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Short communication

Detection of expressed fungal Type I polyketide synthase genes in a forest soil

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ABSTRACT

Secondary metabolites synthesized via the polyketide pathway are thought to mediate interference competition between soil microorganisms as well as cell-to-cell signaling. Although their synthesis has been documented in laboratory culture, it remains uncertain whether these compounds are actually produced under field conditions. Here, we report the presence and expression of fungal Type I polyketide synthase (PKS) genes in the organic horizon of a forest soil. If these expressed PKS genes produce compounds that elicit antagonistic interactions among soil microorganisms, then it could be a factor structuring microbial communities in soil.

Type I polyketide synthase (PKS) genes mediate the production of secondary metabolites of the polyketide (PK) pathway, which include pigments, compounds involved in cell-to-cell signaling, as well those which elicit antagonistic interactions between microorganisms. Ascomycete fungi contain large numbers of PKS genes, which are separated into three main groups: reducing PKS genes, non-reducing PKS genes and 6-methyl salicylic acid synthase genes (6-MSAS; Kroken et al., 2003). Non-reducing PKS genes are involved in the synthesis of pigments and unreduced polyketides (e.g. aflatoxins), whereas reducing PKS genes produce a distinct set of ecologically important metabolites (e.g. signaling compounds, diverse mycotoxins, etc.), which have generated interest for biotechnological application (Schümann and Hertweck, 2006). PKS gene diversity and functionality have been predominantly assessed in culture-grown organisms or in lichens (Grube and Blaha, 2003), and phylogenetic analyses have demonstrated that reducing and non-reducing PKS genes cluster in different subgroups (e.g. Clades I–IV, Kroken et al., 2003). Information on the PKS gene diversity in and among soil microbial communities has been described for actinobacteria (Seow et al., 1997; Wawrik et al., 2005), but we do not understand the complete community of soil microorganisms possessing these genes, let alone whether they are expressed in the soil environment. In this study, we focused on the presence of ascomycete reducing PKS genes and their expression in a forest soil, as an initial step towards a better understanding if their products may be an important factor structuring microbial communities in soil. We also sought to produce high quality RNA from soil samples suitable for generating cDNA libraries containing full-length PKS transcripts, which exceed frequently sizes above 7000 bp.

We investigated the presence and expression of fungal Type I reducing PKS genes in the forest floor of a sugar maple-dominated (*Acer saccharum* Marsh.) forest in Michigan, USA (43°40′ N, 86°09′ W). Sampling of the forest floor was performed in November 2007 after leaf senescence. In three replicate 30 × 30-m plots, we collected forest floor (Oe and Oa horizons) from 10 randomly located 0.01 m^2 areas. These samples were composited within each plot, homogenized in the field, and placed in liquid N_2 prior to nucleic acid extraction. Then, nucleic acids were extracted from homogenized soil samples (each ~1 g). RNA was purified from the samples and cDNA libraries were synthesized for each replicate plot ($n = 3$) according the procedure of Luis et al. (2005); it includes cell lysis with a FastPrep machine (MP Biomedicals, USA), several purification steps, and a DNAse digest before library construction. The method we employed to synthesize cDNA produces full-length

