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Analysis of Growth Characteristics of Candidatus 'Accumulibacter phosphatis' by Using Quantitative PCR Method and Its Application to Isolation

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The growth characteristics of unculturable bacteria *Candidatus* 'Accumulibacter phosphatis' ('Accumulibacter') were analyzed by using quantitative PCR. 'Accumulibacter' has not been isolated yet nevertheless they are thought to be the most relevant polyphosphate accumulating organisms (PAOs) in the enhanced biological phosphorus removal (EBPR) activated sludge process. However, even without its isolation, quantitative PCR targeting 'Accumulibacter' enables elucidate its growth characteristics in complex microbial community, namely activated sludge. In this study, growth characteristics of 'Accumulibacter' under aerobic condition were analyzed by using quantitative PCR method. In addition, we attempted to isolate 'Accumulibacter' under the condition which was confirmed of growth.
Activated sludge samples from a laboratory-scale EBPR reactor were incubated under different conditions (carbon sources, pH and temperature) in at least triplicate. After 48 hours of incubation, amounts of 'Accumulibacter' in these samples were quantified by using quantitative PCR. 'Accumulibacter' showed a statistically significant growth when carbon source in the medium, initial pH and temperature were glucose, 10.0 and 20℃, respectively.
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Characterization of bacteria isolated from an acidophilic nitrification reactor

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Key words: nitrification, acidophiles, bioreactor, taxonomy

In general, nitrifying bacteria are neutrophilic, and their activity is inhibited under acidic conditions. However, our previous study has shown that nitrification seemed to take place at pH 4 and below in a mesh-filter-equipped sequencing batch reactor (SBR). The purposes of this study are to reconfirm acidophilic nitrification and to characterize the bacterial community structure of an acidophilic nitrifying SBR (ANSBR). The ANSBR was constructed by seeding with the sludge from the SBR (initial sludge concentration, 2 g [dry wt] L^{-1}) and by acclimating with ammonium-containing mineral medium in tap water (pH 4). The pH of the reactor was adjusted to 4 at the beginning in each batch cycle. During each batch operation, ammonia was consumed at an average removal rate of 0.53 mmol g^{-1} [dry wt] d^{-1}, nitrate was accumulated, and the pH decreased to around 2.7. The sludge concentration gradually decreased during 60 days of operation, and it became stable thereafter. Cultivable aerobic acidophilic bacteria were isolated from the ANSBR using selective medium for ammonia oxidizers. Most of the isolates thus obtained were affiliated with members of the genera *Acidocella*, *Castellaniella*, *Frateuria*, and *Rhodanobacter* by studying 16S rRNA gene sequence information. One of the acidophilic isolates, strain TUTa204, represented a new lineage possibly at the order level within the class *Betaproteobacteria*. The role of this novel bacterium and the other acidophiles in the ANSBR is now under investigation.
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