Phylogenetic diversity of methanogenic archaea in swine waste storage pits

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Abstract

Total DNA was isolated from swine feces and a swine waste storage pit and used as templates for PCR amplification of archaeal 16 rDNA using specific primers. Only the sample from the center of the waste pit produced a PCR product. DNA sequence analyses of random clones demonstrated a variety of methanogenic archaea. Six groups of sequences were identified, including those similar to Methanobrevibacter sp., Methanocorpusculum sp., and Methanothrix sp. Three groups of sequences represented unidentified organisms. These data suggest that swine waste storage pits may represent an untapped source of novel methanogenic archaea. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

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1. Introduction

The evolution of the swine industry in the USA has resulted in the concentration of animals into fewer, larger facilities where the animals can be managed more efficiently. However, this change has also resulted in the concentration of animal waste naturally associated with such operations. Modern swine production systems generate large quantities of liquid manures (mixed feces and urine) which are commonly discharged into lagoons for treatment or stored in deep pits until they can be applied to surrounding farm land as fertilizer. In either case, the handling of large quantities of waste is associated with the production of a variety of emissions. Many volatile organic compounds are produced as a consequence of anaerobic metabolism of organic and inorganic compounds contained in the manure. Ammonia, inorganic acids and alcohols, amines, and sulfides contribute significantly to odor problems that arise surrounding these facilities. Treatment and storage of swine manure also lead to production of large quantities of methane. Global methane emissions from animal waste have been estimated to be about 28 Tg/year, and of this liquid animal waste...
systems account for over 10 Tg/year [1]. We have initiated a study to identify the bacterial population of swine feces and stored waste, including the archaea. This study uses recent advances in culture-independent microbial ecology techniques exploited in other ecosystems. DNA sequence analysis of 16S rDNA genes isolated by PCR and the development of diagnostic probe and hybridization techniques have been used by others to examine the microbial ecology of other complex microbial ecosystems [2-4]. We now report on the first phylogenetic characterization of the methanogenic population from swine waste pits using 16S rDNA sequence analysis.

2. Materials and methods

2.1. Sample collection

Samples of swine feces and waste pits were collected at a swine facility near Eureka, IL. Waste pit samples were collected from depths of 90 and 180 cm (bottom) in a waste pit under a barn of feeder pigs (approx. weight 23 kg) fed a corn-soybean diet. Samples were collected with a sampling device that opened up at the selected depths (Tank Sampler, Nasco, Fort Atkinson, WI). A fresh fecal sample was recovered from the same animals. All samples were placed in sterile Whirl-Pak sample bags (Nasco, Fort Atkinson, WI) and kept on ice during transfer to the laboratory. Feces (1 g) was added to anaerobic salts buffer (9 ml) in an anaerobic glovebox and suspended by vortexing.

2.2. DNA extraction, PCR amplification, and recovery of PCR product

Aliquots (1 ml) of the waste pit samples and suspended feces were lysed using a bead-beater device (Mini-Bead-Beater; Biospec Products, Bartlesville, OK). The samples were combined with 1 ml of phenol and 10% v/v zirconium beads (0.1 mm) and shaken twice for 2 min at 5000 rpm. The suspension was centrifuged at 14,000×g for 1 min to separate the phases. The aqueous phases were recovered and extracted twice with phenol/chloroform. Total nucleic acids were recovered by precipitation with ammonium acetate and ethanol, centrifuged at 14,000×g for 10 min, then suspended in Tris-EDTA (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) buffer.

The DNA was used for partial PCR amplification of the archaeal 16S rDNA genes using the forward primer A109f, 5′-ACKGCTCAAGTCACAGT-3′ (Escherichia coli 109-125) and the reverse primer A934b, 5′-GTGCTCCCCGCCAATTCC (E. coli 915-934) [5,6]. Conditions for PCR were 25 cycles of: 94°C, 45 s, 52°C, 60 s, 72°C, 90 s, 25 cycles, 72°C, 10 min. A final extension step of 72°C. All PCR reagents obtained from PE Biosystems (Foster City, CA). Reactions were carried out with 1×PCR reaction buffer, 200 mM deoxynucleotide triphosphates, and 3.5 units of Taq DNA polymerase. Aliquots of 16S PCR products were analyzed by gel electrophoresis to confirm presence of the correct size PCR product.

2.3. DNA sequencing and phylogenetic analysis

The PCR products were cloned into the plasmid pCR2.1TOPO (Invitrogen) using the TA Cloning Kit according to the manufacturer’s instruction. Randomly selected clones were analyzed for correct size inserts. The 16S rDNA inserts were sequenced at the W.M. Keck Center for Comparative and Functional Genomics, University of Illinois Biotechnology Center, Urbana, IL, using the m13 forward and reverse primers. DNA sequences were analyzed using the Lasergene software (DNASTAR, Inc., Madison, WI). Similarity analyses were carried out using the Advance BLAST Program of GenBank (NCBI, NIH, Washington, DC). The sequences were aligned using the ClustalW program [7], and phylogenetic dendrograms were prepared using the Treecom software [8] using the distance calculation of Galtier and Gouy [9]. The partial 16S sequences have been deposited in the GenBank database under accession numbers AF157518 through AF157524.

3. Results and discussion

PCR reactions were carried out with varying amounts of DNA purified from the swine feces, 90-cm and 180-cm waste pit samples. Only the 90-cm waste pit DNA provided a positive reaction with the archaeal primers. This would suggest that the con-
centrations of archaea from the feces and 180-cm pit samples were too low for detection using this method. Sixteen clones were selected at random for sequencing and similarity analysis.

All 16 of the cloned sequences were found to group within the methanogenic archaea (Fig. 1). Six groups of sequences were identified, with two or more sequences of at least 99% identity included in each group, along with one individual sequence. The single sequence (Ar32) was most closely related (97%) to the archaean *Methanoculleus marisnigri*. Only one other group, Ar37, was closely related (99%) to an identified methanogen, *Methanocorpusculum parvum*. Three of the groups (Ar26, Ar21, and Ar28) had little similarity to any identified methanogen (Fig. 1), indicating that these sequences represent novel, as yet unidentified methanogenic archaea. The final group (Ar40) was found to have about 95% similarity with *Methanobrevibacter smithii*.

This report represents one of the first analyses of stored animal waste as a microbial ecosystem. The data presented would indicate that the conditions for storage of swine waste in pits allow for increased numbers of methanogenic archaea than those present in the feces, resulting in production of methane along with other fermentation products from the...
pits. The presence of previously unknown methanogens indicates that such pits may be new sources of microbial biodiversity, and new and novel archaea and bacteria.

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References


