (PBPs) do not necessarily each perform a fixed and constant function in cell wall metabolism, but that their functions may vary depending on the physiological status of the cells (9, 10). More recently, support has come for this suggestion in the form of experimental findings in *Streptococcus faecium*, showing that saturation of PBP 3 (alone or together with PBPs 1 and 2) causes growth inhibition when cells are incubated in a chemically defined medium at optimal temperature, but not when they are grown in this medium at lower temperatures (2), where saturation of PBP 5 is necessary for cell growth inhibition. This indicates that PBP 3 changes its functions, no longer proving indispensable for cell growth, when the physiological status of the cells is modified as a result of the changed growth conditions. In other papers, we have also shown that *S. faecium* mutants which overproduce PBP 5 are capable of growing normally in the presence of benzylpenicillin (and other beta-lactam) concentrations that saturate all PBPs except PBP 5 and stop growing when PBP 5 is saturated (2, 11). On the basis of this finding we have suggested that an overproduced PBP 5 may take over the function of other PBPs (2, 11), as previously proposed by other investigators for the PBPs of different microorganisms (13, 17, 26, 27).

Corroboration of the validity of our suggestion that a PBP does not necessarily perform a fixed and constant function would be important, particularly in view of the contribution this could make towards a clear understanding of the mechanisms of cell wall growth and septum formation. This requires a precise knowledge not only of the enzyme activity of each PBP, but also of the regulation mechanism of its activity and function.

* Corresponding author.

Two different experimental systems are often employed for studying functions of PBPs in cell physiology. One involves the use of beta-lactam antibiotics with different affinities for the various PBPs, and the other involves selection and analysis of mutants with conditional or constitutive PBP defects (7, 8, 11–13, 15–18, 23–27).

Our previous studies were mostly performed with experimental systems using beta-lactam antibiotics (2, 9–12). In the wake of the conclusions reached in these studies, we decided to test the validity of our previous proposals by isolating and analyzing the properties of *S. faecium* mutants exhibiting constitutive or conditional PBP alteration.

The properties of these mutants lend a measure of support to our suggestions that the function performed by PBPs may vary depending on the physiological status of the cells and that an overproduced PBP 5 may take over the function of other PBPs.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 209 previously described (3, 4) temperature-sensitive division (or growth) mutants of *S. faecium* PS were used in this study. Bacterial strains were grown in brain heart infusion broth (BHI) (Difco Laboratories, Detroit, Mich.) or in chemically defined medium (21). Cell number was evaluated with a Coulter Counter model ZBI equipped with a 30-μm probe as previously described (3, 4). Optical density was measured using a Beckman model DU6 spectrophotometer.

Evaluation of macromolecular synthesis. DNA, protein, and peptidoglycan synthesis was evaluated in chemically defined medium at 30 and 42°C as incorporation of labeled precursors ([^{3}H]thymidine, 4 μCi/ml; [^{3}H]leucine, 1 μCi/ml; [^{3}H]lysine, 6 μCi/ml; The Radiochemical Centre, Amersham, England) into trichloroacetic acid-precipitable frac-

TABLE 1. Temperature-sensitive mutants of *S. faecium* with PBP alterations

Mutant	Alterations in PBP pattern at:		Effect of shifting to 42°C on cell growth (and class[a])
	30°C	42°C	
NT1/27	PBP 1 with a lower mol wt	PBP 1 with a lower mol wt	Growth inhibited 30 min after temperature shift (class 2)
NT1/20	PBP 2 lacking penicillin-binding ability	PBP 2 lacking penicillin-binding ability	Growth inhibited immediately after temperature shift (class 1)
NT1/119	None	PBP 3 lacking penicillin-binding ability; PBP 5 overproduced	Growth continues for 150 min, then culture begins to lyse (class 4)
NT1/37	None	PBP 3 lacking penicillin-binding ability	Growth inhibited 30 min after temperature shift (class 2)
NT2/72	None	PBP 3 lacking penicillin-binding ability	Growth inhibited 30 min after temperature shift (class 2)
NT1/43	PBP 5 lacking penicillin-binding ability	None	Growth inhibited 15 min after temperature shift (class 2)
NT1/143	PBP 5 lacking penicillin-binding ability	None	Growth inhibited 150 min, then culture lyses (class 4)
NT1/131	PBP 5 lacking penicillin-binding ability	PBP 5 lacking penicillin-binding ability	Growth inhibited 45 min after temperature shift (class 3)
NT1/41	PBP 6 split into two bands	PBP 6 split into two bands	Growth inhibited 15 min after temperature shift (class 2)

[a] Mutants were subdivided into various classes on the basis of the effects of temperature shift on subsequent cell division, as previously described (4).

tions. Before radioactivity was counted in a Beckman LS7000 counter, samples for peptidoglycan synthesis were incubated with pronase, as previously described by Boothby et al. (1).

