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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称： 博　士（農学）　　氏名　渡　邉　統　之

学 位 論 文 題 名

Identification and genetic characterization of microbes which are highly sensitive to hydrogen peroxide in the agar plate

（寒天培地中の過酸化水素に極めて高い感受性を示す微生物の同定および遺伝学的分析）

Background

Majority of the environmental microbes could not be cultured in the laboratory condition, especially when we attempt to culture them into visible colonies on the solid medium. The generation of hydrogen peroxide (H_2O_2) in the agar medium was discovered to be one of the factors which lowers the colony yields, since it diffuses through the cell membranes and damages cell components. However, the critical concentration of H_2O_2 detrimental to the colony formation of microbes was undetermined. This study aimed to determine the critical effect of plate embedded H_2O_2 on the colony formation of microbes, which resulted in the isolation of bacterial strains which showed unprecedented H_2O_2 sensitivities. Furthermore, the genotypic and phenotypic study on these environmental isolates were performed to elucidate the cause of their high H_2O_2 sensitivities.

1. Critical effect of H_2O_2 in the agar plate on the growth of laboratory and environmental strains

The medium embedded H_2O_2 was shown to lower the total colony count of environmental microbes, however, the critical concentrations of H_2O_2 detrimental to colony formation on the agar plate remain largely undetermined. Herein, I elucidated the specific effect of H_2O_2 on microbial colony formation on solid agar medium by external supplementation of varying amounts of H_2O_2. While common laboratory strains formed colonies in the presence of high H_2O_2 concentrations (48.8 μM or higher), microbes from a freshwater sample demonstrated greatly decreased colony counts in the presence of 8.3 μM H_2O_2. This implies that environmental microbes are susceptible to much lower concentrations of H_2O_2 than laboratory strains. During the above experiment, the relative

abundance of betaproteobacterial colonies was found to be lower on plates containing higher amounts of H_2O_2. Further, the growth of the representative betaproteobacterial isolates was completely inhibited in the presence of 7.2 μM H_2O_2.

This study demonstrates that low micromolar levels of H_2O_2 in agar plates critically affect growth of environmental microbes, and large portions of those are far more susceptible to the same than laboratory strains.

2. Whole-genome sequencing analysis of the environmental isolates which are highly sensitive to H_2O_2 in the agar plate

Two *Comamonadaceae* bacterium strain OS-1 and OS-4 showed higher sensitivities than few common laboratory species, however, the cause of their high sensitivities remain uninvestigated. These two strains are aerobic microbes, and aerobic microbes are known to possess various enzymes to degrade endogenous H_2O_2, which possibly provide defenses against exogenous H_2O_2. Since strain OS-1 and OS-4 were highly vulnerable to the H_2O_2, I investigated if strain OS-1 and OS-4 possess the genes of H_2O_2 degrading enzymes or not by performing the whole-genome sequencing analysis. The result revealed that both strains possess the putative genes annotated as commonly conserved H_2O_2 degrading enzymes and H_2O_2 sensory protein. Among these results, the presence of the putative genes of catalases drew my attention since catalase is one of the most powerful H_2O_2 degrading enzymes in microbes. The predict amino acid sequences of the putative catalase genes were compared against the online protein database to predict the functions of these putative genes.

3. Elucidation of the mechanism of H_2O_2 sensitivities by cloning catalase genes

The presence of the putative catalase genes in the genomes of strain OS-1 and OS-4 indicated that microbes could be sensitive to the low micromolar level of H_2O_2 even if they possess genes of H_2O_2 degrading enzymes. However, the functions of the products of these putative genes were uncertain at this point, thus these putative catalase genes were cloned to confirm the functions of their products. This experiment was performed to elucidate the mechanism of high H_2O_2 sensitivities of the environmental isolate OS-1 and OS-4.