Soil Biology & Biochemistry 105 (2017) 71–80

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in coniferous forest soil

Shamina Imran Pathan [a, *], Lucia Žifčáková [b], Maria Teresa Ceccherini [a], Ottorino Luca Pantani [a], Tomáš Větrovský [b], Petr Baldrian [b]

[a] Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy
[b] Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, 14220 Praha 4, Czechia

ARTICLE INFO

Article history:
Received 4 August 2016
Received in revised form
3 November 2016
Accepted 4 November 2016
Available online 22 November 2016

Keywords:
β-Glucosidases
Forest soil
Bacteria
Fungi
Gene pools
Transcript pools

ABSTRACT

Cellulose is the most abundant polysaccharide in the dead plant biomass, and its degradation is an important part of global carbon cycle. β-Glucosidases complete the final step of cellulose hydrolysis by converting cellobiose to glucose. Genetic potential and expression of β-glucosidase genes were studied in the topsoil of a *Picea abies* forest in two contrasting seasons. These seasons were the summer, representing the peak of plant photosynthetic activity, and late winter, after an extended period with no photosynthate input. Fungal and bacterial β-glucosidase genes belonging to glycoside hydrolase families GH1 and GH3 amplified from DNA and RNA and amplicon pools were analyzed. Transcript pool were largely corresponded to gene pools, although some abundant transcripts were not found in the gene pool. The major reservoirs of β-glucosidase genes were the fungal phylum Ascomycota and Basidiomycota and the bacterial phyla Firmicutes, Actinobacteria, Proteobacteria Acidobacteria and Deinococcus-Thermus. This indicates that a diverse microbial community utilized cellobiose. Seasonality influenced both genetic diversity of β-glucosidase genes and their expression. The results indicate that a complex community of bacteria and fungi expresses β-glucosidases in forest soils. Even β-glucosidase genes showing low abundance in DNA may be functionally important as revealed by their high expression especially in basidiomycota. The functional diversity in the studied ecosystem clearly exhibited a seasonal pattern.

1. Introduction

The coniferous forest soils contain more than one third of all carbon stored in terrestrial ecosystems. Hence, understanding organic matter decomposition in the coniferous forest ecosystem is crucial for estimating global C fluxes and their potential future changes (Šťursová et al., 2012; Baldrian et al., 2012). Dead plant biomass accumulating on the forest floor is mostly composed of cellulose, hemicellulose and lignin. Cellulose, a glucose polymer linked by β-1,4-glycosidic bonds, is the most abundant polysaccharide in terrestrial environments and its degradation was the subject of research for decades. This research revealed that microorganisms have the dominant role in this process in soils (Lynd et al., 2002; Baldrian and Valášková, 2008).

Enzymatic hydrolysis of cellulose typically requires the synergic action of three groups of hydrolytic enzymes: endo-β-1,4-glucanases (endocellulases, EC 3.2.1.9.1), cellobiohydrolases (exocellulases, EC 3.2.1.91) and β-1,4-glucosidases (cellobiases, EC 3.2.1.21). Among them, β-glucosidases complete the final step of cellulose hydrolysis by converting the disaccharide cellobiose to glucose molecules and deliver glucose for the central metabolism (Alef and Nannipieri, 1995). Their activity thus plays a vital role in the global C cycle (Knight and Dick, 2004). β-Glucosidases have also attracted considerable attention in recent years due to their important role in diverse biotechnological processes such as bioethanol production, hydrolysis of isoflavone glucoside, detoxification of cassava, elimination of bitter components from citrus products etc. (Singhania et al., 2012; Li et al., 2013).

Large microbial diversity in soils implies various carbon hydrolyzing activities (Nannipieri et al., 2012). In spite of the fundamental role of glycoside hydrolases in nature, their diversity is still poorly understood (Pathan et al., 2015). Recently, Bao et al. (2012) obtained several β-glucosidases from yak rumen metagenome.

* Corresponding author.
E-mail address: shamina.pathan@unifi.it (S.I. Pathan).

http://dx.doi.org/10.1016/j.soilbio.2016.11.003
0038-0717/

Several sets of degenerate primers have been designed to analyze β-glucosidase gene diversity in soils (Kellner and Vandenbol, 2010; Cañizares et al., 2011; Li et al., 2013). Unfortunately, none of these studies used sufficient sequencing depth and only Li et al. (2013) covered both fungi and bacteria using a PCR-DGGE approach to analyze potential diversity of β-glucosidase genes in DNA. Baldrian et al. (2012) expressed the need to analyze the DNA and RNA gene pools to quantify the proportion of expressed genes, and demonstrated this approach on fungal cellobiohydrolase *cbhI* gene. Cellulose is available in both litter and the organic horizon of forest soils (Šnajdr et al., 2011), hence similar cellulose degraders can be present and potentially active in both horizons. However, litter contains higher amounts of cellulose and likely supports a higher diversity of cellulose degraders (Baldrian et al., 2012).

It has been demonstrated that the seasonal variation of climatic conditions and consequent differences in tree physiology in temperate and boreal zone forests are accompanied by changes in microbial biomass content and composition of fungal and bacterial communities, which is a consequence of limitation of the photosynthetic activity to the growing season with optimal temperature and light (Voříšková et al., 2014; López-Mondéjar et al., 2015; Žifčáková et al., 2016). Some previous studies suggest that C allocation through rhizodeposition, during the growing season, shows profound influence on soil microbiota. Seasonality of enzyme processes and plant photosynthate production were also demonstrated to greatly affect microbial expression in temperate coniferous forests (Žifčáková et al., 2016).