Isolation of revertants with growth capability at 42°C. Plates of BHI agar were inoculated with 10^8 cells and incubated at 42°C for 48 h. Colonies appearing on the plates were checked first for growth capability at the above temperature and then for growth on bile-salts agar and for chemical reactions on the API Strep System (Analytab Products, Plainview, N.Y.).

Isolation of penicillin-resistant mutants. Spontaneous mutants resistant to different penicillin concentrations were isolated by serial cultivation on BHI agar containing increasing penicillin concentrations, as previously described (11). Colonies which appeared on the plates containing the suitable penicillin concentration were checked for growth capability on the same penicillin concentration and for biochemical reactions, as reported above.

Membrane preparation and PBP detection. A 500-ml volume of cells exponentially growing (10^8 CFU/ml) at 30°C was divided into two parts; one was reincubated at 30°C, and the other was transferred to 42°C for an additional 60 min. Cultures were then rapidly collected by centrifugation at 4°C and washed twice with cold sodium phosphate buffer (0.01 M, pH 7.2). Cells were lysed with lysozyme, and membranes were collected by differential centrifugation as previously described (5, 9–11). A 20-μl sample of membranes (100 to 200 μg of proteins) in the above-described phosphate buffer was treated with 100 μM of [^{14}C]benzylpenicillin (59 Ci/mol; The Radiochemical Centre, Amersham) for 60 min at the same growth temperature. Protein solubilization, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and PBP detection were performed as previously described (5, 9–11). PBP identification was based on both molecular weight, determined as described by Coyette et al. (5), and affinity for penicillin (11).

In vivo PBP detection. Cultures of the mutants for in vivo binding were prepared as described above, and radioactive penicillin was bound to whole cells as previously described (9).

RESULTS

Mutants showing damage in PBP pattern. Of 209 temperature-sensitive cell division (or growth) mutants analyzed, 9 showed PBP alterations. Altogether, these strains exhibited damage to all PBPs except PBP 4 (Table 1, Fig. 1).

Four mutants showed an altered PBP pattern at both 30 and 42°C (constitutive damage): in one mutant, PBP 1 had a molecular weight lower than the PBP 1 of the parent; in another PBP 2 was absent; in yet another PBP 5 was absent; and in the fourth, PBP 6 was split into two bands. Five mutants were found to have a temperature-sensitive PBP alteration (conditional damage): two lacked PBP 3 at 42°C; one lacked PBP 3 and overproduced PBP 5 at 42°C; and two lacked PBP 5 at 30°C.

The same alterations were also found when PBPs of all these mutants where labeled in whole cells, thus excluding the possibility that the damaged PBP might be active in vivo, but might be so unstable as to be inactivated during membrane preparation (data not shown).

As regards the relationship between PBP damage and cell growth, only in the two mutants with altered PBP 3 was the PBP damage associated with inability to grow (Table 1). In the mutants with temperature-sensitive PBP 5 alterations, this PBP was missing at permissive temperature but appeared normal at nonpermissive temperature. The behavior of the mutant NT1/119 was particularly interesting, showing normal PBPs at 30°C and a double alteration consisting of a lack of PBP 3 and overproduction of PBP 5 at 42°C. As compared to the two strains which lacked PBP 3 and presented a normal PBP 5 at 42°C, this strain grew normally for at least 150 min at this temperature and then began to lyse (Table 1). It is worth noting that growth at 42°C for 150 min followed by lysis is not peculiar to strain NT1/119, since a similar behavior was also observed in strain NT1/143, which showed a normal PBP pattern at nonpermissive temperature. All the mutants showing the same PBP damage at 30 and 42°C grew at 30°C, but not at 42°C (Table 1).

