Hydrogen Generation by Halophilic Photosynthetic Bacterium and its Application

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Summary

Ectothiorhodospira halophila is a salt-loving photosynthetic bacterium, which lives under 2 - 4 M NaCl. We have been studying photoactive yellow protein, a putative photoreceptor of negative phototaxis of this bacterium. During a culture of the bacterium, we noticed that the bacterium produces hydrogen gas. In order to examine the possibility of the application of hydrogen generation by this bacterium, we carried out the quantitative analysis of hydrogen generation, DNA cloning of nitrogenase genes which is a key enzyme for hydrogen generation and the development of the gene handling system with this bacterium.

A trace amount of hydrogen was generated photosynthetically by *E. halophila* with a standard culture medium containing 15mM NH_4Cl. It is known that hydrogen generation by photosynthetic bacteria is mediated by nitrogenases, enzymes for nitrogen fixation. NH_3 acts as an inhibitor of the enzymes. Thus, we examined the effect of various kinds of amino acids as a substitutive nitrogen source for the culture. When the concentration of NH_4Cl was decreased to 5mM, the improvement of hydrogen generation was observed, 80ml/L culture for 1 week. No cell growth was observed with tryptophane, and no hydrogen generation was observed with glycine, methionine, asparagin, glutamin. Among the amino acids tested, aspartate gives the most effective hydrogen generation. Alanine, phenylalanine and arginine gave also good results for hydrogen generation. Hydrogen generation was only observed at the logarithmic growth phase. The total amount of generated hydrogen increased, when the density of cells were controlled to keep the logarithmic growth phase.

It is known with various nitrogen-fixation bacteria that nitrogenase is composed of NifH, NifK and NifD. First, two PCR products for the candidate of nifH were obtained and DNA sequences were determined. Homology search indicated that these two products were close to a gene for bacteriochlorophilide reductase, but not for nifH. Although we designed and examined various kinds of primers, we could not succeed to clone nifH gene. We could succeed to clone a part of nifD. Using the cloned region of nifD, we also succeeded to clone the entire region of nifD, the 771bp of nifH including the C-terminal region of NifH, the 5' end of nifK and the connecting regions for nifH and nifD, and nifD and nifK. We are now trying to clarify the entire sequence of nif genes.

In order to consider the nitrogenase function with molecular level, we tried to develop the genetic engineering system for *E. halophila*. First, a new method was developed to obtain single *E. halophila* colonies without the need for an anaerobic cabinet. Mating experiments of *E.coli* S17/pKT210 with *E. halophila* resulted in a 100-fold increase of streptomycine resistant *E. halophila* colonies compared to control matings with *E.coli* S17. These results indicate that (i) plasmid transfer occurs between *E. coli* and *E. halophila* (ii) appropriate plasmids replicate in the extremely halophilic bacterium *E. halopila* and (iii) streptomycin is a suitable antibiotic. We will develop the gene handling system for *E. halophila* based on these results.