学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

ENRICHMENT, GROWTH KINETICS AND ECOPHYSIOLOGY OF ANAMMOX BACTERIA

(嫌気性アンモニア酸化細菌の集積培養法の確立および生理生態学的解析)

Anaerobic ammonium oxidizing (anammox) bacteria have the unique metabolic ability to convert ammonium and nitrite to dinitrogen gas. This discovery led to the knowledge that activity of these bacteria were responsible for a substantial part of the enormous nitrogen losses observed in the freshwater and marine environments. However, ecophysiology and niche differentiation of anammox bacteria are still the enigma that remains due to the time-consuming enrichment methods, insufficient/inaccurate knowledge of growth kinetics and various environmental factors involved that yet to be identified. In this thesis, I focus on the eco-physiological characteristics of anammox bacteria to address their niche differentiation and ecological significance. In chapter 3, since it is still not possible to achieve pure culture of anammox bacteria, lack of appropriate culture has become the biggest hurdle for physiological study of anammox bacteria requiring high purity planktonic culture. Such culture has once been obtained using membrane bioreactor before, but took considerably amount of time and efforts. This chapter focuses on the development of a novel method for rapid cultivation of free-living anammox cells using immobilization technique. It was demonstrated that active free-living planktonic anammox cells with purity more than 90 percent was successfully developed in the MBR with overall only 35 days operation time. In chapter 4, it was acknowledged that the specific niche of certain microorganism is mostly determined from their kinetics including maximum specific growth rate. Inaccurate information of their kinetics would inevitably disturb the interpretation of their behaviour in complex eco-systems. Specifically, in the case of anammox bacteria, whose maximum specific growth rate is always in debate. In this chapter, a reliable re-evaluation procedure for maximum specific growth rates of three anammox species was developed and showing that anammox bacteria may not appropriate to be considered as slow-growing bacteria any more. In chapter 5, previously the issue of niche differentiation of anammox bacteria has been addressed by analysing the database of so far published 16S rRNA gene sequences, illustrating their niche partitioning and global distribution. However, information regarding their true dynamic behaviour could not be obtained. Enriched culture-dependent competition under given conditions is a direct way to illustrate

their niche differentiation. In this study, the microbial competitions for a common substrate (nitrite) among three anammox species (i.e. "Candidatus Brocadia sinica", "Candidatus Jettenia caeni" and "Candidatus Kuenenia stuttgartiensis") were systematically investigated in nitrite-limited gel-immobilized column reactors (GICR) and membrane bioreactors (MBRs) under different nitrogen loading rates (NLRs). 16S rRNA gene-based population dynamics revealed that "Ca. J. caeni" could proliferate only at low NLRs, whereas "Ca. B. sinica" outcompeted other two species at higher NLRs in both types of reactors, demonstrating NLR was one of factors determining ecological niche differentiation of "Ca. B. sinica" and "Ca. J. caeni". In chapter 6, although nitrogen loading rate has been demonstrated as a factor in the niche differentiation of two freshwater anammox species ("Ca. B. sinica" and "Ca. J. caeni"), a statistical study looking at over 6000 anammox 16S rRNA gene sequences from the public database, indicated that salinity was the most important factor governing anammox bacterial distributions, with "Ca. Scalindua" dominated in saline environments while "Ca. Brocadia" were mostly found in freshwater environments. Though it is strongly suggested, physiological verification of salinity as a niche factor for anammox bacteria has never been conducted. Detailed understanding of the extent of the effect of salinity on adaptation, genetic basis and ecological significance are all completely lacking at this moment. In this chapter, we conducted both batch and continuous experiment combined with mass analysis and genetic identification aiming to address the question whether and how salinity becomes one of the key factor in the niche differentiation between "Ca. Brocadia" and "Ca. Scalindua". Results obtained suggest that trehalose associated different response to osmostress in "Ca. B. sinica" and "Ca. S. japonica" might be one of the reason shaping the distinct niche in between. In the final chapter findings of previously conducted studies were compiled and recommendations for future prospective were highlighted.