EFFECT OF HYDROGENASE 3
OVER-EXPRESSION AND DISRUPTION OF
NITRATE REDUCTASE ON FERMENTATIVE
HYDROGEN PRODUCTION IN Escherichia coli

A Metabolic Engineering Approach

Koji Sode,1 Mika Watanabe,1 Hiroshi Makimoto,1 and Masamitsu Tomiyama2

1Department of Biotechnology
Tokyo University of Agriculture and Technology
2-24-16 Nakamachi
Koganei, Tokyo 184
2National Institute of Agrobiological Resources
2-1-2 Kannondai
Tsukuba City, Ibaraki 305, Japan

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1. SUMMARY

Based on available molecular genetics data on the regulations and enzymes in the E. coli anaerobic metabolism, we are examining approaches for the enhancement of hydrogen production by E. coli through metabolic engineering. A regulation mutant, HD701, in which the repressor gene hycA for the terminal enzyme for hydrogen production (hydrogenase 3) was disrupted, increased the level of expression hydrogenase 3. However, this mutation did not result in the enhancement of hydrogen production efficiency. Subsequently, the E. coli mutant strain RK5265, of which narG, a gene encoding the α-subunit of nitrate reductase, was disrupted, was examined for hydrogen production. The elimination of nitrate reductase resulted in hydrogen production in the presence of nitrate,
although the nitrate reductase active parent strain (RK4353) could not produce hydrogen under the same conditions because of the repression of the formate hydrogenlyase system. These results revealed that the elimination of the branch reaction in formate utilization resulted in the construction of a versatile strain for practical hydrogen production.

2. INTRODUCTION

Bacterial hydrogen production based on anaerobic metabolism has been studied as a means of efficiently using waste biomass, such as molasses from sugar manufacturing. Anaerobic processes can decompose saccharides, recovering molecular hydrogen gas with high efficiency. Simultaneously produced organic acids can be further utilized for photosynthetic bacterial hydrogen production.

Molecular biology studies on the anaerobic metabolism of *Escherichia coli* have identified the majority of enzymes and genes responsible for fermentative hydrogen production (Böck et al., 1996). On the basis of the genomic information, *E. coli* was shown to be an attractive target microorganism for bacterial hydrogen production by redesigning the metabolic pathway. *E. coli* produces hydrogen by mixed acid fermentation, mainly from glucose. In *E. coli*, hydrogen evolves by a formate hydrogenlyase system (FHL) containing the formate dehydrogenase-H (FDH-H, hydrogenase linked), electron carrier intermediate(s), and hydrogenase 3 (Sawers et al., 1985). In evaluating a metabolic engineering strategy to improve bacterial hydrogen production, fermentative hydrogen production must be investigated using adequate mutant strains that show unique properties on the branch point and rate-limiting steps on carbon and/or electron flux from glucose to FHL, for example, the rate of glucose or other saccharide uptake and preference, the utilization of pyruvate in the competition between lactate dehydrogenase and pyruvate formatelyase, the utilization of formate in the competition between FHL and formate dehydrogenase-N coupling with nitrate reductase system, level of FHL expression, contribution of uptake hydrogenases to the rate of hydrogen production, and so on.

In this study, we focused on the following effects on hydrogen production: the expression level of hydrogenase 3 and the mutation of a branch pathway competing for the carbon flux from glucose to hydrogen production, formate dehydrogenase-N (FDH-N), which is coupled with a nitrate reductase system (Figure 1).