JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2416–2417
0021-9193/92/072416-02$02.00/0

Vol. 174, No. 7

Escherichia coli Mutant Y16 Is a Double Mutant Carrying Thermosensitive *ftsH* and *ftsI* Mutations

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Received 15 October 1991/Accepted 15 January 1992

The *Escherichia coli* mutant Y16, which shows thermosensitive colony formation and filamentation with reduced amounts of penicillin-binding protein 3 (PBP3), has mutations in the *ftsI* gene encoding PBP3 and in the *ftsH* gene. The *ftsI* mutation markedly reduces the amount of PBP3 at 42°C, whereas the amount in the *ftsH* single mutant is slightly reduced.

The mutant Y16 was originally isolated as a cell division mutant, and the mutation responsible for the thermosensitivity in colony formation was mapped at 69 min on the *Escherichia coli* chromosome (5). Penicillin-binding assays suggested that thermosensitive filamentation of the mutant was caused by a decrease in the amount of penicillin-binding protein 3 (PBP3) (2). PBP3 is a septum-forming enzyme essential for cell division and is encoded by the *ftsI* gene located at 2 min (7–9). Transformation by plasmids which overproduce PBP3 corrected thermosensitive filamentation of Y16 but not thermosensitive colony formation (2, 4). Genetic analysis showed that the *ftsH* mutation in Y16, which is located at 69 min and is responsible for thermosensitive colony-forming ability, is recessive to the wild-type allele and that the degree of filamentation caused by the *ftsH* mutation is dependent on the host genetic background (1).

In this paper, we report the genetic identification of two mutations in the mutant Y16: one in the *ftsI* gene responsible for thermosensitive filamentation and another in the *ftsH* gene responsible for thermosensitive colony formation. We also report the results of immunological detection of PBP3 in transductants carrying either the *ftsI* or the *ftsH* mutation.

When the *ftsH* mutation located at 69 min [which is referred to as *ftsH1*(Ts) hereafter] was transduced into certain strains, all of the *ftsH1*(Ts) transductants showed thermosensitive colony formation, but, in contrast to the original mutant, Y16, they did not form filaments (2, 4). A plasmid, pPH115, overexpressing PBP3 (3) prevented filamentation of Y16, but the transformant was still thermosensitive for colony formation (4). These results suggested that the lethality of the *ftsH1*(Ts) mutation was not due to decreased PBP3 activity and that Y16 might have a second mutation, in the *ftsI* gene encoding PBP3, which is responsible for filamentation.

We performed P1 transduction experiments by using transposons located near the *ftsI* gene (2 min) and the *ftsH* gene (69 min) to examine whether Y16 has a second mutation located at 2 min in addition to the *ftsH1*(Ts) mutation. Results showed that Y16 has a second mutation at 2 min and that this mutation is responsible for filamentation. The transductant AR736 (Table 1) carrying the mutation located at 2 min filamented partially after 2 h at 42°C (Fig. 1C) but formed colonies at 42°C. On the other hand, the transductant AR754 carrying the *ftsH1*(Ts) mutation showed an inability to form colonies and showed some cell elongation after 2 h at 42°C (Fig. 1D). Filamentation caused by the mutation at 2 min was more evident in some other strains, but all of the transductants carrying the mutation could form colonies at 42°C. Cell elongation caused by the *ftsH1*(Ts) mutation was less evident in some other strains, although all of the *ftsH1*(Ts) transductants of the strains tested showed thermosensitive colony formation.

Complementation tests indicated that filamentation due to the mutation at 2 min was suppressed by plasmids carrying the wild-type *ftsI* gene. Sequencing of the mutated *ftsI* gene of Y16 revealed a substitution of the second base of the codon for the 372nd Pro (C to T), corresponding to the amino

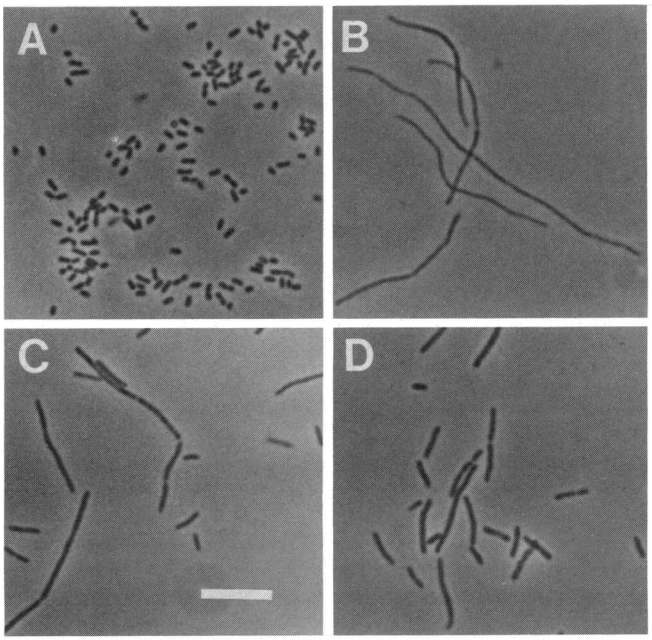

FIG. 1. Phase-contrast micrographs of mutant *E. coli* cells. Cells were grown for 2 h at 42°C. (A) C600 (wild type); (B) Y16 [*ftsI372*(Ts*) *ftsH1*(Ts)]; (C) AR736 [*ftsI372*(Ts*)]; (D) AR754 [*ftsH1*(Ts)]. Bar, 10 μm.

