Microbial Community Analysis in Constructed Wetland Treated with Swine Wastewater Using DGGE

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ABSTRACT

Constructed wetlands have been used to treat diary and swine wastewater. In wetlands microbial communities play a vital role in elemental transformations. The classical microorganisms that are responsible for these biochemical functions are well known. However, enough data is not available on different microbial communities that exist in wetlands. The variation in microbial communities could be influenced by spatial difference, nutrients availability, oxidation-reduction conditions, and other inhibitory substrates. A number of bio-molecular methods have been used to analyze soil microbial communities under various ecological practices. In the present study, PCR-dependent DGGE molecular biological method was used to compare the spatial differences in microbial communities in marsh-pond-marsh constructed wetlands treated with swine wastewater. The DGGE profiles of bacteria from the same soil differed among culture-dependent and culture-independent samples, or culture-independent samples with different temperature. The DGGE patterns from different samples were different. DGGE bands were cloned and sequenced. 21 DNA sequences had been registered in GenBank. Real-time PCR will be performed to identify and quantify the functional important soil microbes .

INTRODUCTION

Wastewater from swine houses typically have significantly higher concentrations of organic matter and nutrients than treated municipal effluent, which pose potential impairment against public health and environmental concerns. Constructed wetlands in association with stabilization ponds have been suggested as a potential treatment of animal waste prior to land application. The diversity of microorganisms in the wetland environment may be critical for the proper functioning and maintenance of the system [1]. Since most environmental bacteria are unculturable, bacterial populations based on cultivation approaches may misrepresent the true diversity of microbial populations in wetland. Culture-independent molecular techniques, such as PCR-DGGE and real-time PCR used in the present investigation provide insights on the environmental microbial communities in the wetland.

OBJECTIVE

- To investigate the spatial comparisons of bacterial communities in constructed wetland using PCR-DGGE method.
- To identify nitrifying bacteria and denitrifying bacteria, and assess their diversity and biological contribution in biogeochemical cycling.

METHODS

1. Site description ::
Marsh-Pond-Marsh wetland was constructed at the swine facility of the North Carolina A & T State University farm in Greensboro, NC. Two marshes planted with Cattail (Typha latifolia, L.) at the influent and effluent ends and one pond section had operating depths of 15 and 75 cm, respectively [2].

2. Sampling :

Soil samples MA, MB and PB collected separately from marsh A, marsh B and pond (PB) at 0-2cm depth

Figure 1. Marsh-Pond-Marsh Wetland

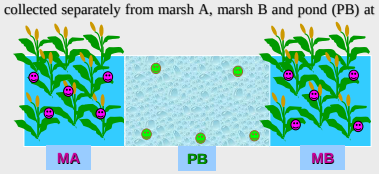

Figure 2. Sampling sites on Marsh-Pond-Marsh Wetland

3. Microbiological enumerations :
Ten-fold serial dilutions of the soil samples were prepared and spread-plated on nutrient agar medium. Bacteria population were determined following 2 days of incubation at 37°C and 28°C. Colony DNA extraction was performed after 4 days culture.

4. Denaturing gradient gel electrophoresis (DGGE):
In this study, DGGE was performed using an 8% denaturant gel with a 35-55% gradient at 60°C and 35 V for 16 hours on a D-Code Universal Mutation Detection System (Bio-Rad Laboratories).

5. Experimental flow chart

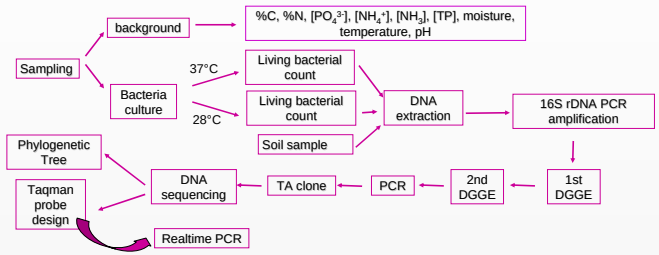

Figure 3. Flow Chart – Experimental Design

RESULTS AND DISCUSSION

1. Background Analysis on Soil Samples

Table 1. Physical and chemical properties of the soil samples

Soil Sample	% C	% N	NH_4 (mg/kg)	% TP	PO_4 (mg/kg)	% moisture	Temperature (°C)	pH
MA	1.44	0.13	77.96	0.20	177.97	22.00	17	7.14
MB	2.11	0.12	46.80	0.10	147.85	12.67	17	7.35
PB	0.87	0.10	70.67	0.14	169.44	18.30	17	7.35

2. Living bacterial count

Bacteria were counted and statistical analysis was performed by using SAS system v 8.1. The bacteria population cultured from PB samples at 37°C was significantly higher than from MA samples at 28°C ($P<0.05$).

