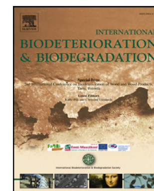
International Biodeterioration & Biodegradation 115 (2016) 64–73

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



Metaproteomic analysis of the microbial community present in a thermophilic swine manure digester to allow functional characterization: A case study

Yi-Wen Lin [a], Nguyen Ngoc Tuan [a], Shir-Ly Huang [a, b, *, 1]

[a] Department of Life Sciences, National Central University, Taiwan
[b] Institute of Environmental Engineering, National Central University, Taiwan

ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form
29 May 2016
Accepted 27 June 2016

Keywords:
Metaproteomics
Thermophilic anaerobic digestion
Microbial community
Pig manure
Acetyl-CoA decarbonylase

ABSTRACT

Anaerobic digestion is a widely accepted approach to the treatment of organic waste and uses a complex consortium of microorganisms. In this study, a metaproteomic approach was used to analyze the proteins expressed in an anaerobic digester that ran on swine manure at 55 °C. The extracted proteins were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis, and identified by LC-ESI-MS/MS. The study identified proteins with functions that are related to hydrolysis, acidogenesis and methanogenesis. The majority of the identified proteins (42%) were involved in the translation, ribosomal structure and biogenesis, which were followed by proteins associated with energy production and conversion (28%). The majority of the above proteins were eubacterial, with about 87% being Proteobacteria. Among the methanogenic archaeal proteins present, acetyl-CoA decarbonylase from *Methanosarcina* spp., which are able to produce methane from both acetate and CO_2, were identified. The present study is the first to investigate the metaproteome of a thermophilic anaerobic digester and to demonstrate the presence in the system of a high bacterial diversity where Proteobacteria are dominant. The study also provides evidence of types of microbial activity taking place during thermophilic anaerobic digestion when swine manure is used as the sole feedstock to produce biogas.

© 2016 Published by Elsevier Ltd.

1. Introduction

Anaerobic digestion has been and is being developed as a means of sustainable waste management and the conversion of waste to useful biogas. Biogas, a mixture of methane, carbon dioxide and some water vapor, is a combustible and renewable source of energy (Weiss et al., 2008). It is generated as a product of the breakdown of organic waste, such as municipal waste, agricultural residues, livestock manure, and food waste (Goberna et al., 2009; Sasaki et al., 2011; Tang et al., 2011; Yabu et al., 2011). In Asia, pig manure is the traditional substrate for biogas production in some countries such as China for many years (Jian, 2009). In addition to recovering energy, anaerobic digestion of pig manure provides a number of advantages including reducing volatile organic compound emissions and the control of unpleasant odors (Bonmati et al., 2001). The process has been described as a multi-step process in which several key groups of microorganisms take part using both serial and parallel (Chouari et al., 2005; Kim et al., 2010). The first step is the hydrolysis of organic polymers such as carbohydrates, proteins and lipids, which is mainly done by eubacteria. These biological macromolecules are broken down into smaller units such as simple sugars, amino acids and short chain fatty acids. Next, the acidogenesis step takes place where involves eubacteria to produce acetate, CO_2 and hydrogen. During the third step, methane and CO_2 are produced from various intermediate products by methanogenic organisms. Several studies have pointed out that the bacterial hydrolysis step is the rate-limiting step of the overall anaerobic digestion system when using complex organic materials as substrates (Noike et al., 1985; Vavilin et al., 1996; Mata-Alvarez et al., 2000; Bonmati et al., 2001; Park et al., 2005). The eubacteria that are involved in the hydrolysis and acidogenesis steps would seem to play a key role in initial digestion because these are the organisms that degrade the complex organic matter present in

* Corresponding author. National Central University, Department of Life Sciences, No. 300, Jhongda Rd., Jhongli City, Taoyuan County 320, Taiwan.
E-mail addresses: slhuang@cc.ncu.edu.tw, sl.huang@ym.edu.tw (S.-L. Huang).
[1] SL Huang's address after August 1st, 2016: National Yang-Ming University, Institute of Microbiology and Immunology, No. 155, Sec. 2, Li-Nong Street, Taipei 112, Taiwan.

http://dx.doi.org/10.1016/j.ibiod.2016.06.013
0964-8305/© 2016 Published by Elsevier Ltd.

the feedstock (Bonmati et al., 2001). However, the actual metabolic relationships within the community and the metabolic functions of the various microbial species present in the digester still remain unclear. In order to improve the substrate utilization efficiency for biogas production, a better understanding of the biological systems within the bioreactor is greatly needed.

