厌氧氨氧化污泥中效应菌的分子生物学研究

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摘要：对具有厌氧氨氧化作用的细菌进行更深入的分析和了解有助于该新型生物脱氮过程在实践中的应用，采用分子生物学方法从已培养的具有厌氧氨氧化活性的污泥中提取细菌总DNA 16SrDNA PCR 16SrDNA 836bp DNA 1 ~ 2 16SrDNA DNA 16SrDNA

关键词：生物脱氮，厌氧氨氧化菌，16SrDNA

中图分类号：U211

文献标识码：I

文章编号：530698&2%26%98%0001-6209 2005 03-0335-04

1990 年荷兰 Delft Kluyver ANAMMOX-anaerobic ammonium oxidation ANAMMOX Brocadia anamnnoxidans Anaerobic ammonium-oxidizing Planctomycete Uncultured anoxic sludge bacterium KUI DNA ANAMMOX 16S rDNA

1

1.1

1.2

pGEM T Easy Vector system EcoRI Promega Kclone Kclone Kclone DNA pAT378 Sangon ANAMMOX Pla46r 5'-GGATAGGCATGCAAGTC-3' Amx820 5'-AAAACCCCTCTACGAGTC-3'

基金项目：国家自然科学基金(2010920101) 通讯作者：3.)4567

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收稿日期：2004-10-21 修回日期：2005-01-07
1.3 DNA

周 [1] 以 50mL H2O 溶解的 DNA, 5mL 12000r/min 4°C 10min, 10mL 12000r/min 4°C 10min, 3 次。将上述样品在室温下以室温离心, 混匀后在室温下沉淀。在水相中加入用冰预冷的 9mL DNA 100mmol/L pH8.0 Tris-HCl [1] 100mmol/L pH8.0 EDTA [1] 100mmol/L pH8.0 EDTA 1.5mol/L NaCl 1% CTAB 100μL K 10mg/mL DNA 1g 1g 37°C 225r/min 30min 1.0mL 20% SDS 65°C 2h 15 20min 1 10min 9000r/min 10min 4.5mL DNA 0.5mL 20% SDS 65°C 10min 9000r/min 10min 50mL 3min 4000r/min 3min

1.5 PCR

A [1] 200μL PCR [1] 1μL 1μL 10 × Taq 1μL 2mmol/L dATP 5μL 200μmol/L 1.5μL Taq [1] 5U/μL DNA 5.5μL 70°C 25min 1

1.6 16S rDNA

ANAMMox [1] 16S rDNA GenBank [1] "AY518553"


2 DNA

16S rDNA [1] 25μL dNTP 12.5μL 200μmol/L MgCl2 0.5mol/L Pla466r 0.5μL 0.534μmol/L Amx820 0.5μL 0.492μmol/L Taq 1μL 0.5μL DNA Taq 1μL 0.5μL PCR 5 min 95°C 4 min 30s 55°C 45s 72°C 1 min 30 s 72°C 5 min 1% DNA

1.4 ANAMMox [1] 16S rDNA

0.7% β 21kb DNA

1 1 DNA

DNA DNA DNA DNA DNA DNA DNA DNA DNA DNA DNA DNA
Fig. 1 Crude and purified DNA extracts obtained from ANAMMOX sludge.

DNA

M. Lambda DNA/EcoR I + Hind III Marker I. Crude DNA II. Purified DNA by DNA gel extraction kit.

16S rDNA

ANAMMOX

Fig. 2 16S rDNA fragment PCR product of the ANAMMOX bacteria.

Fig. 3 Phylogenetic tree of the ANAMMOX bacteria in ANAMMOX sludge with its relatives.

Numbers in parentheses represent the sequences' accession number in GenBank. Numbers in square brackets indicate the clone number out of the total clones. The numbers next to the nodes represent the bootstrap values of 1000 replications. The scale bar represents 5 nucleotide substitutions per 100 nucleotides.
Molecular biology study on the effective bacteria in ANAMMOX sludge

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Abstract A greater understanding of anaerobic ammonium oxidation bacteria will help to pave the way for the new biological nitrogen removal process application in practice. To this end, this study used molecule biology methods. Crude DNA of the total bacteria in a cultivated sludge with ANAMMOX capability was extracted and purified. Then PCR amplification using specific primers clone and sequencing processes were performed. The partial 16S rDNA sequence of cultivated ANAMMOX bacteria is 836bp. Some clones have one to two base mutations. Phylogenetic analysis indicates that the cultivated ANAMMOX bacteria in this study close to Candidatus Brocadia anammoxidans anaerobic ammonium-oxidizing Planctomycete and uncultured anoxic sludge bacterium KU1 with the same function whereas the cultivated ANAMMOX bacteria are relatively low DNA sequence similarity to the aforementioned bacteria using alignment analysis. The results suggest that there is a kind of bacterium which has never been found before with ANAMMOX capability existing in nature.

Key words Biological denitrification, Anaerobic ammonium oxidation bacteria, 16S rDNA

References