Table 2. Living bacterial count – Statistical comparison

Sample	MA (37°C)	MA (28 °C)	MB(37°C)	MB (28 °C)	PB (37°C)	PB (28 °C)
Bacterial count	1.02×10^8 AB	1.77×10^8 A	5.90×10^5 B	1.00×10^8 AB	3.51×10^6 B	5.33×10^7 B

3. Denaturing gradient gel electrophoresis (DGGE)

Since most environmental bacteria are unculturable, the culture-independent PCR-DGGE technique provides an useful method to research soil bacterial community.

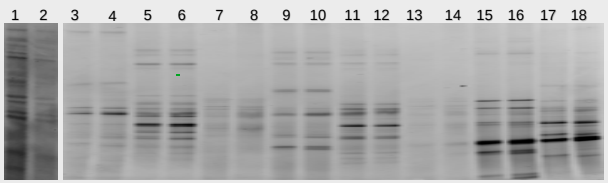

Figure 4. DGGE analysis of 16S rRNA fragments of total bacterial population from wetland soil samples and cultured bacterial samples. Lane 1-2, lane 7-8 and lane 13-14 are PCR products amplified from soil DNA samples MA, PB, MB separately. Lane3-4, lane 9-10 and lane 15-16 are PCR products from 28°C cultured soil bacteria from MA, PB, MB separately. Lane5-6, lane 11-12, lane 17-18 are PCR products from 37°C cultured soil bacteria from MA, PB, MB separately.

The patterns of the DGGE profiles of bacteria from the same soil differed among culture-dependent and culture-independent samples, or culture-independent samples with different temperature. DGGE patterns from different samples were different.

4. Sequence analysis

The major DGGE bands were cloned, sequenced and matched with the GenBank database. Sequence chimera were checked by the use of CHECK_CHIMERA program and no sequence was chimeric. 21 sequences were deposited in the GenBank database and were assigned NCBI accession numbers EU220701–EU220721.

Table 3. Sequence Analysis of Bands excised from DGGE gels derived from bacteria 16S rDNA extracted from wetland samples

Representative clone (GenBank accession number)	Best match database (GenBank accession number)	Similarity (%)
P1 (EU220701)	*Pseudomonas sp.* (AY663434)	100
P3 (EU220702)	Unclultured soil bacterium (AM884681)	100
P4(EU220703)	*Bacillus megaterium* (EU124555)	99
P5(EU220704)	*Flavobacterium sp.* (EF523606)	100
P6 (EU220705)	Uncultured Janthinobacterium sp. (EF072930)	99
P8(EU220706)	Unclultured soil bacterium (AM884681)	99
P9(EU220707)	Uncultured bacterium (DQ860045)	99
P11 (EU220708)	*Lysinibacillus sphaericus* (AB363739)	100
P12 (EU220709)	Bacillus cereus (EU163266)	100
P14 (EU220710)	Bacillus sp. (DQ985362)	98
P15 (EU220711)	*Exiguobacterium undae* (AB334767)	100
P16(EU220712)	Uncultured bacterium clone (DQ264647)	96
P17(EU220713)	Uncultured planctomycete clone (AY494689)	91
P18 (EU220714)	Uncultured bacterium clone (EF157114)	96
P19(EU220715)	Uncultured bacterium (AM259174)	98
P20 (EU220716)	Uncultured Clostridiales bacterium (AB234496)	97
P21(EU220717)	Uncultured bacterium (AM181828)	98
P22(EU220718)	Uncultured Bacteroidetes bacterium clone (EF417645)	99
P23 (EU220719)	Uncultured planctomycete clone (AY494689)	91
P24 (EU220720)	Uncultured bacterium clone (AY193194)	95
P25 (EU220721)	Uncultured proteobacterium clone (AF402974)	87

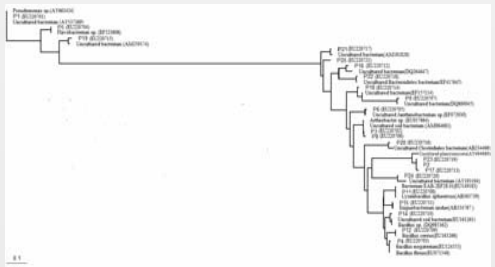

Figure 5. : Phylogenetic tree of partial 16S rDNA sequences from selected DGGE bands. The tree is based on a neighbour-joining method. The scale bar represents 0.1 substitutions per nucleotide position.

FUTURE WORK

Functional important soil microbes, for example nitrifying bacteria and denitrifying bacteria, will be identified. Their diversity and biological contribution in biogeochemical cycling will be assessed.

REFERENCES

1. A. Mark Ibekwe, Catherine M. Grieve, et al. 2003. Characterization of Microbial Communities and Composition in Constructed Dairy Wetland Wastewater Effluent. Appl. Environ. Microbiol. 69: 5060–5069
2. G.B.Reddy, P.G Hunt, et al. 2001. Treatment of Swine Wastewater in Marsh-Pond-Marsh Constructed Wetlands. Water Sci. Tech. 44(11-12):545-550.