About 90% microorganisms in the environment cannot be cultured by artificial methods (Ward et al., 1990). It is important to note that the microbial community involved in anaerobic digestion is extremely complex and dynamic. Furthermore, to date, relatively little research has been conducted on how microbial communities function in environments like anaerobic digesters. Nonetheless, there has been increasing interest in directly understanding such microbial communities and their functioning within their respective environments. Recently, a number of studies have described the structure and functions of microbial communities in various anaerobic digesters. This has mostly involved analysis of the 16S rRNA genes present in the populations (Chouari et al., 2005; Tang et al., 2008; Weiss et al., 2008; Goberna et al., 2009; Kim et al., 2010; Sasaki et al., 2011; Tang et al., 2011; Yabu et al., 2011; Tuan et al., 2014). However, rather than the bacterial species themselves, it is the proteins they produce that play a key role in catalyzing the digestion processes. Metaproteomics can be used to study protein expression from mixed cultures and therefore this approach is able to provide direct evidence of the metabolic and physiological activities occurring in a given system (Lacerda et al., 2007; Wilmes et al., 2008). This approach has been used elsewhere to study the relationship between the structure of microbial communities and the functioning of an ecosystem (Wilmes and Bond, 2004). This method has been applied to environmental samples such as soils (Benndorf et al., 2007; Wang et al., 2011), activated sludges (Wilmes and Bond, 2006; Wilmes et al., 2008; Kuhn et al., 2011), groundwater (Kan et al., 2005; Benndorf et al., 2007), wool fabrics (Solazzo et al., 2013) and wastewater treatment bioreactors (Lacerda et al., 2007). Furthermore, recently, the metaproteomic approach has been used to investigate a low temperature (15 °C) anaerobic wastewater treatment bioreactor that used glucose-based wastewater (Abram et al., 2011) as well as a thermophilic (55 °C) anaerobic digester that used agricultural biomass (Hanreich et al., 2012). In our previous study, the first survey of the microbial community in thermophilic anaerobic digester that uses swine manure as sole feedstock was performed by using 16S rRNA gene analysis (Tuan et al., 2014). The results revealed that *Clostridia* from the phylum Firmicutes were the dominant eubacteria. Firmicutes are widely present in various natural and constructed anaerobic habitats. They are able to degrade a variety of complex organic macromolecules (Weiss et al., 2008). However, our fundamental understanding of thermophilic anaerobic digesters still remains limited. The aim of this study was to enhance our fundamental understanding of microbial functionality and community involvement at the protein level.

