Construction of engineered Azotobacter vinelandii for increased ammonia production in culture medium

ShT-02.1-2

Y. Kang[I], R. Takimoto[I], K. Kuroda[II]

[I]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, [II]Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto, Japan

The ammonia synthesis process developed by Haber and Bosch has environmental sustainability issues. Therefore, researchers have been exploring alternative methods to address these problems. Among the biological methods, the nitrogenases of *Azotobacter* spp. and *Klebsiella* spp. have been attracting significant attention. However, ammonia production through heterologous expression of nitrogenase gene clusters in *E. coli* and yeast is challenging due to the complexity of the nitrogen fixation mechanism and the oxygen sensitivity of nitrogenase. In this study, we attempted to improve the ability of the nitrogenfixing microorganism *Azotobacter vinelandii* to produce ammonia by modifying its function. To this end, we focused on the *nifL* gene of *A. vinelandii*, which is a negative regulator of nitrogen fixation in the presence of a nitrogen source, and the *amtB* gene, which encodes an ammonia accumulation channel. We created a strain by knocking out these two genes responsible for nitrogen fixation and ammonia release into the culture medium. The target gene was replaced with an antibiotic resistance gene using standard homologous recombination methods, resulting in the deletion of the target gene. The deletion of genes in *A. vinelandii* was confirmed through a series of steps, which included antibiotic selection, PCR confirmation, and DNA sequencing analysis. To evaluate ammonia production, the engineered strain was incubated for 48 h at 30°C and 300 rpm in the nitrogen-free modified Burk's medium. The ammonia concentration in the supernatant was measured using an Ammonia Assay Kit through a colorimetric reaction. The measurement of ammonia concentration in the culture supernatants of Δ*amtB*, Δ*nifL*, Δ*amtB*Δ*nifL*, and wildtype strains revealed a significant increase in ammonia concentration in the Δ*amtB*Δ*nifL* strain compared to the WT strain. These results suggest that *A. vinelandii* has the potential for ammonia production as a biological method.