THERMOSENSITIVE MUTANT OF ESCHERICHIA COLI REQUIRING NEW PROTEIN SYNTHESIS TO RECOVER CELLULAR DIVISION ABILITY

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SUMMARY: Several thermosensitive mutants deficient in cell division were derived from E. coli by means of membrane filters. Two of the mutants showed normal growth at 30°, filament formation at 42° and rapid increase in cell number when shifted back from 42° to 30°. After the temperature shift, the one could not divide in the presence of 150 μg/ml of chloramphenicol while the other could irrespective of the antibiotic.

For the purpose of analysing the biochemical pathways involved in cellular division of bacteria various thermosensitive mutants have been isolated (1,2,3), Kohiyama et al. (2) and Hirota et al. (3) finely classified those mutants on DNA synthesis, occurrence of septation, cell morphology etc. and showed that the process of the cellular division cycle is controlled by several genetical loci. However, the attempt of biochemical resolution of the materials directly or closely related to septum formation or cellular division has not been successful. In this respect we have attempted to isolate new kinds of thermosensitive mutants by a simplified method of Van de Putte et al. (1). The common characteristics of the mutants selected by this method are to divide normally at 30° but not at 42°, to form filamentous cells at 42°, to retain viability at 42° at least for 120 min, to recover colony forming ability when shifted back to 30° after 120 min of incubation at 42°.

* Abbreviations: NBT, nutrient broth containing thymine; NTG, N-methyl-N'-nitro-N-nitrosoguanidine; CAP, chloramphenicol.

MATERIALS AND METHODS

Bacterial strain and media: E. coli JE1011 (F^- thr^- leu^- trp^- his^- thy^- thi^- ara^- lac^- gal^- xyl^- mtl^- str^r) was used as a parent strain. The strain was derived from E. coli K12 and kindly obtained from Dr. H. Matsuzawa of our department. NBT* contained 10 g of polypeptone, 7 g of meat extract, 3 g of NaCl and 10 mg of thymine per liter of distilled water. For nutrient agar, NBT was solidified with 1.5 per cent agar. Tris-maleic buffer contained, per liter of distilled water, Tris-HCl 6.057 g, maleic acid 5.804 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $(NH_4)_2SO_4$ 1 g, sodium citrate 0.5 g, pH was adjusted to 6.0 with n-NaOH.

Chemicals: NTG was purchased from Aldrich Chemical Co., Milwaukee, Wis. CAP was generously gifted by Dr. H. Okazaki, Sankyo Co. Ltd.

Isolation of mutants: Preparation of NTG solution and treatment of cells with NTG were made following the little modified method of Adelberg et al. (4). 0.1 ml of the overnight culture of E. coli JE1011 was introduced into 10 ml of fresh NBT and incubated at 30° with reciprocal shaking. When the culture reached the logarithmic phase of growth, the cells were filtered on a 25 mm membrane filter (Sartrius Membranfilter GmbH, 34 Goettingen, West Germany), pore size 0.45 μ. After washing on the filter with 10 ml of Tris-maleic buffer, the cells were suspended in 2 ml of Tris-maleic buffer. NTG was added to the suspension to a final concentration of 100 μg/ml. The final concentration of treated cells was 10^9/ml. The mixture was kept at 30° for 30 min. After incubation, the sample was filtered on a membrane filter of 0.45 μ pore size, washed with 10 ml of cold saline and resuspended in 2 ml of saline. 0.1 ml of the suspension was inoculated in 10 ml of NBT and incubated at 42° for 120 min with reciprocal

shaking. Then the culture was poured onto a 25 mm membrane filter, 8 μ pore size, and filtered gently. The cells retained on the filter were washed with 10 ml of saline and resuspended in 10 ml of saline. The suspension was diluted to 5 x 10^3 cells/ml with saline. 0.1 ml of the sample was pipetted on the surface of solidified agar in petri dishes and overspread with 3.0 ml of melted 0.75 per cent agar at 45°. After 24 hours of incubation at 30° the colonies were replica plated on a couple of plates. One of the plates was incubated at 30° and the other at 42° for 24 hours. The colonies formed at 30° but not at 42° were obtained.

