Bioresource Technology 134 (2013) 51–58

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Molecular analyses of the functional microbial community in composting by PCR-DGGE targeting the genes of the β-glucosidase

Hongtao Li, Xiuhong Xu *, Huihai Chen, Ying Zhang, Jie Xu, Jing Wang, Xinying Lu

College of Resources and Environmental Sciences, Northeast Agricultural University, Harbin 150030, China

HIGHLIGHTS

- The β-glucosidase-producing microbial communities during composting is studied.
- Analysis of microbial community by PCR-DGGE targeting the β-glucosidase gene.
- The relation between community composition and its function is studied.

ARTICLE INFO

Article history:
Received 22 November 2012
Received in revised form 12 January 2013
Accepted 16 January 2013
Available online 9 February 2013

Keywords:
β-glucosidase
Cellulase
Microbial community
DGGE
Compost

ABSTRACT

The study investigated the β-glucosidase-producing microbial communities and the enzymatic dynamics of CMCase and β-glucosidase during the process of cattle manure-rice straw composting. In order to analyze the succession of functional community by PCR-denaturing gradient gel electrophoresis (DGGEs), three sets of PCR primers were designed to amplify the family 1 and 3 β-glucosidase genes from both bacteria and fungi. The results showed in general that the stable functional community composition as well as for the high level enzymatic activities of both cellulase and β-glucosidase occurred during the last phase (days 14–31) of composting. In the process of composting, that functional groups were determined by the stable bands (GH1-F, GH1-H, GH1-G, GH3E-D and GH3E-E) may significantly contribute to the increase of β-glucosidase activities in the later phase. Especially, the bands from the family 1 β-glucosidase genes were appeared before that from the family 3 β-glucosidase genes from fungi, then the former was substituted for the latter gradually in the cooling phase. We found significant correlations between the β-glucosidase activity and the communities of the functional bacteria and fungi. The results indicated that different β-glucosidase-producing microbe release different amounts or activities of β-glucosidase, and that the composition of microbial communities may play a major role in determining overall β-glucosidase activity during the composting process.

1. Introduction

Composting helps in managing large quantities of organic wastes in a sustainable manner. It is one of the technologies of integrated waste management strategies, used for the recycling of organic materials into a useful product. Cellulose is by far the most abundant carbohydrate available from plant biomass, which can be used as a carbon source during composting. All organisms that can degrade cellulose secrete more or less complex cellulase systems. The enzymatic hydrolysis of cellulosic material into glucose involves the synergistic action of at least three different enzymes: endoglucanase or endo-β-1,4-glucanase (EC 3.2.1.4), exoglucanase or exo-cellobiohydrolase (EC 3.2.1.91), and β-1,4-glucosidase or cellobiase (EC 3.2.1.21). Cellulose fibers are firstly cleaved in between by endoglucanase releasing small cellulose fragments with free reducing and non reducing ends which are attacked by exoglucanase to release small oligosaccharides, cellobiose; and is finally hydrolysed into glucose monomers by β-glucosidase. β-glucosidase completes the final step of hydrolysis by converting the cellobiose (an intermediate product of cellulose hydrolysis) to glucose, hence; is the rate limiting enzyme (Alef and Nannipieri, 1995).

β-glucosidases are a heterogeneous group of hydrolytic enzymes and have been classified according to various criteria. There is no single well-defined method for the classification of these versatile enzymes. The most accepted method of classification is by nucleotide sequence identity scheme. The β-glucosidases are mostly placed in either family 1 or family 3 of glycosyl hydrolases

* Corresponding author. Tel.: +86 451 5519 1137; fax: +86 451 5510 3336.
E-mail address: xuxiuhong@neau.edu.cn (X. Xu).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.077

though these enzymes are also found in families 5, 9 and 30 of glycosyl hydrolases (Henrissat, 1991; Cantarel et al., 2009; Opassiri et al., 2007). Family 1 comprises nearly 62 β-glucosidases from archaebacteria, plants, mammals, and also includes 6-phosphoglycosidases and thioglucosidases. Most family 1 enzymes, also show significant β-galactosidase activity (Singhania et al., 2013). Family 3 of glycosyl hydrolases consists of nearly 44 β-glucosidases and hexosaminidases of bacterial, mold, and yeast origin. Most of the fungal β-glucosidases studied belong to the family 3 of glycosyl hydrolases (Singhania et al., 2013).

β-glucosidases have attracted considerable attention in recent years due to their important roles in various biotechnological processes such as hydrolysis of isoflavone glucosides, the production of fuel ethanol from agricultural residues, the release of aromatic compounds from flavorless precursor, etc. (Singhania et al., 2013). Glucose tolerant β-glucosidases can circumvent the problem of feedback inhibition in bioethanol production. Few species of *Aspergilli* are known to produce glucose tolerant β-glucosidases. It is expected that more of such glucose tolerant β-glucosidases may be prevalent in nature especially in filamentous fungi. Isolation of such enzymes and knowledge about their properties, sequences and expression patterns can help in design of better enzyme cocktails for biomass hydrolysis as well as in targeted approaches for modifying the glucose tolerance of existing β-glucosidases (Singhania et al., 2013). Recently, an article shows the significant role of β-glucosidase produced by *Aspergillius niger* in degrading soil organic matter and the effect of soil minerals in enzymatic activity (Lammirato et al., 2010).

