Proceedings of the 2nd International Seminar on Chemistry 2011 (pp. 247-250)
Jatinangor, 24-25 November 2011
ISBN 978-602-19413-1-7

Amplification 16S rRNA of methanogenic archaeal directly during cattle manure composting

Safika[1,2], Darmawi[1], Fida Madayanti[2], Pingkan Aditiawati[3], Akhmaloka[2*]

[1] Faculty Of Veterinary Medicine, Syiah Kuala University
[2] Biochemistry Research Group, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung.
[3] Microbiology Genetics and Moleculer Biology research Group, School of Life Science and Technology, Institut Teknologi Bandung.
[*]Corresponding author**:** loka@chem.itb.ac.id

Abstract

The polymerase chain reaction (PCR), has become a popular method among microbial ecologists to study the diversity of natural microbial populations such as manure composting. *Archaea* can thrive in various natural and engineered environments, and many of them can grow in habitats at the extreme limits. Composting is one of the most successful methods for treating organic waste such as cattle manure. However the composting treatment can reduce these problems and can be applied to agricultural soil fertilizer. This research was focused to determine the dynamics of microorganisms, especially *Archaea*, during manure compost period. Measurement of physicochemical parameters such as temperature, pH, and water content were performed during the composting. The diversity analysis of microorganism was carried out using culture-independent by membrane filtration method. The diversity was analyzed through variation of 16S rRNA of methanogenic archaeal gene fragment resulted from PCR amplification. Variation of pH during stages of composting were detected in the range of 7 – 8.9. Water content was decreased from 89,66% to 43,46% at cooling stage. The total DNA were isolated from samples and used as PCR template to amplify 16S rRNA of methanogenic archaeal gene fragments using a set of primer of 400 bp long fragments incorporated with 40 bp GC-clamp. Total DNA successfully amplified 16S rRNA of methanogenic archaeal from all stages of composting. PCR results were used for further DGGE analysis to determine the dynamics of microorganisms during the process of manure composting.

Keywords: Manure compost, Methanogenic archaeal, Stages of composting, PCR

Introduction

Methanogenic archaea belong to the Euryarchaeota together with halophilic and thermophilic archaea. Five orders (Methanobac-teriales, Methanococcales, Methanomicrobiales, Methanopyrales and Methanosarcinales) of methanogenic archaea have been described so far and each of them forms a distinct lineage within the Euryarchae (Watanabe, et al, 2004).

Composting is defined as the biological conversion organic wastes into a stable material that can be used to fertilize. Decomposition of organic wastes in the composting process is carried out by a succession of microbial communities (Marshall, *et al*. 2003). It consist three major phases: mesophilic, thermophilic and maturation stages (the compost stabilization). The efficiency of composting stages depends on a variety of parameters, including aeration, temperature, content of moisture in the waste (Neklyudov and Ivankin 2008; Ishii. K and S. Takii. 2003). The most important, however, are the microorganism species involved and the activity.

Manure composting process in Indonesia is generally carried out traditionally. A result of the procces lost a lot of nutrients as well take a long time. Microorganisms is an essential factor in the success of composting. The understanding of the structure and dynamics of microorganisms that play a role in the degradation of organic compounds in each phase of composting is needed to effectively control of the composting process, especially the special roles of microorganisms during degradation of organic materials compost.

One of the most important phase of composting is termophilic phase characterized by the presence and activity of thermophilic microorganisms. Unders-tanding of commu-nity structure and dynamics of microorganisms is necessary to control the composting process effectively, especially the role of microorganisms degrading organic materials of the compost.

Whilst traditional culturing techniques have indicated microbial succession, the relatively recent advances in molecular, DNA based, approaches offer certain advantages over these techniques. Ribotyping techniques have been shown to describe diversity in compost environments that has not been detected by culturing techniques.

Alternative profiling techniques, such as 16S–23S rRNA intergenic spacer amplification (ARISA),