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference, source, or construction
C600	*thr-1 leu-6 thi-1 supE44 lacY1 tonA21*	H. Hara
Y16	Same as for C600 except for *ftsH1*(Ts) *ftsI372*(Ts*)[a]	5
CAG12153	*zha-6*::Tn*10*	6
BW7261	HfrC *leu-63*::Tn*10 tonA22* Δ(*argF-lac*)*U169 ompF627 relA1 spoT1* T2[r]	A. Nishimura
AR442	Same as for Y16 except for *zha-6*::Tn*10*	P1*vir*/CAG12153→Y16
AR586	Same as for Y16 except for *leu-63*::Tn*10*	P1*vir*/BW7261→Y16
AR754	Same as for C600 except for *zha-6*::Tn*10 ftsH1*(Ts)	P1*vir*/AR442→C600
AR736	Same as for C600 except for *leu-63*::Tn*10 ftsI372*(Ts*)	P1*vir*/AR586→C600

[a] Y16 was found to have an *ftsI* mutation in addition to the mutations described (see text).

acid substitution Pro-372 to Leu, in the coding sequence of the *ftsI* gene. Therefore, we refer to the mutation as *ftsI372*(Ts*) hereafter.

A constructed double mutant carrying both the *ftsH1*(Ts) and *ftsI372*(Ts*) mutations filamented extensively at 42°C, as did the original double mutant, Y16 (data not shown). This suggests that the combination of the two mutations may cause a more pronounced thermosensitive block to cell division than does the *ftsI372*(Ts*) mutation alone.

To measure the amount of PBP3 in the mutants, immunoblotting analyses of total cell extracts were carried out by using an antiserum against PBP3. The anti-PBP3 antiserum (obtained from J. A. Ayala) was partially purified by using total protein samples prepared from Y16 [*ftsI372*(Ts*) *ftsH1*(Ts) mutant] cultivated at 42°C for 3 h, which included no detectable amounts of PBP3, to minimize signals for other proteins. In the *ftsI372*(Ts*) mutant, AR736, the amount of PBP3 was markedly reduced after 2 h at 42°C (Fig. 2, lane 2) compared with that in the wild-type strain C600 (Fig. 2, lane 1). On the other hand, in the *ftsH1*(Ts) mutant, AR754, the amount of PBP3 was slightly reduced after 2 h at 42°C (Fig. 2, lane 3).

Strains carrying the plasmid pAR201, which is a chloramphenicol-resistant derivative of pPH115 and overproduces PBP3, were also examined. The decrease in the amount of PBP3 caused by the *ftsI372*(Ts*) mutation was fully suppressed by the presence of pAR201, as expected (Fig. 2, lane 5). The presence of pAR201 also increased the amount of PBP3 in AR754 (Fig. 2, lane 6), but the amount of PBP3 in mutant cells was reduced compared with that in wild-type C600 cells carrying pAR201 (Fig. 2, lane 4).

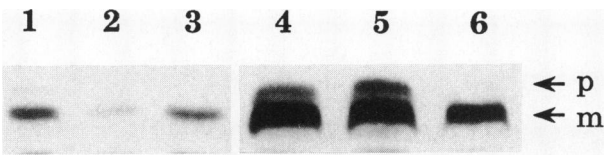

FIG. 2. Immunological detection of PBP3. Cells were grown for 2 h at 42°C. Lanes: 1, C600 (wild type); 2, AR736 [*ftsI372*(Ts*)]; 3, AR754 [*ftsH1*(Ts)]; 4, C600(pAR201); 5, AR736(pAR201); 6, AR754 (pAR201). Abbreviations: p, pre-PBP3; m, mature PBP3.

We previously measured PBP3 expression in the *ftsH1*(Ts) mutant and found no decrease in either transcription or translation of *ftsI* (4). It is noteworthy that pre-PBP3 is completely absent in the *ftsH1*(Ts) mutant, even when the overproducing plasmid is put into the mutant (Fig. 2, lane 6). This is not due to the lower level of expression, since pre-PBP3 is visible in the wild-type C600 expressing low levels of PBP3 (Fig. 2, lane 1). Unpublished results (10) indicate that the *ftsH1*(Ts) mutation may have an effect on the insertion of PBP3 into the cell membrane. Evidence suggests that most pre-PBP3 observed in C600 has already been inserted into the membrane and that a significant fraction of pre-PBP3 expressed in the *ftsH1*(Ts) mutant cells has been rapidly degraded before insertion, causing no accumulation of pre-PBP3 in the mutant cells (10).

We thank J. A. Ayala, H. Hara, A. Nishimura, U. Schwarz, B. G. Spratt, D. R. Edward, and P. Bouloc for generously supplying antiserum, bacterial strains, and plasmids and for technical suggestions and stimulating discussion. We also thank A. Matsusaka, C. Ichinose, S. Fukunaga, and I. Yoshioka for assistance.

This work was supported by grants from the Ministry of Education, Science and Culture of Japan and from the Medical Research Council of Great Britain.

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