This study was performed in two contrasting seasons; summer (July) when plant photosynthetic activity peaks, and late winter (March) after a prolonged period with no photosynthate input. The aim was to demonstrate how the β-glucosidase genes and transcript pools of bacteria and fungi differ among horizons with different cellulose content, and which members of the soil microbial community express the corresponding genes in the two different seasons. Kellner and Vandenbol, 2010 reported that β-glucosidase genes are expressed in forest soils by both the Ascomycota and Basidiomycota. Berlemont and Martini (2013) reported that β-glucosidase genes are present in nearly all bacterial phyla, and recently López-Mondéjar et al. (2016) showed that cellulolytic bacteria are common in forest litter and soil. These facts demonstrate that both bacteria and fungi likely participate in the last step of enzymatic cellulose hydrolysis, but it is unclear, what is the relative contribution of the various taxa to this process.

2. Materials and methods

2.1. Study site, sample collection

Study area was located in the highest altitudes (1170–1200 m) of the Bohemian Forest mountain range (Central Europe) and was covered by an unmanaged spruce (*Picea abies*) forest (49 °02.64 N, 13 °37.01 E). The mean annual temperature is 5 °C, and the mean annual precipitation is 1000 mm. The site was previously studied with respect to the composition of total and active microbial community in the soil and differences in gene expression in litter and soil among seasons (Baldrian et al., 2012; Žifčáková et al., 2016). Samples used here were from the study of Žifčáková et al. (2016) which also provide the information about the composition of fungal and bacterial communities in the studied samples. Samples were processed as described previously (Žifčáková et al., 2016). Briefly, sampling was done in two contrasting seasons, summer (July) when plant photosynthetic activity peaked and late winter (March) after a prolonged period with low photosynthate input and soil being insulated by a deep snow cover. Soil temperature was around 15 °C during July and 2 °C in March. Soil samples were collected from 6 sites, located 250 m from each other, and eight soil cores (4.5 cm diameter) were collected from around the circumference of a 3 m diameter circle. The Litter horizon (2–4 cm) and the organic horizon of soil (3–6 cm) were pooled separately. After removal of roots, litter was cut into 0.5 cm pieces and mixed; soil was passed through a 5-mm sterile mesh and mixed. A total of 24 samples were collected (six sites × two seasons × two horizons). Aliquots for nucleic acids extraction were immediately frozen and stored at −80 °C, samples for chemical analysis and enzyme activity measurement were freeze-dried and stored at −45 °C. Enzyme assays were performed in soil homogenates (Štursova and Baldrian, 2011).

2.2. Nucleic acid extraction and reverse transcription

Total DNA was extracted in triplicate from all samples using a modified Miller method (Sagova-Mareckova et al., 2008) and cleaned with a Geneclean Turbo kit (MP Biomedicals). RNA was extracted using RNA PowerSoil Total Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) combined with the OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA). Three soil aliquots (3 × 2 g of material) were extracted per sample. Triplicates of extracted RNA were pooled and RNA was purified using the RNA Clean & Concentration kit (ZymoResearch) on a column treated with DNase I (Fermentas) according to manufacturer's instructions. These products were checked for quality (RIN number) and length distribution on an Agilent 2100 Bionalyser (Agilent Technologies). Approximately 1 μg of RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Life Technologies) using random hexamer primers. Samples were designated as LSD = litter summer DNA, LWD = litter winter DNA, SSD = soil summer DNA, SWD = soil winter DNA, LSR = litter summer cDNA, LWR = litter winter cDNA, SSR = soil summer cDNA, DWR = soil winter cDNA.

2.3. Primer design and tag-encoded amplicon sequencing

To identify β-glucosidase genes, two sets of degenerate primers were designed based on the sequences of β-glucosidase genes from a broad spectrum of soil fungi. All protein sequences annotated as β-glucosidase in the glycoside hydrolase (GH) families GH1 and GH3 in the CAZy database (http://www.cazy.org/) were selected, and their nucleotide counterparts were retrieved from the GenBank (http://www.ncbi.nlm.nih.gov/). All nucleotide and protein sequences were de-replicated, multiple sequence alignments were conducted with MAFFT (Katoh et al., 2002) and alignments curated manually. The primer pairs bglFGH1F/bglFGH1R and bglFGH3F/bglFGH3R were designed to amplify partial conserved fragments of GH1 and GH3 β-glucosidase genes from fungi, respectively (Table 1). Designed primers were tested *in silico* in SEED 1.2.1 (Větrovský and Baldrian, 2013). Against available metatranscriptomic data from the same soil. The specificity of primers was tested using the Primer-Blast tool from NCBI against the GenBank dataset. The primer pairs βgluF2/βgluR4 (Cañizares et al., 2011) and GH3BF/GH3BR (Li et al., 2013) were used to amplify partial conserved fragments of GH1 and GH3 β-glucosidase genes from bacteria, respectively. Primers for tag-encoded sequencing contained, in addition, sample tags separated from primers by spacers. Spacer sequences were designed to have a trinucleotide, which was absent in all GenBank sequences at this position to avoid overrepresentation of some target sequences (Parameswaran et al., 2007). Tagged primers were tested for self-dimmer, hetero-dimer and hairpin formation using the online tool OligoAnalyzer 3.1 (http://eu.idtdna.com/calc/analyzer).

All PCR amplifications were performed in five replicate 25 μl PCR reactions. Reaction mixtures contained 1 μl of template DNA/cDNA,