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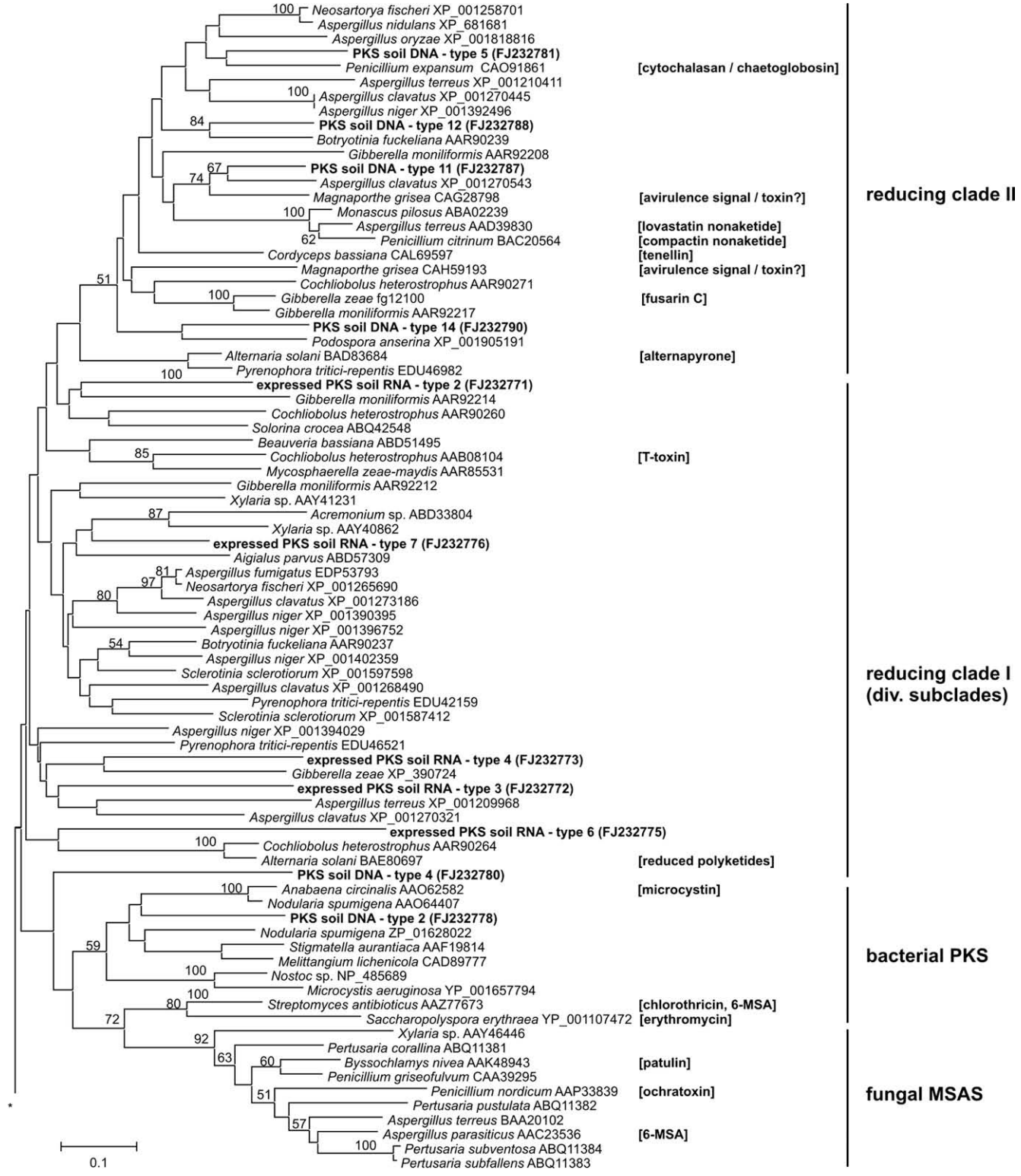

Fig. 1. Phylogenetic tree of partial PKS amino acid sequences using Neighbor-Joining method and Poisson's corrected distances. Branch support was estimated by calculating 2000 bootstrap replicates and the result is shown above the branches. Additional information for product formation of single PKS genes is reported in brackets, and clade structure information is based on Kroken et al. (2003). Discarding hypervariable alignment proportions, effectively 71 positions were used to generate the phylogenetic inference. PKS genes of *Gibberella zeae* fg12100 and fg12125 were obtained from the Munich information center for protein sequences (mips.gsf.de/genre/proj/fusarium).

gene transcripts, because adapter sequences at both ends of the transcripts allow amplification of all cDNAs by a long-distance PCR; to accomplish this we used the Advantage 2 PCR kit (Chenchik et al., 1998; SMART technology, Clontech, USA). Soil DNA was separated from RNA using columns provided in the RNA/DNA Midi kit according to manufacturer's protocol (Qiagen, Germany; a modified step of RNA extraction in Luis et al., 2005), loaded on agarose gel and then gel-purified using the QIAquick Gel Extraction Kit