All the cell division (or growth) mutants were previously grouped into different classes depending on the timing of the division block in the cell division cycle (4). No relationship

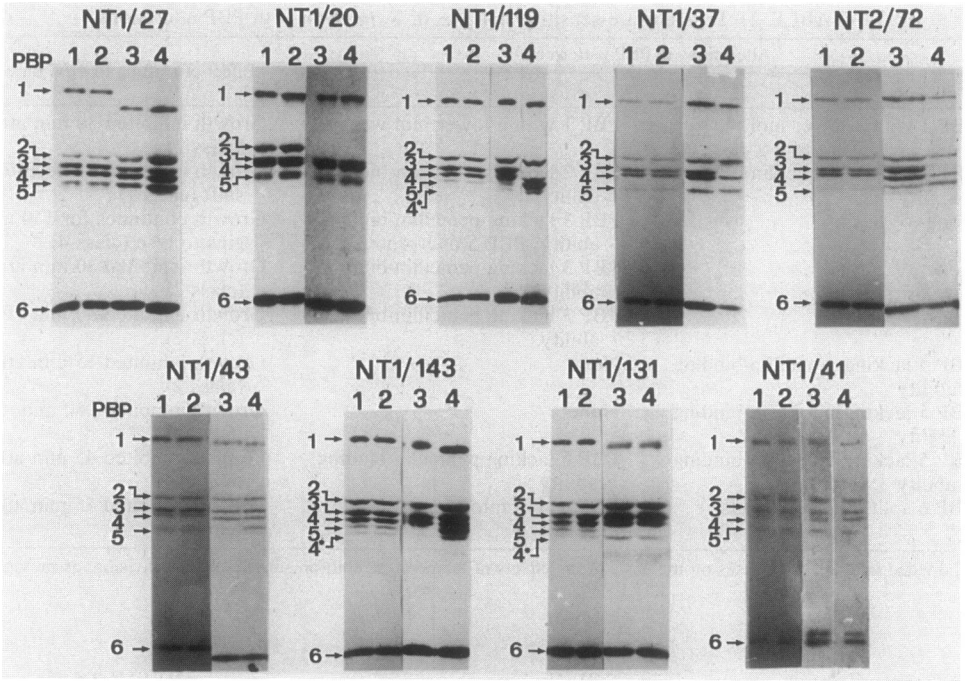

FIG. 1. Electrophoretic pattern of PBP-altered *S. faecium* mutants. In each group, lanes 1 and 2 represent the PBP pattern of the parental strain grown at 30 and 42°C, respectively; lanes 3 and 4 represent the PBP pattern of the mutant grown at 30 and 42°C, respectively. Nomenclature of PBPs is in accordance with Coyette et al. (5). PBP 4* represents a proteolytic degradation of PBP 3 and was not consistently observed in all experiments.

