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## Assessment of a biogas-generating microbial community in a pilot-scale anaerobic reactor

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**In this work bacteria and methanogenic archaea utilizing agricultural wastes in a pilot-scale biogas reactor were examined using sequencing and terminal restriction fragment length polymorphism analysis. Based on the analyses of 16S rRNA genes, *Clostridia* represented the most diverse group in the digester. Of the *Clostridia*, unclassified *Clostridiales* and the members of the genera *Anaerotruncus* and *Tissierella* were detected at high abundances. The representatives of the bacterial phyla *Bacteroidetes* and *Proteobacteria* were also defined, but in minor proportions, and were assigned to non-dominant communities. Within the phylum Euryarchaeota, the members of the orders *Methanosarcinales* and *Methanomicrobiales* were found at high levels. Methanogenic archaea were analyzed using both 16S rRNA and *mcrA* genes. Actually good results were received using both approaches; however, the rRNA gene method missed the non-dominant order *Methanobacteriales*.**

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[**Key words:** Biogas; Agricultural wastes; 16S rRNA genes; *mcrA* genes; Terminal restriction fragment length polymorphism]

Many research works have been devoted to the development of new clean and renewable alternative energy sources due to the increase of gas and oil prices, the depletion of energy resources, and the necessity to protect the environment from global warming. One of the perspective alternative energy sources is biomass. Large amounts of organic wastes, such as agricultural and municipal wastes, become valuable sources of energy. In this connection, it is necessary to create new processing and recycling technologies, including biotechnology for microbial conversion of organic residues with biogas production, which is considered as one of the most efficient and environmentally attractive methods (1–3).

Anaerobic treatment of different organic wastes by microbes, involving the members of the Bacteria and Euryarchaeota, appears to be the effective biotechnological method to convert biowastes into bioenergy. Intensive process of anaerobic digestion of biowastes requires the maintenance of certain optimal microbial processes. As a consequence, many researchers have focused their works on the production of biogas from biodegradable materials (e.g., agricultural, municipal, and industrial wastes as well as sewage sludge) and have made successful efforts on the investigation of the structure and dynamics of biogas-producing microbial associations in various bioreactors (2,4–10).

Except for the cultured collection of bacterial and methanogenic strains, in the last years a large group of uncultured microorganisms was discovered. Since it is difficult to study anaerobic microorganisms with culture-based methods, culture-independent molecular methods were intensively developed to investigate complex bacterial and archaeal communities in the environment.

These methods allow the studying prokaryotic communities diversity based on 16S rRNA gene and some functional genes. Methanogens can be effectively analyzed based on  $\alpha$  subunit of methyl coenzyme M reductase (*mcrA*) gene, molecular metabolic marker of methanogenesis (11–14). Methyl coenzyme M reductase, which is peculiar to methanogenic archaea, catalyzes the reduction of methyl coenzyme M with coenzyme B to heterodisulfide and methane under anaerobic conditions (15). Molecular biology techniques developed for the characterization of microbial populations, which carry out the anaerobic treatment of biomass, allow researchers to monitor microbial interactions and ultimately will help to improve the efficiency of the whole anaerobic digestion process.

Within the main research topics, to achieve a more stable anaerobic digestion process and to avoid its failure, should be the selection of well-adapted microbial populations based on the substrate composition as well as the investigation of the key biochemical pathways for various organic compounds degradation with biogas production. Scientific advances in the establishment of highly active and well-adapted members of biogas-producing microbial communities should be a breakthrough in solving the problem of biowastes disposal and, at the same time, should provide a scientific basis for regulation of the anaerobic process with biogas generation. Recently we investigated bacteria and archaea involved in anaerobic digestion of multifarious organic waste materials in lab-scale digesters (10,16), and in this research we set a goal to determine bacterial and methanogenic archaeal community diversity in a pilot-scale biogas reactor. The feedstock for the bioreactor was composed primarily of cattle manure and plant biomass. The diversity of the bacterial community involved in this anaerobic process was assessed by creating clone libraries for the bacterial 16S rRNA genes and by terminal restriction fragment

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