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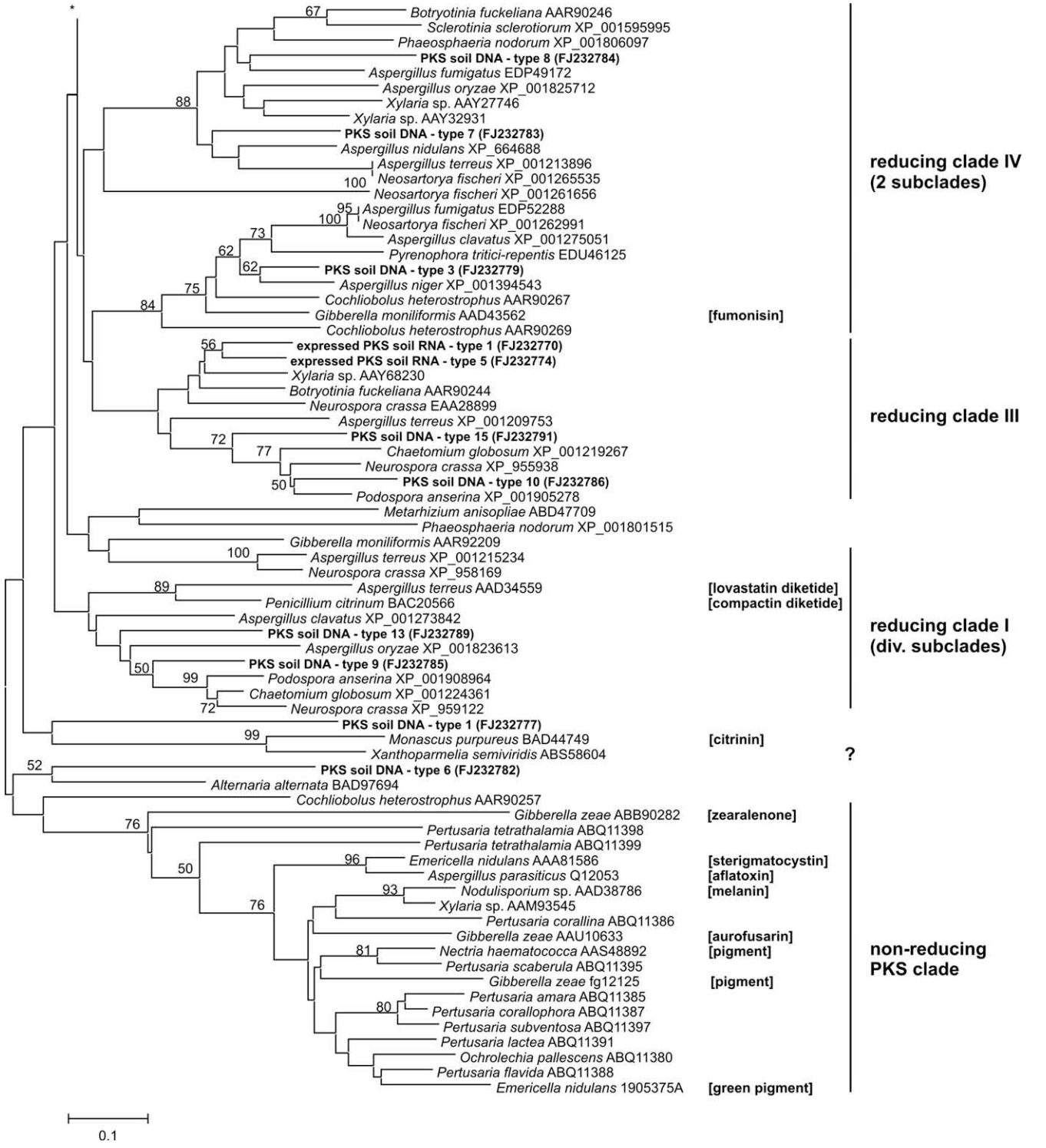

Fig. 1. (*continued*).

(Qiagen) to remove residual humic substances. By extracting and analyzing DNA and mRNA (cDNA) in parallel, we were able to assess the presence of fungal Type I PKS genes in genomic DNA, as well as the community of organisms expressing those genes in the soil environment. Partial reducing Type I polyketide synthase genes, spanning part of the ketoacyl synthase (KS) and acyl transferase (AT) domain, were amplified using the KAF1 (5′-GAR KSI CAY GGI ACI GGI AC-3′) and KAR2 (5′-CCA YTG IGC ICC YTG ICC IGT RAA-3′) primer pair (Amnuaykanjanasin et al., 2005). Amnuaykanjanasin et al. (2005) were able to amplify a considerable diversity of PKS genes in the ascomycete *Xylaria* sp. using this primer set, making it an obvious first choice to explore the presence and expression of these genes in soil. PCR products (~600–900 bp) were gel-purified using the QIAquick Gel Extraction Kit (Qiagen) and cloned into the

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pCR 2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen, Carlsbad, USA), according to manufacturer's protocol. A total of 60 (20 clones for each library) were randomly selected, cultured overnight in liquid Luria broth, and sequenced at the Laboratory for Genomics and Bioinformatics at the University of Georgia using M13 primers (GenBank accession numbers: FJ232770–FJ232791). An amino acid alignment of the amplified genes (TreeBASE study no. S2290 http://www.treebase.org/treebase/index.html), including references obtained from GenBank, was performed with ClustalW. Phylogenetic analyses were conducted using MEGA4 (Neighbor-Joining method, Poisson's corrected distances, 2000 bootstrap replicates; Tamura et al., 2007).

