Comamonadacae: the important poly-β-hydroxybutyrate-degrading denitrifiers in activated sludge

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Introduction Poly-β-hydroxybutyrate (PHB) degradation is a well-known process mediated by a number of microorganisms. It is highly desirable to find some additional characteristics associated with PHB biodegradation and to assign PHB-degrading bacteria to their correct phylogenetic positions. In our study we have found some bacteria that degrade PHB under anaerobic conditions with nitrate as the terminal electron acceptor. Such strains will be used for the development of a nitrogen removal system using PHB as an electron donor. Here we report the phylogenetic affiliation of these PHB-degrading, denitrifying isolates.

Methods Five different activated sludge samples were collected from two different sewage treatment plants at different interval of time. The population of PHB degraders was obtained anaerobically using two different methods, the MPN and plate counting methods. The medium used was minimal medium supplemented with 0.2% PHB granules as the sole carbon source and 0.2% potassium nitrate as terminal electron acceptor. The strains thus isolated were subjected to 16S rDNA analysis. The sequences were assembled using the GENETYX-MAC program, and phylogenetic tree based on the 16S rDNA sequences was constructed using the neighbour-joining method. Quinone profiling and FISH analyses of PHB-acclimated activated sludge were performed as described by Hiraishi (1999) and Amann (1995), respectively.

Results and Discussion The population of PHB-degrading denitrifiers recorded using the MPN method was 100 folds higher than that with the plate count method. Twelve different isolates capable of PHB degradation along with their capability to denitrify were isolated. All of the isolates were identified as being members of the family *Comamonadaceae* on the basis of 16S rDNA sequences. However, all isolates showed a sequence similarity of less than 98% to any established species, suggesting them to be taxonomically new at the species or higher taxonomic level. Quinone analysis of PHB-acclimated activated sludge showed that the Q-8 content increased from 35 to 72 mol% of the total quinone content during PHB acclimation under denitrifying conditions. It is most likely that Q-8 in the activated sludge is originated from the class *Betaproteobacteria*. The quinone analysis of individual isolates also showed the same quinone profile. The FISH using the group specific probes and for total cell count revealed the predominance of the *Betaproteobacteria* in the PHB-acclimated sludge.

Thus direct 16S rDNA isolation and characterization, indirect analysis of quinone profiling and FISH lead us to conclude that *Comamonadaceae* members are the important PHB degrading denitrifiers in activated sludge samples in our study.