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Effect of high-CO_2 on the growth of the thermophilic cyanobacterium on plates

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Introduction

As single cell micro-alga such as cyanobacteria have original and simple photosynthetic systems, they have been noted in recent years as model organisms in studying higher plants. We have studied on the structure and function of photosystem 2 proteins (Miyairi et al. 1992) in the thermophilic cyanobacterium *Synechococcus elongatus*, which was isolated from hot springs and grows vigorously at 55 °C (Yamaoka et al. 1978). For the isolation of desired strains or mutants of cyanobacteria, cultivation on solid media is an essential technique. Solid cultures of various cyanobacteria have usually been performed with agar plates under air (Allen 1968, Castenholz 1988). At high temperature suitable for the growth of thermophiles, syneresis and drying of solid media are liable to occur. For the cultivation of thermophilic bacteria above 55 °C, gellan gum was used as a solidifying agent with good thermal stability and clarity (Lin and Casida Jr. 1984). As cyanobacteria including thermophilic species generally require much longer time for the growth than bacteria, acceleration of growth is desirable to decrease the thermal effects on gels. From these standpoints, effects of CO_2-enriched air on the growth of *S. elongatus* on plates of various gelling agents was examined.

Materials and Methods

The medium for liquid culture of the cyanobacterium was basically the mineral medium (Dyer and Gafford 1961) with some modifications. The medium pH was adjusted to 7.5 with NaOH. Liquid culture was conducted in a 650 ml flat bottle of 6 cm thickness at 52 °C, under illumination from tungsten lamps at an intensity of 50 E/m^2 /s. Air or CO_2-enriched air was provided from the bottom of the bottle at a rate of 200 cc/min. Growth was followed by absorbance at 750 nm.

Solid culture medium was prepared at 60 °C by mixing the solutions of the mineral medium and solidifying agent autoclaved separately. Solidifying agents were agar (DIFCO Lab., USA), agarose (Pharmacia, Sweden) and gellan gum (Kelco Co., USA). Final concentrations of agar, agarose and gellan gum were 1.6, 0.7 and 0.8% (w/v), respectively. To increase mechanical strength of the gellan gum gel, $MgCl_2$ solution autoclaved separately was added to the medium at a concentration of 0.075%. The medium was dispenced by 30 ml per a plate and the plate were placed in an incubator at 30 °C for a day. The cyanobacterial cells cultivated in the liquid medium under 2% CO2 were spread on the plates, which were then inverted and incubated in a chamber at 48 °C, at a humidity of 90%, under illumination from fluorescent lamps of 30 E/m^2 /s. A small amount of water was placed on the inverted top of each plate to prevent drying of the gels. CO_2 concentration in the chamber was controlled by supplying CO_2-enriched air. Growth of colonies was followed by measurement of diameter at

intervals with a microscope and expressed as an average of 100 colonies, with a standard error of less than 10% in any case.

Results and Discussion

Time course of the cyanobacterial growth in the liquid medium supported with air or 2% CO_2 is shown in Fig.1. Doubling times of the cells during the growth of the first 12 h were 4.7 and 3.3 h under air and 2% CO_2, respectively.

Rate of colony growth was showed by the mean values of colony diameters (Fig. 2). Colonies grew 3, 4 and 2 times faster under 2% CO_2 than under air, after 2 weeks incubation on agar, agarose and gellan gum plates, respectively. Gellan gum plates were reported to be comparable to agar plates in solid culture of thermophilic bacteria (Lin and Casida Jr. 1984,

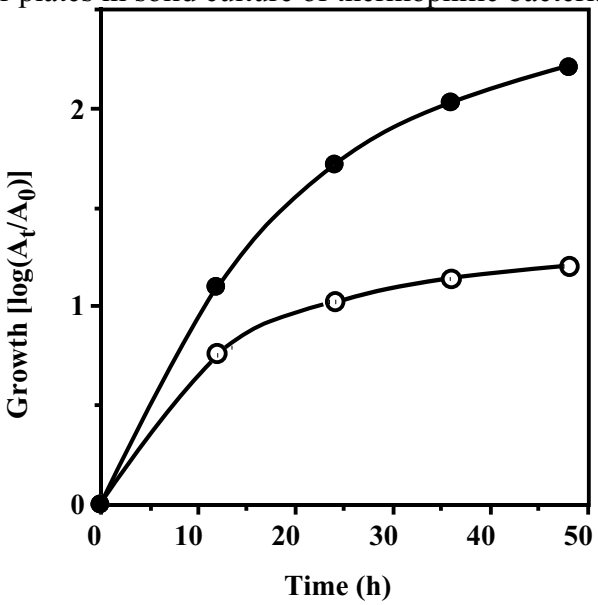

Fig. 1. Time course of the cell growth in the liquid medium.
Supported gases were air (open circles) and 2% CO2 (closed circles).

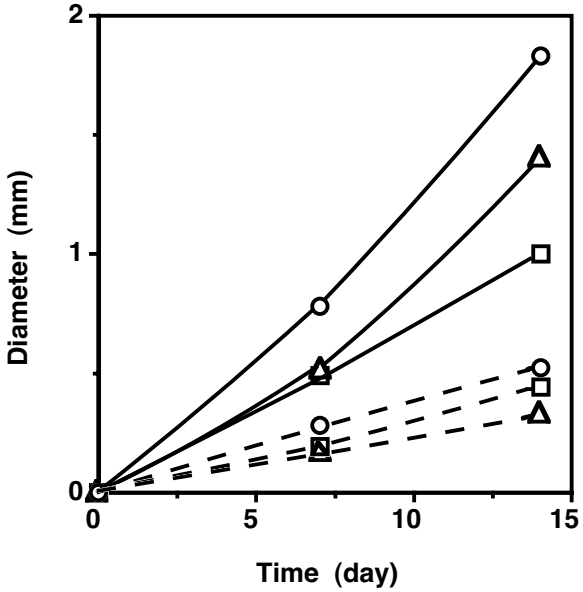

Fig. 2. Time course of the colony growth on the plates.
Colonies were grown on the agar (circles), agarose (triangles) and gum (squares) plates under air (dotted lines) and 2% CO2 (solid lines).

Shungu et al. 1983). Above results show that agar plates are most competent for the cyanobacterial growth under either CO_2 condition. Syneresis of the gel was observed in agar and agarose plates after 2 weeks. 1.5%-gellan gum plates without $MgCl_2$ were comparable to 0.8%-gellan gum plates wth 0.075% $MgCl_2$ in breeding the colonies (figure not shown). Although no colonies grew on 0.8%-gellan gum plates containing 0.075% $CaCl_2$ in place of $MgCl_2$, $CaCl_2$ of this concentration did not show inhibitory effect on the growth in the liquid medium. Colonies continued to grow on any plate more than 2 weeks. Agarose and gellan gum plates were liable to lose moisture of the gel surface, compared with agar plate. Without a portion of water on an inverted top of a plate, any plate began to dry earlier. The cells of the colonies on any plate, which were cultivated for 2 weeks under either CO_2 condition and stocked in sealed plates for 4 weeks at room temperature under dim light, started to grow when inoculated in the liquid medium. While, the cells, cultivated in the liquid medium and stored at room temperature for 3 weeks, could not grow in the (fresh) liquid medium. Above results show that use of CO_2-enriched air gives some advantages for plate culture of thermophilic cyanobacteria.

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