2. Materials and methods

2.1. Protein extraction and separation

The conditions and operation of the anaerobic reactor have been described previously by Tuan et al. (2014). The sample was collected from the digester and then stored at −80 °C. For protein extraction, the frozen sample (30 g) was suspended in 100 ml of 67 mmol l^{-1} phosphate buffer (pH 7), which consisted of $Na_2HPO_4 \cdot 12H_2O$ (2.38 g l^{-1}) and KH_2PO_4 (8.17 g l^{-1}). The mixture was then shaken (150 rpm) on ice for 1 h. After ultrasonication at room temperature for 1 h, the suspension was centrifuged for 20 min at 6000 × g and 4 °C. The supernatant was filtered through No. 1 filter paper (Advantec Toyo, Japan) and then passed through a 0.22 μm pore size PVDF membrane (Millipore, USA). The filtrate was concentrated by ultrafiltration using a 1 kD Ultrafilitrate cellulose membrane PL-1 (Millipore, USA) using a stirred cell (8400- Normal Filtrate Driven Device 350 ml, Millipore, USA). The concentrated solution was precipitated using trichloroacetic acid. The filtrate was then stored at −80 °C for overnight. Next the sample was centrifuged at 4000 × *g* for 30 min at 4 °C and washed twice with two volumes of ice-cold acetone containing 0.07% 2-mercaptoethanol. The Bradford Assay (Bio-Rad) was used to measure the protein concentration. In order to separate the extracted proteins, the proteins (50 μg) were dissolved in 10 μl sample buffer, heated to 95 °C for 5 min and then subjected to 12% SDS-PAGE with molecular weight standards (Precision Plus, Bio-Rad). Finally, the PAGE gel was stained with Coomassie Brilliant Blue R250 before being cut into strips.

2.2. Protein digestion

After protein separation, the proteins were digested to allow detection by LC-ESI-MS/MS analysis. Each gel lane was divided into 18 slices (Fig. S1). Each protein gel slice was individually cut into small squares (~1 mm^2) and transferred into separate 0.5 ml Eppendorf tubes. The pool of gel pieces were destained in 100 μl of 25 mmol l^{-1} ammonium bicarbonate solution following by 100 μl of 25 mmol l^{-1} ammonium bicarbonate in water/acetonitrile (50/50 v/v) solution; this process was repeated three times. Next the pools were treated with 100 μl of 10 mmol l^{-1} dithiothreitol solution at 56 °C for 1 h to reduce the proteins. After the gel fragments had cooled to room temperature, the dithiothreitol solution was replaced with the same volume of 55 mmol l^{-1} iodoacetamide solution at room temperature for 45 min in dark to allow alkylation. The gel pieces were then washed with 100 μl of 25 mmol l^{-1} ammonium bicarbonate solution (pH 8) for 10 min with vortexing, and dehydrated with 100 μl of 25 mmol l^{-1} ammonium bicarbonate in water/acetonitrile (50/50) solution. After dehydratation with acetonitrile and drying in a laminar flow hood, the gel pieces were rehydrated in a minimum volume of trypsin (Promega), 0.1 μg ml^{-1}, with incubation at 37 °C overnight. Next the pieces were centrifugated and the supernatants were transferred into a 200 μl microcentrifuge tube. The peptide extracts from each individual gel pool were completely dried using a Speed-Vac centrifuge (Eppendorf, Germany). Finally, the extracted protein fragments obtained from the protein digestions were redissolved in 5 μl of 0.1% formic acid before nanoLC-MS/MS analysis was carried out.

2.3. LC-ESI-MS/MS analysis and data analysis

For protein identification, LC-ESI-MS/MS analysis was performed using an integrated nanoLC-MS/MS system (Mircomass, UK). This system comprised a 3-pumping Micromass/Waters CapLC™ system with an autosampler, a stream select module configured for precolumn together with a analytical capillary column, and a Micromass Q-Tof Ultima™ API mass spectrometer fitted with nano-LC sprayer. The system was operated under MassLynx™ 4.0 control. Injected samples were first trapped and desalted isocratically on an LC-Packings PepMap™ C18 μ-Precolumn™ Cartidge (5 μm, 300 μm I.D. × 5 mm; Dionex, Sunnyvale, CA, USA) for 2 min using 0.1% formic acid delivered by the auxillary pump at 30 μl min^{-1}. After desalting, the peptides were eluted off from the precolumn and separated using a 40 min fast gradient of 5%–80% acetonitrile in 0.1% formic acid at 300 nl min^{-1} on an analytical C18 capillary column (15 cm × 75 μm i.D., packed with 5 μm, Zorbax 300 SB C18 particles,; Micro-Tech Scientific, Vista, CA, USA) that was connected inline to the mass spectrometer.

Download English Version:

https://daneshyari.com/en/article/4364161

Download Persian Version:

https://daneshyari.com/article/4364161

Daneshyari.com