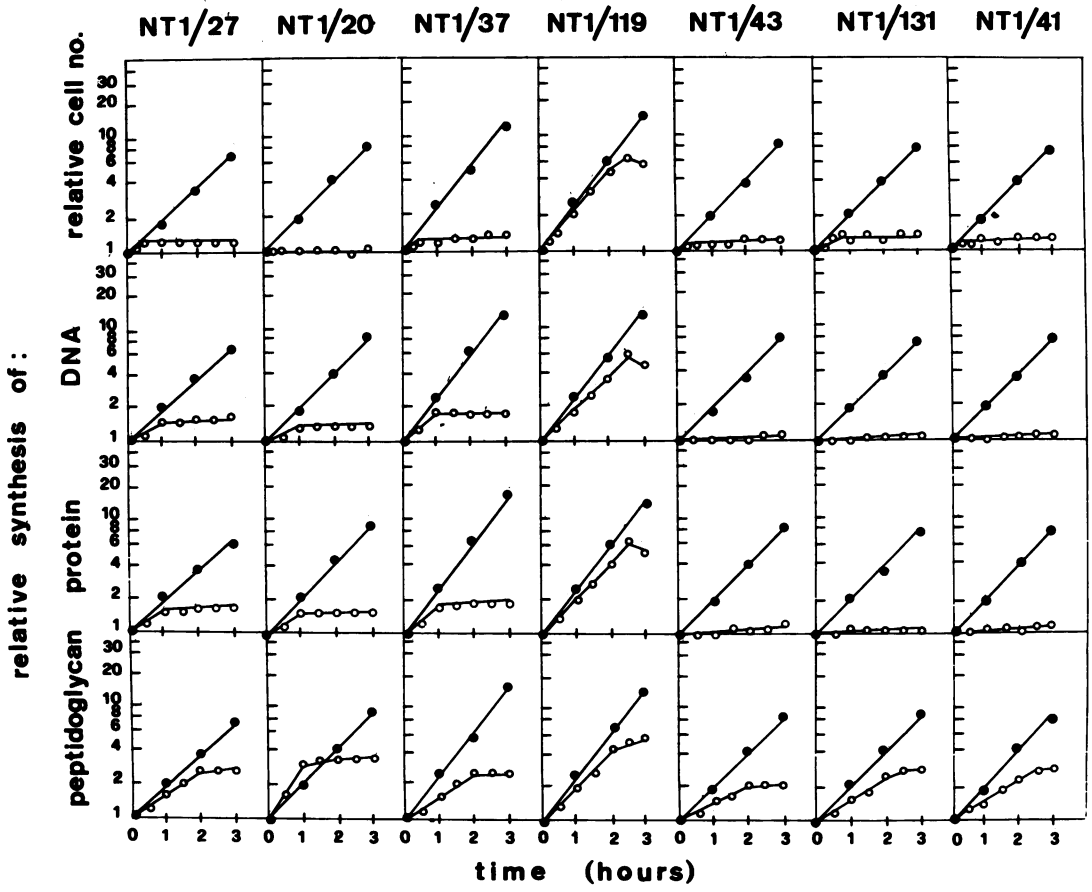

FIG. 2. Cell number increase and macromolecular synthesis in some PBP-altered mutants of *S. faecium* grown at 30°C (●) and 42°C (○).

TABLE 2. Some properties of revertants selected for growth capability at nonpermissive temperature from temperature-sensitive PBP-altered mutants

Mutants	PBP alteration	No. of revertants studied[a]	PBP damage at 30 and 42°C[b]
NT1/27	PBP 1 with a lower mol wt at 30 and 42°C	3	PBP 1 has a normal mol wt at both 30 and 42°C in all revertants
NT1/20	PBP 2 lacking penicillin-binding ability at 30 and 42°C	3	PBP 2 binds penicillin at both 30 and 42°C in all revertants
NT1/37	PBP 3 lacking penicillin-binding ability at 42°C	2	PBP 3 binds penicillin at both 30 and 42°C in both revertants
NT2/72	PBP 3 lacking penicillin-binding ability at 42°C	2	PBP 3 binds penicillin at both 30 and 42°C in both revertants
NT1/131	PBP 5 lacking penicillin-binding ability at 30 and 42°C	2	PBP 5 lacks penicillin-binding ability at 30 and 42°C in both revertants
NT1/43	PBP 5 lacking penicillin-binding ability at 30°C, but not at 42°C	3	PBP 5 lacks penicillin-binding ability at 30°C, but not at 42°C, in all revertants
NT1/41	PBP 6 split into two bands	2	The PBP 6 alteration is maintained at 30 and 42°C in both revertants

[a] Unless otherwise indicated, all revertants isolated from a given mutant showed equal properties.
[b] PBPs not mentioned were normal at both 30 and 42°C. Revertant growth was normal at both temperatures.

was found between the specific PBP alteration and the timing of cell division block.

Macromolecular synthesis and residual division after shift to nonpermissive temperature. Effects of nonpermissive temperature on macromolecular synthesis were similar in all mutants with different alterations (Fig. 2). In general, in all mutants, cell division and protein and DNA synthesis stopped within the first hour of incubation at nonpermissive temperature. In particular, in the strains showing damage to PBPs 1, 2, and 3, a complete block of DNA and protein synthesis occurred only 60 min after the shift to nonpermissive temperature, whereas in the mutants with altered PBP 5 or 6, this block was virtually immediate (Fig. 2). In contrast, peptidoglycan synthesis continued at 42°C in almost all strains for at least 3 h. In most cases the rate of synthesis was (30 to 50%) lower than at 30°C, but in the mutant carrying a damaged PBP 2 it was faster for the first hour and then gradually stopped increasing. Residual division continued for 30 min in the mutants with altered PBPs 1 or 3 and for 15 to 150 min in the strains with altered PBP 5 (at 30 and 42°C) or 6. The mutant with altered PBP 2 was the only one in which cell number increase was blocked immediately after shifting to 42°C.