RESULTS

Isolation of mutants: When E. coli JE1011 was treated with 100 μg/ml of NTG in Tris-maleic buffer at 30° for 30 min, 50 per cent of the cells survived. This condition has been reported by Adelberg et al. (4) to be optimal for mutagenesis by NTG in E. coli K12. After 120 min of incubation at 42° in NBT following NTG treatment, the cell morphology was studied microscopically. 3 to 5 per cent of the cells formed filaments. The filtration on a filter of 8 μ pore size increased the frequency of filamentous cells by 5 to 10 times. The percentage of thermosensitive mutants to the filtered cells was 0.1. Among 59 strains obtained by this procedure 23 strains (group 1) formed filamentous cells at 42° and normal cells at 30°, 21 strains developed into a mixture of filamentous and normal cells only at 42° or at both 42° and 30°, and the rest did not reveal any morphological change at any temperature. Repeating filtration for three times on the filter of 8 μ pore size resulted in the microscopic appearance of filamentous cells to the frequency of 70 to 80 per cent. However,

the number of viable cells decreased and the final yield of thermosensitive mutants was less than 10^{-5}.

Strains in group 1 were examined by a microscope directly or after Giemsa staining. Most of them including ts 1 and ts 20 formed multinucleated filaments after incubation at 42°

Ts 1 and ts 20: The increases in optical density at 30° and 42° were compared on two of the strains in group 1, ts 1 and ts 20. Fig. 1 shows that after temperature shift to 42° increase of optical density was more rapid for 60 min, but slower after that, than it was at 30°. Cells grown at 42° for 60 min formed filaments 2 to 4 times as long as normal cells.

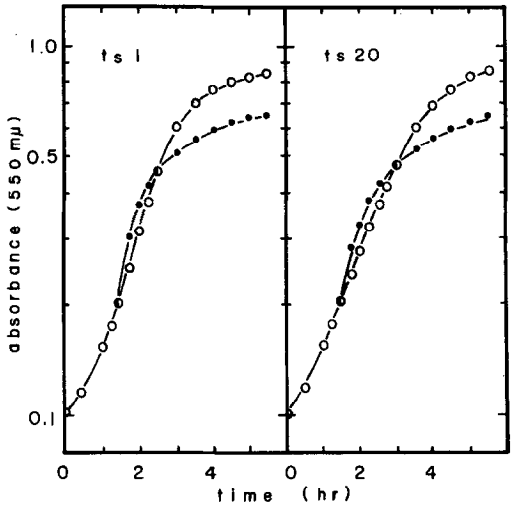

Fig. 1. Increase in optical density of ts 1 and ts 20 grown at different temperature conditions.
The culture was grown at 30°. When optical density at 550 mμ reached 0.20 the culture was divided into two parts: one (•) was shifted to 42° and the other (O) was kept at 30°.

As shown in Fig. 2 both strains showed exponential growth at 30°. However, when the cells were shifted to 42° cell division stopped immediately. The cell number of ts 1 at 42° gradually decreased and then increased. In the case of ts 20, viable count remained constant as long as 6 hours. When the cells

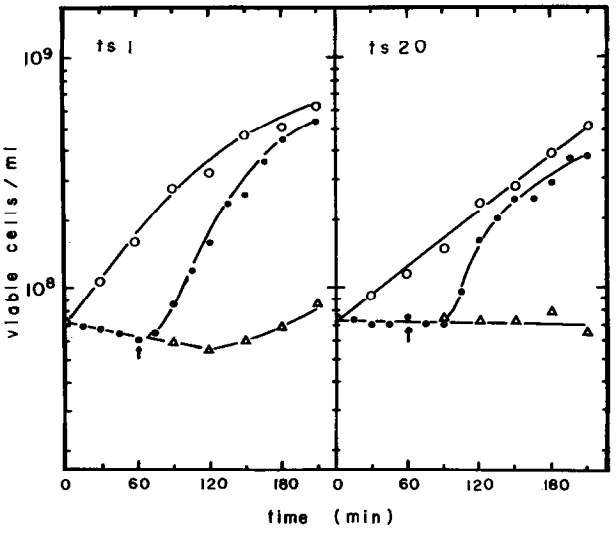

Fig. 2. Viable count of ts 1 and ts 20 grown under different temperature conditions.
The culture grown at 30° in NBT was divided into two parts: one (O) was kept at 30° and the other was shifted to 42°. After 60 min of incubation at 42° the culture (•) was shifted back to 30° while a part (Δ) was kept at 42°. Viable cells were counted after 24 hr of incubation at 30° on NBT agar plates.

