Molecular characterization of eukaryal biodiversity in the thermophilic phase of composting

Baiq Vera El Viera¹, Fida Madayanti¹, I Nyoman Pugeg Aryantha², Akhmaloka¹*

¹Biochemistry Research Group, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Indonesia
²Microbiology, Genetics and Molecular Biology Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia
*Corresponding Author: loka@chem.itb.ac.id

Abstracts

Thermophilic (60-70°C) eukaryal community during the traditional composting of domestic waste was analysed through denaturing gradient gel electrophoresis (DGGE) combined with 18S rRNA gene fragment sequences. The analysis was based on culture-independent and culture-dependent strategies. PCR amplification were performed with a set of eukaryotic primer, EF-GC and ER (Saccharomyces cerevisiae position 1423-1438 and 1641-1627) resulting a fragment with a length around 210 bp. The DGGE analysis showed that there were some difference patterns between bands from culture-independent and culture-dependent samples. The selected DGGE gels were excised, re-amplified, and directly sequenced. Phylogenetic and homologous analysis of the sequences showed that the selected bands were affiliated to Ascomycota, Basidiomycota, Chordata and Uncultured eukaryote.

Keywords: Culture-Dependent, Culture-Independent, DGGE, 18S rRNA, Thermophilic

Introduction

Composting refers to biological conversion of organic waste into stable material under self heating and aerobic condition. It was not only a waste treatment technique but also a recycling method as the end product can be used in agriculture as fertilizer, in gardening or in landscaping. On the basis of temperature, the composting process can be divided into four different phases (Gray et al. 1971), that was, mesophilic, thermophilic, cooling and maturation. Microorganisms play key roles in all the phases of the composting process as active decomposers.

One of the important steps of composting was a thermophilic phase resulting from microbial activity. The presumption has been made that the thermophilic fungi play an important role in the composting process as agents of cellulose decomposition (Tuomela et al., 2000). Thermophilic fungi have also been studied in other self-heating environments (Fergus, 1964; Kane & Mullins, 1973; Ryckeboer et al., 2003; Anastasi et al., 2004; Cahyani et al., 2004; Hatamoto et al., 2008; 2010; Hultman et al., 2010), but of these the studies based on molecular methods have been rather limited.

In this report we would like to present eukaryal biodiversity in the thermophilic phase of composting. Biodiversity characterization was based on PCR amplification of 18S rRNA gene fragments combined with denaturing gradient gel electrophoresis (DGGE) techniques. DGGE method has been widely used in the characterization of microorganisms from various environments. DNA fragments of the same length but of different sequence can be separated (Muyzer and Smalla, 1998). Separation was based on the melting behavior of fragments with different sequence composition under increasing gradients of denaturants.

Materials and Methods

Compost sampling

Compost sample was taken from composting process organized by LPW Cibangkong, a Non Government Organization, Bandung, with the distance at around 5 km from the Laboratory. The composting process use domestic waste as compost material with traditional process. 1-2 kg of compost of thermogenic phase at temperature around 60-70°C was collected from about 30 cm of compost surface. Samples were immediately used for further analysis.

Isolation and cultivation of microbes

Water extracts of the compost samples were prepared by shaking approximately 30 grams of the fresh sample in 270 ml of sterile distilled water with horizontal shaker for 30 minutes and then filtered. Water extract was re-filtered through a 0.22-µm-pore-size cellulose membrane filter (Sartorius, Germany). Microbes pellet in the membrane was resuspended in STE buffer (10 mM Tris-HCl pH 8.0, 0.1 M NaCl, 1