Short Paper

Confirmation of a Sequence of the Events Associated with Trimethylamine Formation by *Escherichia coli**¹

Recently we reported that trimethylamine N-oxide (TMO) could promote anaerobic growth of *Escherichia coli* by functioning as an electron acceptor.^{1,2)} Molecular oxygen, if present around cells, was inhibitory for the formation as well as activity of TMO reductase catalyzing trimethylamine (TMA) production by cells.³⁾ In the course of cell proliferation, it would thus be suggested that TMA is produced by cells respiring on TMO after thoroughly consumed oxygen dissolved in cultures and that cells in turn grow well, depending upon the oxide. This paper describes confirmation of a sequence of these events occurring in earlier growth stage of *E. coli*.

E. coli (IFO 3301) was statically grown at 37°C on the medium reported previously¹⁾ in a rubber-stoppered cylinder without any introduction of argon. When cell density reached to 0.031 of optical density at 660 nm (O.D. 660),¹⁾ TMO was added to cultures at 7.0×10^{-2} M. TMO-dependent growth was expressed as difference in cell density of the cultures added with and without the oxide. Dissolved oxygen (D.O.) was measured on a Beckman Fieldlab oxygen analyzer. TMA in the culture was determined by the picrate method modified by TOZAWA *et al.*⁴⁾

As presented in Fig. 1, the culture rapidly decreased its D.O. level and finally attained to an anaerobic state about 30 min after the addition of

TMO. TMA appeared at about 50 min, increased logarithmically thereafter, and was followed by TMO-dependent growth which began at 70–75 min. So far as D.O. is found in the culture, cells can hardly produce TMA because of the inhibitory effect of oxygen on TMO reductase formation and activity.⁵⁾ Time required for the appearance of TMA was about 20 min after accomplished the anaerobiosis, much longer than that for the beginning of TMO reductase formation in highly grown cells which could promptly produce the enzyme on the addition of TMO.¹⁾ This finding seems to indicate that a factor like specific redox potential inside or outside cells is necessary for commencing the enzyme synthesis, as suggested in nitrate reduction by *E. coli*.^{6,6)} Cells thus used up D.O. in the culture, deriving energy from the respiration on TMO with concomitant accumulation of TMA, can exhibit the resulting well growth by following after the amine evolution.

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Fig. 1. Decrease in D.O. level and subsequent increase of TMA in the culture preceding TMO-dependent growth of *E. coli*. TMO was added to the culture at 0 min. The air-flushed medium before inoculation was taken as a sample with 100% saturation of D.O.