

Enhancing the colonization of environmental microbes by introducing catalase genes

(環境微生物へのカタラーゼ遺伝子導入によるコロニー形成の促進)

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1. Introduction

Previous studies showed that when phosphate and agar are being autoclaved together, high amount of hydrogen peroxide (H_2O_2) would be generated in the agar medium. Since H_2O_2 causes oxidative stress, it is being said that H_2O_2 decreases the number and variety of colonies and thus impeding the exploration of novel microbial resources. In this research, concentration depending effects of H_2O_2 on the colonization efficiency of environmental microbes were studied. H_2O_2 sensitive microbes were discovered and isolated; furthermore, catalase genes were introduced to them to enhance the colonization efficiency.

2. Materials and Methods

To compare the concentration depending effect of H_2O_2 , PYG (Peptone-Yeast extract-Glucose) plates with different H_2O_2 concentrations were prepared by adding external H_2O_2 . Water sample from Ono pond in Hokkaido University was spread on these plates and incubated in 20°C for 10 days, then CFU and the microbial variety were compared. Bacterial strains only colonized on low concentration of H_2O_2 but not on high concentration were identified and further utilized as H_2O_2 sensitive microbes. To observe if externally supplied catalase genes could help microbes to overcome H_2O_2 in the agar plate or not, *katG* and *katE* genes from *Escherichia coli* were introduced to the sensitive strains by using broad host range plasmid vectors.

3. Results and Discussion

PYG plates were prepared with following H_2O_2 concentrations, 1.8 μM , 3.2 μM , 8.3 μM and 17.3 μM . CFU on the plate decreased inversely to the amount of H_2O_2 . Compared to 1.8 μM plate, 17.3 μM plate had only about 1/6 of CFU. The 16S rRNA gene sequencing analysis revealed that the count of class *Betaproteobacteria* decreased with increasing amount of H_2O_2 . By contrast, the count of class *Gammaproteobacteria* showed most obvious increase in the direct proportion to the amount of H_2O_2 . These results taken together showed that H_2O_2 in the agar plate biases the variety of cultivatable microbes.

Catalase genes *katGE* loaded on plasmid pBHR1 was introduced into one highly H_2O_2 sensitive strain P-1. Transformed P-1 was cultivated on agar plates with 0.6 μM , 11 μM and 28 μM of H_2O_2 . Currently, the effects of introduced catalase genes are being examined.

4. Conclusion

Results showed that H_2O_2 in the agar plate has effect on the number and variety of colonies. Introducing catalase genes to environmental microbe is highly challenging, development of the novel genetic tool is required. Further experiments on the transformation of catalase gene should be performed to apply this technique on novel microbe cultivation.