Preservation and cultivation of ammonia-oxidizing bacteria

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Abstract:

Ammonia oxidizing bacteria (AOB) have an essential role in the global cycling of nitrogen, being responsible for the aerobic oxidation of ammonium to nitrite. Despite their industrial and research importance, AOB have not been preserved in culture collections because of its difficulty in maintenance of the pure cultures of AOB for a long period. Aiming development of the collection of AOB in NBRC, we examined (1) the condition of their stable preservations, (2) the application of non-cultural methods to check purity such as DGGE and (3) a PCR-based method to identify the kinds of RubisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase EC.4.1.1.39) in betaproteobacterial AOB, that provide important information related to the cultivation of AOB.
(1) Preservation of AOB L-drying has been the essential method of preservation for NBRC and applicable to particular strains such as *Nitrosomonas europaea* NBRC 14298. However, the result varied greatly among strains. In this study, the most effective method was cryopreservation with dimethylsulfoxide (DMSO) or glycerol by slow cooling at -80C, provided that the cryoprotective agent was removed from revival stocks by centrifugation.
(2) Detection of microbial purity: It is difficult to confirm the purity of AOB culture by cultivation-dependent methods. Colony development of AOB on a solid medium typically takes several months, and the colonies are small and difficult to transfer. The contaminations by other bacteria including other AOB strains were precisely detected by DGGE method targeted at the V3 region of 16S rDNA.
(3) RubisCO forms of AOB: RubisCO is a key enzyme in CO2 fixation in the Calvin Benson Bassham (CBB) reductive pentose phosphate pathway. The enzyme exists in several forms with different structural and kinetic properties, and the corresponding genes exist either singly or in multiple combinations within a particular bacterial genome. By our analyses, we demonstrated that the combinations of the RubisCO forms among the betaproteobacterial AOB group were heterogeneous, and that the growth of AOB in the non-bicarbonate-containing media varied depending on the presence or absence of the carboxysomal RubisCO (Form IAc RubisCO) in their cells. Keywords: ammonia-oxidizing bacteria, AOB, Cryopreservation, RubisCO, Carboxysomes

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