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Introduction

Grobal warming caused by greenhouse gas like CO_2 is an urgent environmental problem. Although methane is one of the greenhouse gases, it is the most important energy resource for natural gas-fired plant. Moreover, methane is recyclable energy resource and methane production using fecal waste from human or livestock is a low environmental load method . However the problem is that its production rate and volume are not stable. Therefore, we aim to find an effective bacterial flora for methane production from livestock waste. In this report, we analyzed and compared bacterial flora in the methane fermentation tank and bovine rumen used as the plant microbial resource by 16S rRNA gene sequencing with PCR-DGGE and PCR-pyrosequencing.

Materials & Methods

Bacterial flora of methane producing plant was gotten directly from methane fermentation tank in Field Science Center at Tohoku University (Osaki-shi, Miyagi). Bovine rumen were donated from a meet center and stored at -80°C. Genome DNA was extracted from the bacterial flora and rumen by modified Venter method (Morita et al. 2009). Purified DNA was used as a template for polymerase chain reaction in PCR-DGGE and PCR-pyrosequencing analysis. PCR-DGGE was used denaturing gradient of 30-60% under 8% acrylamide gel and electrophoresis was running at 130V for 5 h at 60°C. A 100% denaturant was the mixture of 7 M urea and 40% formamide. The Gel was stained by SYBR Gold (invitrogen) and band pattern was visualized by LAS-4000 (Fuji Film). PCR-pyrosequencing was carried out by GS Junior System (Roche). Sequencing results were analyzed by Pyrosequence Pipeline in Ribosomal Database Project (http://pyro.cme.msu.edu/).

Results & Discussions

PCR-DGGE analysis showed a different band pattern for major bacteria between methane fermented tank and bovine rumen, and the results indicated that bacterial flora from methane production was much different from that from bovine rumen. To know the result details of PCR-DGGE, PCR-pyrosequencing is now in progress.

Acknowledgements

This work was supported by a grant of Tohoku recovery Next-generation Energy Research and Development Project.