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Bacterial ecology of abattoir wastewater treated by an anaerobic digestor

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ABSTRACT

Wastewater from an anaerobic treatment plant at a slaughterhouse was analysed to determine the bacterial biodiversity present. Molecular analysis of the anaerobic sludge obtained from the treatment plant showed significant diversity, as 27 different phyla were identified. *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Thermotogae*, *Euryarchaeota* (methanogens), and msbl6 (candidate division) were the dominant phyla of the anaerobic treatment plant and represented 21.7%, 18.5%, 11.5%, 9.4%, 8.9%, and 8.8% of the total bacteria identified, respectively. The dominant bacteria isolated were *Clostridium*, *Bacteroides*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfovibrio* and *Desulfotomaculum*. Our results revealed the presence of new species, genera and families of microorganisms. The most interesting strains were characterised. Three new bacteria involved in anaerobic digestion of abattoir wastewater were published.

Introduction

For hygienic reasons, abattoirs use copious amounts of water in their processing operations (slaughtering and cleaning), which creates significant wastewater. In addition, the increased use of automated machines to process carcasses, along with the incorporation of washing at every stage, has increased water consumption in slaughterhouse facilities. The high fat and protein content of slaughterhouse waste makes wastewater a good substrate for anaerobic digestion, due to its expected high methane yield.[1] Numerous microorganisms are involved in the anaerobic degradation of slaughterhouse waste, any step of which may be rate-limiting depending on the waste being treated as well as the process involved.

The microorganisms involved in anaerobic digestion have not been fully identified; however, at least four groups of microorganisms are involved in this process.[2] The first group

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are the hydrolytic bacteria that degrade complex compounds (protein, carbohydrates, and fat) into simpler compounds, such as organic acids, alcohols, carbon dioxide (CO_2) and hydrogen. The second group are the hydrogen producing acetogenic bacteria that use organic acids and alcohols to produce acetate and hydrogen. The third group contains homoacetogenic bacteria that can only form acetate from hydrogen, CO_2, organic acids, alcohols, and carbohydrates. The fourth group comprises methanogens that form methane from acetate, CO_2, and hydrogen. Hydrolytic, acetogenic, and methanogenic microorganisms play an equally important role in anaerobic digestion and methane production. Optimal methane production is only achieved via the interaction of multiple microorganisms,[3] and therefore, biodegradation of molecules in wastewater depends on the activity of all microbial groups involved.

Common fermentative bacteria include *Lactobacillus*, *Eubacterium*, *Clostridium*, *Escherichia coli*, *Fusobacterium*, *Bacteroides*, *Leuconostoc*, and *Klebsiella*. Acetogenic bacteria include *Acetobacterium*, *Clostridium*, and *Desulfovibrio*.[2] Methane producing organisms are classified under domain Archaea and phylum Euryarchaeota.[4]

In order to better understand the function of a bacterial population, a detailed description of the microbial ecosystem is necessary. One method is via molecular biology techniques.[5] Recent advances in the molecular analysis of bacterial ecosystems allow a better understanding of the specific microorganisms involved in wastewater treatment. There are only a few studies focused on microbial populations, diversity and evolution in reactors fed with complex organic wastes.[1] Therefore, little is known about the composition of these reactors. The development of advanced molecular biology techniques has contributed to the detection, quantification, and identification of the bacterial populations involved in the treatment of abattoir wastewater. For example, cloning and sequencing of 16S rRNA gene fragments provide information about the phylogeny of the microorganisms. Additionally, single stranded conformation polymorphism (SSCP) offers a simple, inexpensive and sensitive method for detecting whether or not DNA fragments are identical in sequence, and can greatly reduce the amount of sequencing necessary.[6]

This work aimed to study the bacterial ecology of an anaerobic digestor through both bacterial culture and molecular biological techniques. The bacteria involved in the anaerobic digestion of abattoir wastewater were identified using classic microbiology techniques and molecular tools (sequencing of 16S rRNA and SSCP). Additionally, our results were compared with those of Gannoun et al.[6] to evaluate the effect of storage at 4 °C on the bacterial diversity of the sludge.

Material and methods

Origin of the sludge

Anaerobic sludge samples were collected from an upflow anaerobic filter that treats abattoir wastewater in Tunisia.[7] The digestor operated under both mesophilic (37 °C) and thermophilic (55 °C) conditions. Samples were taken at the end of thermophilic phase and stored at 4 °C. The sludge was then analysed to determine the bacterial diversity present, first via bacterial culture.

DNA extraction, PCR and SSCP analysis of the digestor sludge

Four milliliters of the anaerobic sludge sample were centrifuged at 6000 rpm for 10 min. Pellets were re-suspended in 4 mL of 4 M guanidine thiocyanate–0.1 M Tris pH 7.5 and 600 μL of N-lauroyl sarcosine 10%. Two hundred and fifty microlitres of treated samples were transferred in 2 mL tubes and stored at −20 °C.

Extraction and purification of total genomic DNA was implemented according to the protocol developed by Godon et al.[8]

Highly variable V3 regions of bacterial 16S rRNA genes were amplified by PCR using bacterial (w49–w34) primers (Table 1). Samples were treated according to the protocol previously described by Delbès et al.[9]

For electrophoresis, PCR–SSCP products were diluted in water before mixing with 18.75 μL formamide (Genescan-Applied Biosystems) and 0.25 μL internal standards (ROX, Genescan-Applied Biosystems) according to the protocol of SSCP described by Delbès et al.[9]

SSCP analyses were performed on an automatic sequencer abi310 (Applied Biosystems). RNA fragment detection was done on the fluorescent w34 primer. The results obtained were analysed by GeneScan Analysis 2.0.2 Software (Applied Biosystems) as specified by Gannoun et al.[6] For bacterial identification, pyrosequencing of the DNA samples using a 454 protocol was performed (Research and Testing Laboratory, Lubbock, USA).

Methods of analysis for pyrosequencing data used herein have been described previously.[10–14] Sequences are first depleted of barcodes and primers. Then, short sequences under 200 bp are removed, sequences with ambiguous base calls are removed, and sequences with homopolymer runs exceeding 6 bp are removed. Sequences are then denoised and chimeras removed. Operational taxonomic units were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity).[15–21] Operational taxonomic units

Table 1 – Sequences and target positions of primers used in this study.

Primer	Sequence	Target	Reference
w34[a]	TET-TTACCGCGCTGCTGGCAC	16S rRNA universal	Gannoun et al.[6]
w49	ACGGTCCAGACTCCTACGGG	16S rRNA bacteria	Gannoun et al.[6]

[a] The primer w34 is marked at 5′ end with fluorescent phosphoramidite-TET (Applied Biosystems).