Finally, it should be noted that the mutant lacking PBP 3 and overproducing PBP 5 synthesized its macromolecules and showed virtually normal division for 150 min, and then began to lyse (Table 1 and Fig. 2).

Role of PBP damage in inability of mutants to grow at nonpermissive temperature. To establish in which mutants PBP damage was responsible for inability to grow at nonpermissive temperature, we isolated, from the strains showing altered PBPs at 42°C, revertants selected for growth capability at this temperature and analyzed their PBP patterns. All revertants isolated from mutants with altered PBPs 1, 2, or 3 showed a normal PBP pattern, suggesting that these mutants might carry a single mutation governing the phenotype observed and that alterations in these PBPs might be directly responsible for the inability of the cell to grow at 42°C. By contrast, all revertants isolated from mutants lacking PBP 5 or carrying an altered PBP 6 continued to exhibit the PBP damage shown by the parent, which was unable to grow at nonpermissive temperature (Table 2 and

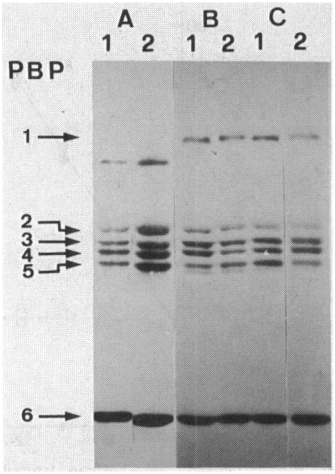

FIG. 3. Electrophoretic pattern of PBP 1-altered mutant NT1/27 (A) and two of its revertants able to grow normally at 42°C (B, C). Each strain was grown at 30°C (lane 1) and 42°C (lane 2).

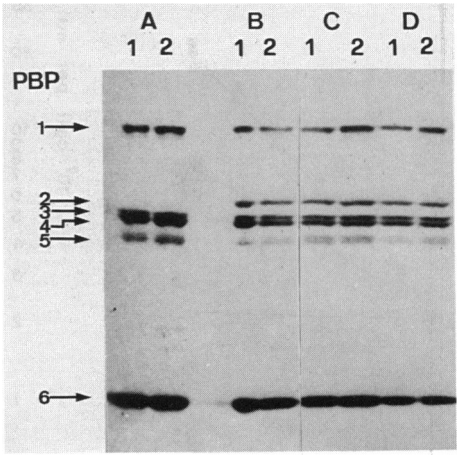

FIG. 4. Electrophoretic pattern of PBP 2-altered mutant NT1/20 (A) and three of its revertants able to grow normally at 42°C (B through D). Each strain was grown at 30°C (lane 1) and 42°C (lane 2).

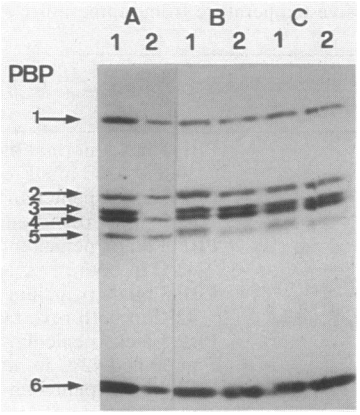

FIG. 5. Electrophoretic pattern of PBP 3-altered mutant NT1/37 (A) and two of its revertants able to grow at 42°C (B, C). Each strain was grown at 30°C (lane 1) and 42°C (lane 2).

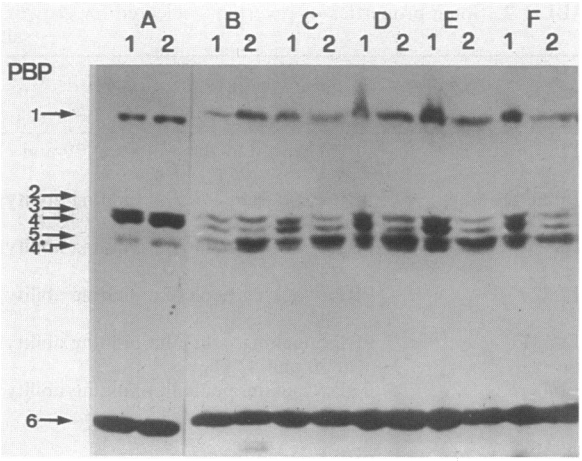

FIG. 7. Electrophoretic pattern of PBP 2-altered mutant NT1/20 (A) and five of its penicillin-resistant derivatives (B through F). Each strain was grown at 30°C (lane 1) and 42°C (lane 2).

