B311 Occurrence of Rumen-Associated Archaeal Sequences in the Subsurface Aquifer by Livestock Wastewater Input

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Archaeal communities of two confined aquifers and livestock wastewater within a stock-farming area were characterized by 16S rDNA sequences analyses. Universal archaeal primers (Arch21F-Arch958R) were used to amplify DNA extracted from groundwater and livestock wastewater. One hundred thirty clones containing 16S rDNA genes for each of the five clone libraries were analyzed by reamplification with Arch21F and Arch958R primers. The analysis of *Rsa*I and *Hae*III-digested RFLP patterns of total 615 clones resulted in 35 RFLP phylotypes. In groundwater clone libraries, most of sequences were assigned to the kingdom *Crenarchaeota*, while most of sequences in livestock wastewater clone library were assigned to the kingdom *Euryarchaeota*. All sequences affiliated to the *Euryarchaeota* were related to the members of methanogens. The sequences assigned to the *Crenarchaeota* showed a higher diversity and were related to the sequences obtained from other studies. Interestingly, sequences not affiliated to the cultivated *Methaonobacterium* were closely related to the environmental methanogen sequences cloned from bovine rumen fluid in GenBank database with strong support by the bootstrap analysis. Therefore, it is concluded that sequences related to the rumen microorganisms found in subsurface aquifer were originated from livestock wastewater containing a variety of rumen microorganisms.

B312 Detection of the Genes Encoding Aminoglycoside Acetyltransferases and Aerolysin in Water Samples from Juam Lake

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The *aacC1, aacC2, aacC3,* and *aacC4* genes encoding aminoglycoside acetyltransferase AAC(3)-Ⅰ, AAC(3)-Ⅱ, AAC(3)-Ⅲ, and AAC(3)-Ⅳ, respectively, and aerolysin genes were surveyed in the water samples from Juam lake by polymerase chain reaction. Surface water was collected from January, 1996 to December, 1998 and followed by DNA extraction. Twelve samples were tested by PCR for the presence of the genes for aminoglycoside acetyltransferases and aerolysins. The presence of the *aacC2* gene was appeared in 75.0% (9/12) of tested DNA samples. Among them, 77.8%(7/9) of *aacC2* positive samples was associated with Tn3 sequence, which was known to increase the expression of *aacC2* gene. However, none of the 12 samples amplified the expected DNA fragment for *aacC1, aacC3,* and *aacC4* genes. PCR primer used for the detection of aerolysin was designed using conserved region of aerolysin and hemolysin of Aeromonas spp. This primer set amplified expected 414-bp PCR product successfully with the DNA samples from lake water.