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Growth Characteristics of *Tetrahymena paravorax*

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ABSTRACT

The ciliate *Tetrahymena paravorax* strain S in a monoxenic culture was sterilized by treatment with antibiotics and then was cultured axenically in a proteose peptone-containing (PYG) medium. The optimal temperature of the cell growth was at 26 to 28 °C. The initial cell density is not an important parameter for the early stage of the cell growth in the PYG medium because the cells started proliferation and grew at the same rate independent of the initial cell density. The culture medium in the logarithmic phase had activities to enhance the growth rate of the ciliate, while the culture medium in the stationary phase had growth-inhibitory activities. These results show release of factors from *Tetrahymena* to regulate its proliferation.

INTRODUCTION

In recent years, many kinds of growth factors have been suggested to have functional roles in cell growth and/or differentiation in invertebrates including nematodes (Greenwald 1985) and sea urchin (Yang et al. 1989) and vertebrates (for review see Burgess and Maciag 1989). In the parasitic protozoa, several reports suggest that mammalian growth factors are involved in regulating growth of trypanosomes (Hirumi et al. 1977; Hide et al. 1989).

As for free-living protozoa, Hosoya et al. (1995a) reported that fetal bovine serum has activities to enhance the growth rate of *Tetrahymena*. While this activity was purified and identified as α2-macroglobulin, it is presently unknown the existence of α2-macroglobulin or functional homologue in *Tetrahymena*.

Tanabe et al. (1990) reported purification of a Paramecium growth factor (ParGF) in the culture medium of *Paramecium tetraurelia*. Although ParGF restored the fission rate of a mutant with a short clonal life-span up to the level of wildtype, whether it promotes the cell division of normal cells remains unknown.

While much information has accumulated on the growth of *Tetrahymena pyriformis* and *T. thermophila*, little is available on other species of the genus *Tetrahymena*. Thus, we describe the growth characteristics of *T. paravorax*. Interestingly, culture medium in the logarithmic phase had activities to promote the growth rate of the ciliate. While the culture medium in the stationary phase had growth-inhibitory activities as observed in *T. pyriformis* (Suzuki et al. 1996). In this report, we suggest that *Tetrahymena* cells produce and release factors that act on the normal cells and regulate the cell proliferation.

MATERIALS AND METHODS

Cell Culture

The ciliate *Tetrahymena paravorax* strain S was used. Cells were cultured axenically in a PYG medium (Watanabe et al. 1981) consisting of 2% (w/v) proteose peptone (Difco Labs., Michigan, USA), 1% (w/v) yeast extract (Oriental Yeast Co. Ltd., Tokyo, Japan), and 0.6% glucose. In some experiments, cells were cultured monoxenically in a lettuce infusion inoculated with *Klebsiella pneumoniae* 24 hrs before use. The infusion was prepared by boiling with 0.05% (w/v) dried lettuce powder in water as described previously (Hosoya et al. 1995b). Unless otherwise described, logarithmically growing cells were inoculated into the culture medium and kept without shaking.

Sterilization of the Monoxenic Culture

Cells in bacterized lettuce infusion were washed three times with an inorganic solution (34 mM NaCl, 1.1 mM KCl and 0.8 mM $CaCl_2$) and then were inoculated into a PYG medium. To remove bacteria, penicillin (Meiji Seika Kaisha, Ltd., Tokyo, Japan) and streptomycin (Meiji Seika Kaisha) were then added to a final concentration of 100 units/ml and 0.1 mg/ml, respectively, and kept overnight at 26 °C. The sterilized cells were washed with the inorganic solution, inoculated into the PYG medium and used in the axenic culture.

Assay for Growth-Promoting and Growth-Inhibitory Activities in the Culture Medium

Cells were cultured in the PYG medium. Conditioned media in the logarithmic phase (1 day after start of cell culture) and stationary phase (4 days

after start of cell culture) were prepared as described previously (Suzuki et al. 1997). Cells were inoculated in 10-ml Erlenmyer flasks containing 2 ml of a fresh culture medium, which was diluted with the conditioned medium in various ratios, and kept without shaking at 26 °C. Cell density was measured as described previously (Suzuki et al. 1997).

RESULTS AND DISCUSSION

Growth Characteristics of Tetrahymena paravorax

The cells in the bacterized lettuce infusion were sterilized by treatment with antibiotics, and were then cultured in the PYG medium axenically. As shown in Fig. 1, the maximal cell density was increased markedly in the axenic culture than in the monoxenic culture, though the lettuce infusion supported higher growth rate in the logarithmic phase than the PYG medium.