Fig. 3, 4, and 5). In these mutants, more than one mutation might be responsible for the phenotype observed, and inability to grow at 42°C might be unrelated to alteration in PBPs 5 and 6. In addition, the finding that revertants of mutants with altered PBPs 1 and 2 carry normal PBPs at both 30 and 42°C indicates that these PBPs are necessary for growth at 42°C, but not at 30°C.

Selection of penicillin-resistant derivatives from mutants carrying damaged PBPs. Since the lack of PBP 3 appeared to cause inability to grow, the fact that strain NT1/119, lacking PBP 3 and overproducing PBP 5, grew normally at 42°C for at least 2 h suggests the possibility that an overproduced PBP 5 may compensate for lack of PBP 3 and may also take over the functions of other PBPs. We then attempted isolation of spontaneous penicillin-resistant derivatives (which we had previously shown [11] to overproduce PBP 5) from mutants NT1/20, NT1/37, and NT1/43. With the exception of the first of these, several strains with relatively high-level resistance to penicillin were obtained from these mutants. Each of the five strains isolated from the mutant lacking PBP 2, and the two strains isolated from the mutant lacking PBP 3, overproduced PBP 5 and grew at 42°C, though still lacking PBPs 2 and 3, respectively (Table 3 and Fig. 6, 7, and 8). By contrast, the three strains isolated from mutant NT1/43, lacking PBP 5 at 30°C but not at 42°C, did not grow at the higher temperature (Table 3 and Fig. 6).

DISCUSSION

The genetic and biochemical characterization of the temperature-sensitive mutants showing damaged PBPs allows us to establish, for five of the six PBPs of *S. faecium*, which do, and which do not, need to be unaltered for the cells to grow. Lack of PBP 5 or alteration of PBP 5 or PBP 6 does not prevent bacterial growth, as revertants of strains NT1/131, NT1/43, and NT1/41 that grow at 42°C still showed the same PBP damage as the parents. In contrast, unaltered PBPs 1, 2, and 3 are indispensable for cell growth, as revertants of strains NT1/27, NT1/20, NT1/37, and NT2/72 capable of growth at 42°C showed a normal PBP pattern at that tem-

