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SUMMARY OF DOCTORAL THESIS

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Title: **STUDIES ON THE MICROBIAL DEGRADATION OF HOMOCHOLINE**
（微生物によるホモコリンの分解に関する研究）

エラー! Microbial degradation and metabolism of quaternary ammonium compounds such as choline, TMA-butanol, and L-, and D-carnitine have been well investigated. Furthermore, the degradation pathways of these compounds in microorganisms have been studied. Although extensive research has been conducted on the microbial degradation of choline and its structurally related compounds, no research has been done on homocholine, a compound that resemble choline in many aspects of cholinergic metabolisms. Therefore, this research was conducted to investigate the degradation of homocholine by soil microorganisms and to elucidate its degradation pathway.

Chapter 1 defines the aim of the work, provides a literature review of the relevant studies on the distribution and function of quaternary ammonium compounds, and also presents the microbial degradation of quaternary ammonium compounds.

In chapter 2, the isolation, characterization and identification of homocholine degrading microorganism were described. Thirty strains with high growth on homocholine medium were selected from 142 strains grown on homocholine. From these strains, four strains showed the highest growth and homocholine degrading rate were selected. Strains belong to the genus *Arthrobacter*, *Rhodococcus* and *Pseudomonas* were identified and characterized by morphological, biochemical and genetical analysis. With few exceptions, most of the bacteria isolated and identified so far that degrade quaternary ammonium compounds were from the genus *Pseudomonas* and *Arthrobacter*. However, *Rhodococcus* species capable of degrading quaternary ammonium compounds have not yet been reported, in the present study we are able to isolate *Rhodococcus* strains that metabolized homocholine as a sole source of carbon and nitrogen.

In chapter 3, this study was attempted to characterize and identify the metabolites of homocholine degradation by *Arthrobacter* sp. strain E5, *Rhodococcus* sp. strain A2 and *Pseudomonas* sp. strain A9 as well as to elucidate the degradation pathway of the compound by these isolates. The degradation of homocholine by the resting cell suspensions and growing cell cultures of these strains and the detection of formed metabolites were tested by capillary

electrophoresis, GC-MS and FAB-MS methods. During the degradation of homocholine by growing and resting cells of these strains, the amount of homocholine decreased concomitantly with the increase of metabolites, identified as trimethylaminopropionaldehyde, β–alanine betaine and trimethylamine. These findings demonstrate the sequential oxidation of homocholine by these isolates. Thus, the degradation pathway of homocholine was revealed to be through consequent oxidation of alcohol group (-OH) to aldehyde (-CHO) and acid (-COOH), and thereafter cleavage of C-N bond of β–alanine betaine to give trimethylamine and alkyl chain (C3-moiety).

The study in chapter 4 was carried out to investigate the enzymatic activities in the degradation pathway of homocholine by the isolated strains. Screening of the homocholine oxidation activity in the isolated strains by replica staining and spectrophotometer assays showed that NAD^+-dependent dehydrogenase enzymes are predominant in all isolates. Furthermore, dried cell reaction of *Pseudomonas* sp. strain A9 cells with homocholine in the presence and absence of NAD^+ demonstrated that the enzymes responsible for the metabolism of homocholine are alcohol and aldehyde dehydrogenases that require NAD^+ as electron acceptor. Moreover, in the cell free extract of *Pseudomonas* sp. strain A9 an inducible NAD^+-dependent homocholine dehydrogenase was detected. The crude preparation of this enzyme has broad substrate specificity. Although various buffering conditions and stabilizing reagent were applied to stabilize the enzyme activity, the enzyme was found to be unstable *in vitro* and lose its activity soon after and during the purification processes. Furthermore, an inducible NAD^+-dependent 3-hydroxypropionate dehydrogenase activity was also detected in the cell free extract of *Pseudomonas* sp. strain A9. This result indicated the presence of 3-hydroxypropionate as an intermediate metabolite in the degradation pathway of homocholine by this strain. Thus, in *Pseudomonas* sp. strain A9, homocholine is oxidized to trimethylaminopropionaldehyde by a NAD^+-dependent homocholine dehydrogenase, and consequently, trimethylaminopropionaldehyde oxidized to β-alanine betaine by a NAD^+-dependent aldehyde dehydrogenase. Thereafter, cleavage of β-alanine betaine C-N bond yielded trimethylamine and 3-hydroxypropionate (C-3 moiety). Thereafter, 3-hydroxypropionate was further oxidized to malonate semi-aldehyde by a NAD^+-dependent 3-hydroxypropionate dehydrogenase.

Overall, this study provides basic information on the microbial degradation pathway of homocholine and illustrates its degradation metabolites and the enzymes involved in. This information is important in order to explore these metabolites and enzymes in biotechnology to overcome hyperosmotic environmental stresses. Further research will be focused on isolation, characterization and possible application of homocholine-degrading enzymes.