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were shifted back to 30° after 60 min of incubation at 42° the cell number increased more rapidly for 60 min than it did at 30° and later almost as slow as the normal growth.

It was examined whether this rapid increase in cell number was influenced by CAP. Ts 1 could divide in the presence as well as in the absence of 150 μg/ml of CAP. On the other hand, division of ts 20 was completely inhibited when CAP was added at the time of the shift. However, a little increase in cell number was observed when the cells were exposed to the antibiotic 15 min after the shift. The results are shown in Fig. 3.

DISCUSSION

An experiment stated above shows that the thermosensitive mutants deficient in cell division which form filaments at 42°

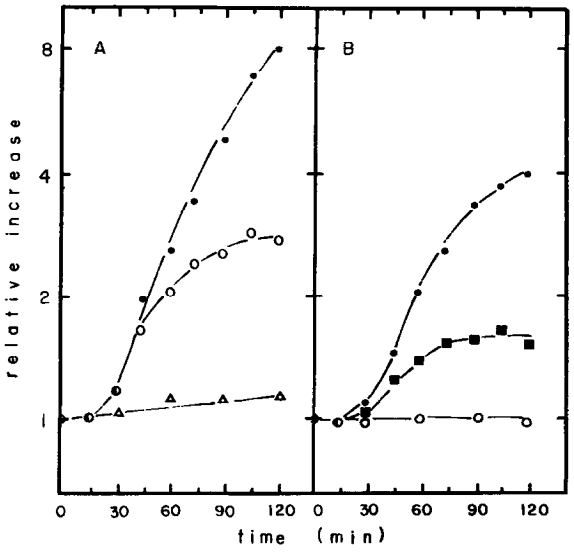

Fig. 3. Relative increase of colony formers grown with or without CAP.
A) Ts 1 grown at 30° to a density of 7.0 x 10^7 cells/ml was shifted to 42°. At the time of the shift (▲) or 60 min after the shift (○, ●), the culture was shifted back to 30°. To (▲) and (○) CAP was added to a final concentration of 150 µg/ml at the time of temperature shift to 30°.
B) Ts 20 grown at 30° to a density of 5.5 x 10^7 cells/ml was shifted to 42°. After 60 min the culture was shifted back to 30° (●). CAP was added to a final concentration of 150 µg/ml at the time of (○) or 15 min after (■) the temperature shift to 30°.

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and can divide normally at 30° were isolated at a good yield. The method using 8 µ pore size membrane filter successfully selected the filamentous cells formed during the incubation following NTG treatment. The process is very simple and easy except that a gentle filtration is required. The low frequency shown at the experiment of repeated filtration might be ascribed to physiological damages on cells, especially on filamentous cells, caused by the repetition.

The nature of two of the mutants were studied. Both of them formed multinucleated filaments at 42° and showed rapid increase in cell number for a while when shifted back to 30°. In ts 1 the division process after the shift was not influenced by CAP.

Therefore, the thermosensitive mechanism was not only reversibly blocked but accumulated at 42° and new protein synthesis after the temperature shift was not necessary in recovering activity. However, in ts 20, the thermosensitive protein was irreversibly blocked and new protein synthesis was indispensable for the cell to divide because CAP added at the time of the shift completely inhibited the cell division. Moreover, the fact that the cell number increased when CAP was added 15 min after the shift indicates that the new protein was synthesized in 15 min to the amount at least sufficient for a part of the cells to divide. The nature of this protein is not clear but it may be closely related to septum formation or cellular division process. Biochemical analysis of this protein will be possible by means of this type of mutants.

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