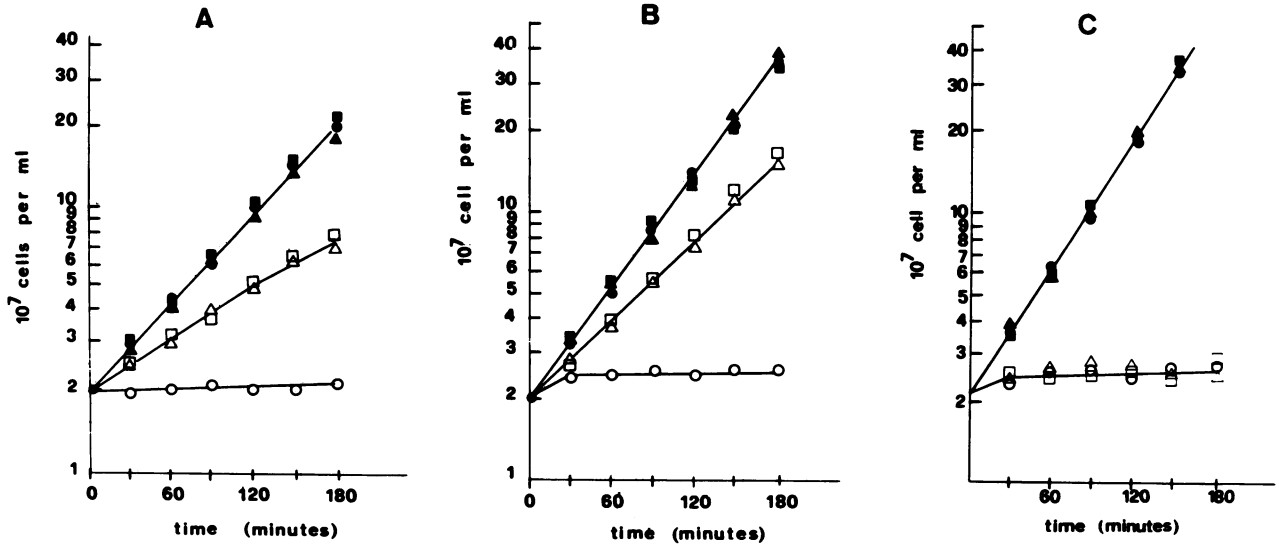

FIG. 6. Cell number increase of PBP-altered mutants NT1/20 (A), NT1/37 (B), and NT1/43 (C) and two of their penicillin-resistant derivatives growing at 30°C (●, mutants; ■, ▲, derivatives) and 42°C (○, mutants; □, △, derivatives).

TABLE 3. Penicillin-resistant derivatives isolated from temperature-sensitive PBP-altered mutants

Mutants	MIC of penicillin (μg/ml)	PBP alteration	No. of penicillin-resistant derivatives studied	Main characteristics of penicillin-resistant derivatives[a]
NT1/20	2	PBP 2 lacking penicillin-binding ability at 30 and 42°C	5[b]	Growth occurs at 42°C; PBP 2 alteration maintained; PBP 5 overproduced
NT1/37	2	PBP 3 lacking penicillin-binding ability at 42°C	2[c]	Growth occurs at 42°C; PBP 3 alteration maintained; PBP 5 overproduced
NT1/43	0.6	PBP 5 lacking penicillin-binding ability at 30°C, but not at 42°C	3[c]	Growth inhibited 15 min after temperature shift; PBP 5 overproduced at both 30 and 42°C

[a] Penicillin-resistant derivatives were isolated by serial cultivation on BHI agar containing increasing penicillin concentrations (11). Each strain studied was obtained in a separate experiment. Growth was followed for 180 min, and in no case was cell lysis observed within this period of time.
[b] These strains were selected after growth in the presence of 10 μg/ml. Their penicillin MIC ranged from 15 to 25 μg/ml.
[c] These strains were selected after growth in the presence of 40 μg/ml. Their penicillin MIC ranged from 60 to 90 μg/ml.

perature. However, lack of PBP 3 may cause inability to grow in all conditions, whereas lack of PBP 2 or alteration of PBP 1 may be compatible with growth at 30°C, but not at 42°C. In addition, the mutants described in this study show that lack of, or damage to, one of the PBPs essential for growth may be compensated for by an alteration in another PBP. In fact, overproduction of PBP 5 enables mutants lacking either PBP 3 or PBP 2 to grow at nonpermissive temperature. In a previous paper, we have shown that for *S. faecium* grown in BHI (the medium used in this study) the optimal temperature is 42°C (10). PBPs 1 and 2 appear to be necessary, then, for cell growth at optimal, but not at suboptimal temperature (and probably for cells to grow at the maximum, but not at a reduced rate; see references 2 and 10). An overproduced PBP 5 may also take over the function that PBPs 2 and 3 perform in cells grown at optimal temperature (and probably at maximal rate). Due to this capability of the overproduced PBP 5, alteration of PBP 1, or lack of PBP 2, may not prevent growth at 30°C because a normally produced PBP 5 can compensate for such alterations in cells growing at a submaximal rate.

The findings of this study lend support to our previous proposal that PBPs of *S. faecium* do not each perform a specific, fixed function, but that their function may vary depending on the physiological status of the cells (10). In fact, PBPs 1 and 2 have proven to play an essential role in cells incubated at optimal but not at suboptimal temperature. In addition, these PBPs, as well as PBP 3, are no longer essential even for growth at optimal temperature when *S. faecium* cells overproduce PBP 5. Our proposal was originally based on (i) the observation that in *S. faecium* cells growth medium and temperature greatly influenced both sensitivity to beta-lactams and PBP electrophoretic pattern (10), and (ii) studies in which specific PBP saturation by various beta-lactams and growth inhibition at optimal and suboptimal temperature were evaluated (2, 11, 12). The fact that the studies performed here, employing a completely different experimental system, have confirmed our previous conclusions adds strength to our claim and makes *S. faecium* one of the most useful microorganisms in understanding peculiar aspects of PBP physiology. It also accords well with our previous model regarding the mechanism of growth inhibition by beta-lactams, in which we suggested that these antibiotics inhibit bacterial growth by an indirect mechanism; this mechanism may be different for the various antibiotics in the group and for bacterial strains of different species, depending on the physiological role played by the PBP(s) essential for growth which is inactivated by the antibiotic (10).

