## Studies on isolation and characterization of long-chain hydrocarbon-degrading microorganisms, and their application for bioremediation of petroleum hydrocarbon-contaminated soil.

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Environmental problems have been occurred by using fossil fuels and become serious problems. Since long-chain hydrocarbons and cyclic alkanes (*c*-alkanes) are remained for a long term in petroleum hydrocarbon-contaminated soil, removal of these hydrocarbons is essential. In order to solve the problems, isolation and identification of long-chain hydrocarbon-degrading bacteria were carried out, and an efficient bioremediation system for petroleum hydrocarbon-contaminated soil was constructed in this study.

In chapter 1, 36 strains of long-chain hydrocarbon-degrading bacteria were isolated and identified. Exhaustive analyses of hydrocarbon degradation of the isolated strains were carried out by the 2,6-dichlorophenol indophenol (DCPIP) assay, and the relationship between phylogenesis and hydrocarbon-degradation was clarified. Hydrocarbon degradation was similar in each genus, and the bacteria belonging to the genera *Rhodococcus* and *Gordonia* were suitable for *c*-alkanes degradation.

In chapter 2 and 3, *c*-alkane degradation and the degradation pathway by *Rhodococcus* sp. NDKK48 or *Gordonia* sp. NDKY76A were analyzed to apply for the hydrocarbon-degrading bacteria belonging to the genera *Rhodococcus* and *Gordonia* for bioremediation. Both strains NDKK48 and NDKY76A degraded *c*-alkanes without co-oxidation. The strains NDKK48 and NDKY76A degraded *c*-alkane via alkyl side chain oxidation pathway and ring oxidation pathway, respectively. Therefore, degradation pathway of *c*-alkane by the genus *Rhodococcus* was different from the genus *Gordonia*.

In chapter 4, an efficient bioremediation system for petroleum hydrocarbon-contaminated soil was investigated by using *c*-alkane-degrading bacteria, *Rhodococcus* sp. NDKK6 and *Gordonia* sp. NDKY76A. Optimum medium condition and inoculation method were determined, and the bioremediation was carried out under the optimum conditions. Furthermore, an additional inoculation of the bacteria based on the monitoring of bacterial biomass in soil enhanced the hydrocarbon degradation. Finally, an efficient bioremediation system was constructed.

In the final chapter, the findings of this study were concluded, and the perspective based on the findings was described.