Temperature is one of the most important factors for the cell growth. Fig. 2A shows the relation between temperature and generation time for *T. paravorax* when cultured in the PYG medium. The cell growth was optimal at 26 to 28 °C. The temperature optimum for cell growth seems species and strain-dependent: *T. pyriformis* strain GL (Prescott 1957; Cameron 1973) and W (Watanabe 1963) show an optimum at 29 °C and 26 °C, respectively, and *T. thermophila* has an higher temperature optimum (Holz et al. 1957).

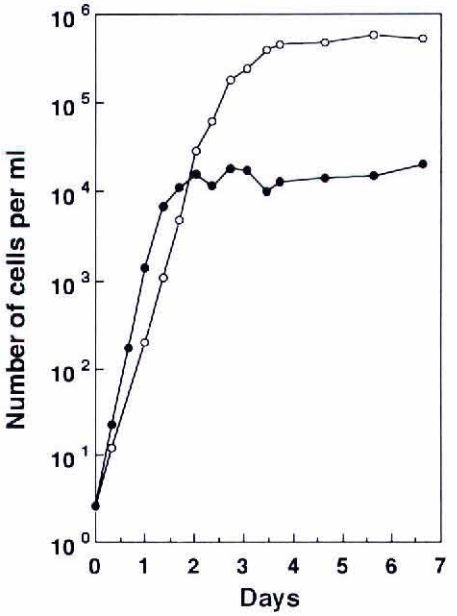

Fig. 1 Growth curves of *T. paravorax* cultured axenically or monoxenically. Cells were cultured axenically in a PYG medium (○) or monoxenically in a bacterized lettuce infusion (●). Cells were inoculated in 200-ml Erlenmyer flasks containing 30 ml of the culture medium. An initial cell density was 2.5 cells per ml and was kept at 24 °C. The mean of three experiments was shown.

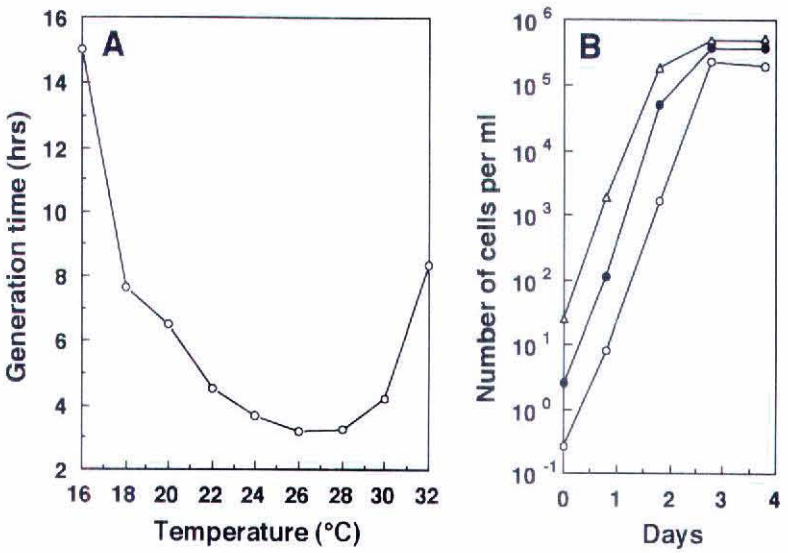

Fig. 2 Effects of temperature and initial cell density on the growth of *T. paravorax*. Cells were inoculated in 200-ml Erlenmyer flasks containing 30 ml of the PYG medium. (A) The relation between temperature and generation time. Cell culture was started at an initial cell density of 25 cells per ml and kept at various temperature. Generation time was estimated from the logarithmic portion of the growth curve. The mean of three experiments was shown. (B) Growth curves of *T. paravorax* at various initial cell densities. Cells were cultured at an initial cell density of 0.26 (○), 2.6 (●) and 26 (△) cells per ml at 26 °C. The mean of three experiments was shown.