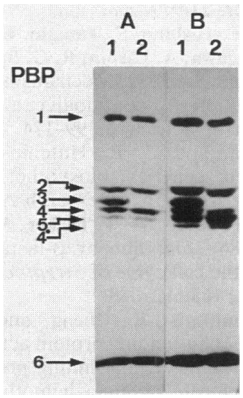

FIG. 8. Electrophoretic pattern of PBP 3-altered mutant NT1/37 (A) and its penicillin-resistant derivative (B). Each strain was grown at 30°C (lane 1) and 42°C (lane 2).

In *Escherichia coli*, the microorganism whose PBPs have been studied most extensively, some of these proteins have been found to be essential for cell growth and some were not (15, 16, 18, 24, 27, 29). A number of the essential PBPs have been described as specifically involved in glycan chain extension, necessary for septum formation and for lateral wall elongation. In *S. faecium* we have identified a situation similar to *E. coli* in that some PBPs do, and some do not, appear essential for growth under any of the conditions tested. It is interesting that, in both microorganisms, the essential PBPs are those presenting the highest molecular weights. Another physiological property of *S. faecium* PBPs shown by the mutants described in this work was, however, peculiar to this microorganism: the high-molecular-weight PBPs of *S. faecium* are not always essential for cell growth, but only for growth at the maximum rate. In addition, one of the "nonessential" PBPs (PBP 5) becomes the only essential one, both in cells grown at suboptimal temperature and in cells that overproduce it. Phenomena of apparent compensation between different PBPs have been described in other microorganisms (13, 16, 26, 27), but such phenomena are clearly different from the case reported here, particularly since PBPs which are essential in some, but not in other, growth conditions, have not been previously reported. This situation is also quite different from that of methicillin-resistant staphylococci, in which it is a novel PBP which, when present, takes over the functions of all other PBPs regardless both of growth conditions and of the amount of PBP produced (14, 20).

The mutants described here are the first example of

temperature-sensitive cell division or cell growth mutants that constitutively or conditionally show damaged PBPs isolated from cocci. Mainly on the basis of studies with beta-lactam antibiotics, it has been suggested that PBP 3 is involved in septum formation, while PBP 2 is necessary for cell size control or for peripheral surface growth (6, 19). The properties of our mutants confirm that PBP 3 is essential for cell division, but do not indicate whether PBP 2 plays a role in peripheral cell surface growth or in cell size control. As regards the function of this PBP, they would tend rather to suggest that it is also necessary for septum formation. The fact that, after a shift to nonpermissive temperature, mutants lacking PBP 3 undergo a significant number of residual divisions, while the strain lacking PBP 2 does not divide at all, indicates that PBP 3 acts at an early stage of septum formation, while PBP 2 acts at a very late one. This has also been proposed by others on the basis of studies on cell division inhibition in the presence of beta-lactams showing different affinities for the various PBPs (19). The properties of the *S. faecium* temperature-sensitive mutants do not correspond entirely to those considered peculiar to cell division mutants, as conceived in the standard definition. These mutants should continue to exhibit increasing cell mass when cell division is inhibited (22). However, the mutants described here, in which inability to grow was shown to depend on PBP damage, have to be considered cell division mutants, in spite of the fact that they do not match the conventional definition. In these mutants, owing probably to the peptidoglycan synthesis defect following PBP damage, the cell wall peripheral surface cannot be expanded (or can be expanded only to a limited extent), and DNA and protein synthesis stops as a result of lack of intracellular space to accommodate the new macromolecules. In forthcoming papers we intend to present data showing that, in cocci, prevention of bacterial cell surface expansion invariably leads to macromolecular synthesis inhibition. In this respect, it is also important to note that, in the temperature-sensitive mutants in which growth inhibition was most likely to be dependent on PBP damage (strains NT1/27, NT1/20, and NT1/37), DNA and protein synthesis continued for at least 30 min after the shift to nonpermissive temperature, whereas it stopped immediately in all other mutants. Most probably, the definition of cell division mutants proposed for rod-shaped bacteria is not also directly applicable to cocci, which do not extend the cell surface when septum formation is inhibited.

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