We amplified, sequenced, and identified (Blastx, 44–76% identity of conserved parts of the KS domain) 15 PKS genes present in forest floor DNA (Fig. 1). The length of the amplified partial genes ranged from 663 to 941 bp, and some sequences contained one (PKS soil DNA – Types 9 & 15, Fig. 1) or two putative introns (PKS soil DNA – Types 1 & 14). The newly obtained genes clustered in previously defined reducing PKS Clades I–IV (Kroken et al., 2003) of mainly ascomycete references, few bacterial references (subclade), and no basidiomycetes. However, using a smaller proportion of the KS domain (Kroken et al., 2003), we could only partly resemble their monophyletic structure (Fig. 1). This was already observed analyzing the phylogenetic inference of *Xylaria* sp. PKS genes (Amnuaykanjanasin et al., 2005). None of the observed genes could be related to particular taxa, indicating the present limitation of public databases or the need to use another conserved gene fragments in the KS domain which contain more phylogenetic information, or for which a greater number of reference sequences reside, in molecular databases. Among the PKS genes amplified from forest floor DNA, one type (Type 2, Fig. 1) was related to bacterial PKS genes, indicating that the primers, situated in conserved parts of the KS domain, might also amplify few bacterial PKS genes from soil.

Seven different types of expressed reducing PKS genes could be amplified and sequenced from the three cDNA libraries, ranging in length from 603 to 813 bp (Fig. 1). None of these sequences matched PKS genes that we amplified from forest floor DNA or to references in databases. However, a similar disparity between expressed and genomic genes has been observed in fungal laccase genes (Luis et al., 2005). Development of single-gene specific PKS primer might solve this problem in future studies, thereby providing a link between the genes present and their transcripts. The PKS genes amplified from cDNA clustered in two defined clades, reducing Clades I and III. PKS RNA-Type 1 was the most frequent, and it was present in two of our three cDNA libraries, whereas all other types appeared exclusively in one library (i.e., singletons).

Our observation of expressed PKS genes has relevance for biotechnological applications. The amplified partial PKS genes from our cDNA libraries indicate the presence of full-length PKS transcripts, because our cDNA synthesis procedure enriches specifically for full-length mRNA transcripts (Chenchik et al., 1998). These full-length transcripts could be cloned into expression vectors, which would subsequently allow for the production and characterization of the resulting PKS gene products, without the interference of introns (Schümann and Hertweck, 2006). Moreover, our approach accessed the whole soil community, rather than isolated single cultures, providing the opportunity to study uncharacterized PKS sequences from unknown fungal organisms, which are overlooked by conventional approaches. For example, no PKS gene obtained by our survey was a perfect match to known fungi (Fig. 1).

In addition to the mere presence of fungal Type I PKS genes in soil, we could clearly demonstrate their expression in the soil environment. Our observation suggests that soil ascomycetes deploy this metabolic pathway under field conditions, and the products of the PKS pathway may be used to enhance their competitive ability in soil. Reducing PKS gene products are plausibility involved in the ecological processes of cell signaling and antagonistic interactions with other soil microorganisms (Karlovsky, 2008). In the latter case, competitive interactions for niches and substrates (e.g. succession of leaf litter in autumn by ascomycetes) are consistent with the production of PKS products in the environment. Database entries also mention basidiomycete PKS-like genes (e.g. *Laccaria bicolor* EDR13531), but we have incomplete knowledge of their function, gene products or the ecological processes their products may mediate. However, future studies are needed to better understand temporal and spatial differences in the expression and to identify produced metabolites, which would provide further insight into their importance for microbial community dynamics in soil. Although we understand very little regarding the production and efficacy of these compounds in the soil environment, our results point to their potential importance in mediating ecological interactions among soil microorganisms, especially between ascomycete fungi and other organisms. If expressed PKS genes produce compounds that elicit antagonistic interactions among soil microorganisms, then they may be an important factor structuring soil microbial communities.

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