Effects of initial cell density on the cell growth in the PYG medium were examined. *Tetrahymena paravorax* was inoculated into the PYG medium at various initial cell densities (2.6×10^{-1} to 10^1 cells per ml). It was observed that the cells started proliferation independent of the initial cell density, while the final cell density was increased in a starting density-dependent manner (Fig. 2B). Christensen and Rasmussen (1992) reported that *T. thermophila* cells in the cultures having 250 cells per ml or less initial cell density die without cell growth when cultured in a synthetic medium. In contrast to their report, our results show that cells can grow even at an initial density of 0.2 cells per ml. It should be noted that the cells grow at the same rate in the logarithmic phase as at a higher cell density when the culture starts at a low cell density (Fig. 2B). This is consistent with the previous report in which *T. thermophila* proliferate at the same doubling times once they survive and start proliferation (Christensen & Rasmussen, 1992). These results show that the initial cell density (2.6×10^{-1} to 10^1 cells per ml) is not an important parameter for the early stage of the cell growth in the PYG medium.

Effects of Conditioned Media on the Growth of Tetrahymena paravorax

To determine whether the cell division of *T. paravorax* is regulated by some factors released from itself, effects of the conditioned media of *T. paravorax* on the growth were examined. Although the lettuce infusion supported higher growth rate in the logarithmic phase than the PYG medium (Fig. 1), cells were cultured in the PYG medium axenically because bacteria and/or its derivative should be excluded as responsible factors for the growth regulation. As shown in Fig. 3A, the culture medium in the stationary phase of *T. paravorax* had activities to reduce the growth rate of the ciliate markedly. In the previous paper, we have described the same activities in the culture medium of stationary phase of *T. pyriformis* (Suzuki et al. 1996).

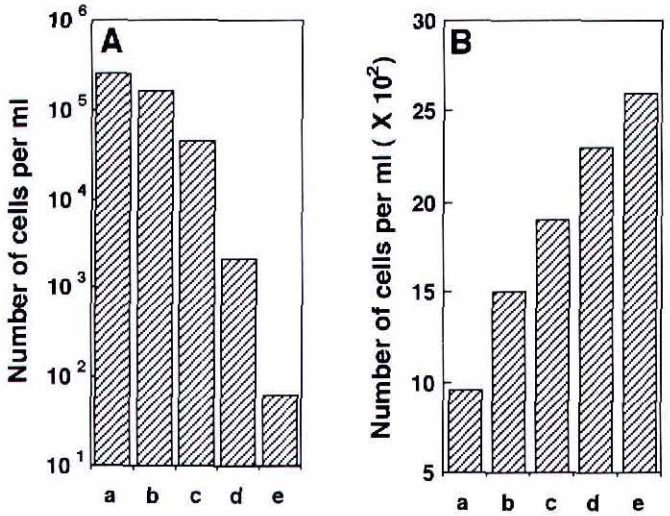

Fig. 3 Effects of conditioned media on the growth of *T. paravorax*. Cells were cultured at 26 °C in the fresh PYG medium, which was diluted in various ratios with the conditioned medium of the stationary phase (A) or the logarithmic phase (B). The ratios of the conditioned medium were 0% (a), 25% (b), 50% (c), 75% (d) and 100% (e), respectively. (A) Growth-inhibitory activities in the culture medium of the stationary phase. Cells were cultured for 36 hrs and then the cell density was measured. The mean of three experiments was shown. (B) Growth-promoting activities in the culture medium of the logarithmic phase. Cells were cultured for 12 hrs and then the cell density was measured. The mean of three experiments was shown.

Next, effects of conditioned medium in the logarithmic phase on the cell growth were examined. Cells were cultured in the fresh culture medium which was diluted with the conditioned medium in various ratios. When the ratio of the conditioned medium was raised, the maximal cell density was not increased (data

not shown), however, measurement of cell density at 12 hrs after start of cell culture confirmed that a modest but reproducible increase in the growth rate occurred in a dose-dependent manner (Fig. 3B). Similar growth-promoting activities were observed when *T. pyriformis* strain W was used as a material (data not shown). These results suggest the release of factors from *Tetrahymena* to accelerate cell division in the logarithmic phase.

It has been reported that *T. thermophila* produce and release factors that stimulate the cells to leave the lag phase and start cell proliferation in a synthetic medium (Christensen and Rasmussen 1992). In *Paramecium,* ParGF was purified as a factor for restoration of the fission rate of a mutant with a short clonal life-span up to the level of wildtype (Tanabe et al. 1990). The fission rate of this mutant was also restored in response to the conditioned medium of *T. thermophila* and *T. pyriformis* (Tokusumi et al. 1996). These factors are found to be different from those reported in the present study because our results suggest that unknown factor(s) acts on the normal cells at the logarithmic phase, although the mechanisms in regulating the cell growth by such factor(s) are not presently known. It is necessary to characterize and identify the factor(s) to elucidate these mechanisms.

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