β-Glucosidase are widely distributed in the living world and they play pivotal roles in several biological processes. In cellulolytic microorganisms, β-glucosidase is involved in cellulase induction (due to its transglycosylation activities) and cellulose hydrolysis. As such, it is interesting to understand the diversity of the β-glucosidase-producing organisms and their activities. There are several reports available for β-glucosidase productions from filamentous fungi such *A. niger*, *Aspergillus oryzae*, *Penicillium brasilianum*, *Penicillium decumbens*, *Phanerochaete chrysosporium*, *Paecilomyces* sp., etc., though there are also various reports of β-glucosidase production from yeasts (majority of them from *Candida* sp.) and few bacteria. Microscopic fungi are the most important source of β-glucosidase (Singhania et al., 2013). Several studies on extracellular β-glucosidase in environmental samples have focused on cultivated microbes (Hayano and Tubaki, 1985; Krogh et al., 2010). However, less than 1% of the microorganisms are only culturable in laboratory conditions. Thus, these approaches have not been very successful in fully understanding β-glucosidase diversity in composite environment samples (Cristóbal et al., 2009). Recently, several β-glucosidase have been obtained via metagenomic strategies (Bao et al., 2012). Cañizares et al. (2011) successfully designed and used a set of degenerate primers for real-time PCR to quantify genetic determinants of β-glucosidase in DNA extracts out of the soil bacteria. Whereas these approaches have not been very fruitful in understanding β-glucosidase diversity in composite environmental samples.

There have been several reports of PCR-DGGE targeting "functional genes" to analyze the microbial communities in environmental samples without any culture step. Before using a "functional gene" as a marker for PCR-DGGE, specific primers employed to amplify targeted gene from various organisms have to be designed. In the present study, sets of degenerate primers were designed to amplify the GH1 and GH3 β-glucosidase genes from bacteria and fungi in compost. Additionally, the CMCase and β-glucosidase activities were determined to investigate the influence of the community composition on cellulase activity during the composting.

2. Methods

2.1. Composting process and compost samples

The aerobic composting of cow manure and straw was performed at the horticulture station of northeast agricultural university in China. The characteristics of the raw materials were 38.62% of water contents, 31.8% of TOC, 1.33% of total nitrogen contents, 23.91 of C/N ratio in the cow manure, and those were 11.91% of water contents, 42.3% of TOC, 0.72% of total nitrogen contents, 58.75 of C/N ratio in the straw. At the beginning of composting, the moisture content of the pile was adjusted to 60%. The temperature in the centre of the pile was monitored every day. The indoor composting process took about 35 days. The composting samples were collected at days 3, 4, 6, 8, 12, 14, 20, 22, 26 and 31.

2.2. Enzymatic analysis

Ten grams of sample were transferred to a flask containing 50 ml acetate buffer (0.1 M, pH5.0). The flask was shaken at 200 r/min for 1 h. The homogenate was centrifuged (1315 g) at 4 °C for 20 min, and the supernatant was filtered through filter papers (Whatman No. 1) and then used for measurement of enzymatic activity. The assay of CMCase activity was carried out by measuring the reducing sugars by the method as described by Zeng et al. (2010). The activity of β-glucosidase was measured by using *p*-nitrophenyl β-D-glucoside (PNPG) as described by Herr (1979).

2.3. DNA isolation

DNA was extracted from compost samples (3 g) using a procedure described previously (Liu et al., 2011). After DNA extraction, the crude DNA was purified using the E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, Inc., Georgia, USA).

2.4. Design of primers to detect β-glucosidase genes

To identify β-glucosidase genes, three sets of oligonucleotide primers (Table 1) were designed on the basis of the evaluation of a broad spectrum of bacteria and fungi. Sequences were analyzed using the BLAST program and the Genbank databases (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignments were conducted with Clustal W algorithm. The primer pair GH1F–GH1R was designed to amplify partial conserved fragments of the family 1 β-glucosidase genes from bacteria and fungi. The primer pair GH3BF-GH3BR was designed to amplify partial conserved fragments of the family 3 β-glucosidase genes from bacteria. The

Table 1
Primers used in this study.

Name	Sequence (5′–3′)
BGH1F	CCT ACC AGA TYG ARG G
BGH1R	GAG GAA GRT CCC ART G
BGH1F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC TAC CAG ATY GAR GG
BGH3BF	TTC GGC GAA GAY CC
BGH3BR	ACG CCT TYR WAR CC
BGH3BF-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GTT CGG CGA AGA YCC
BGH3EF	GGT GGT CGC RRY TGG GA
BGH3ER	CCA GGC ATC GGW CAT RTC
BGH3EF-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG TGG TCG CRR YTG GGA

Modified bases: Y = CT, R = AG, W = TA.