The moss metagenome: A treasure chest for enzymes

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Introduction

Sphagnum mosses (Fig. 1A) are colonized by highly diverse and species-specific microbial communities.[1,2] Analysis of the *Sphagnum* metagenome revealed that several genetic features distinguish it significantly from comparable microbiomes (Fig. 1B). Key microbial functions are involved in nutrient supply and pathogen defense along with the production of bioactive compounds.[3] The antimicrobial activity of mosses has also been reported.[1] *In silico* data mining in the moss metagenome indicated high abundance of different enzyme classes (e.g. nonribosomal peptide synthetases (NRPS), polyketide synthases (PKS), hydrolases, decarboxylases).

Here we explore the *Sphagnum* microbiome as a source of industrially interesting enzymes for biotechnological and biomedical applications.

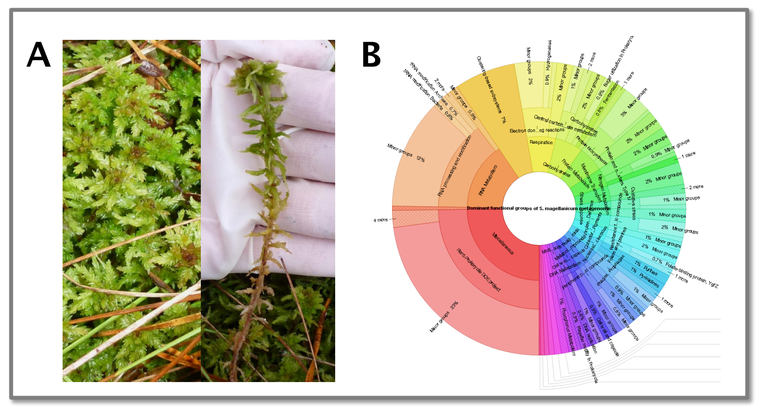

Fig. 1: *Sphagnum magellanicum* (A) and distribution of functional groups in the metagenome (B).[3]

Methodology

A screening platform for proteins of valuable biotechnological potential was established (Fig. 2):

Metagenomic DNA *Sphagnum* moss microbiome → **Fosmid library** (~3.2 Gb) → **Screening** Sequence-based, Activity-based → **Isolation / characterization** Sequencing, ORF finding, cloning, biochemical tests

Fig. 2: Screening platform for identification of metagenomic sequences of interest in an *E. coli* fosmid clone library

Esterases: 'putative polymer hydrolases'

Screening of 90,000 fosmids led to identification of 83 clones showing esterase activity. The most active clones (Tab. 1) were selected for subcloning and sequencing. BLASTx analysis revealed different levels of homology to esterases from microbial sources.

Tab. 1: Homology of metagenomic sequences and specific activities with the surrogate substrate *p*-nitrophenyl butyrate.

Identity (%)	Closest hit (nr protein sequence database)	Specific activity (U $g^{-1}_{total\ protein}$)
36	Fatty acyltransferase-like protein (uncultured bacterium)	3350 ± 165
54	1,4-Butanediol diacrylate esterase (*Rhodopseudomonas palustris*)	2892 ± 193
57	Alpha/beta hydrolase (*Acidocella* sp. MX-AZ02)	5812 ± 245
62	Esterase/lipase (*Caulobacter vibrioides*)	518 ± 2
74	1,4-Butanediol diacrylate esterase (*Bradyrhizobium* sp.)	1250 ± 66

The novel esterase genes were isolated for recombinant expression in *E. coli* and the kinetic characterization is ongoing (Fig. 3). As a promising application we are currently studying the polymer hydrolase activity of the enzymes for biocatalytic degradation of polyesters.

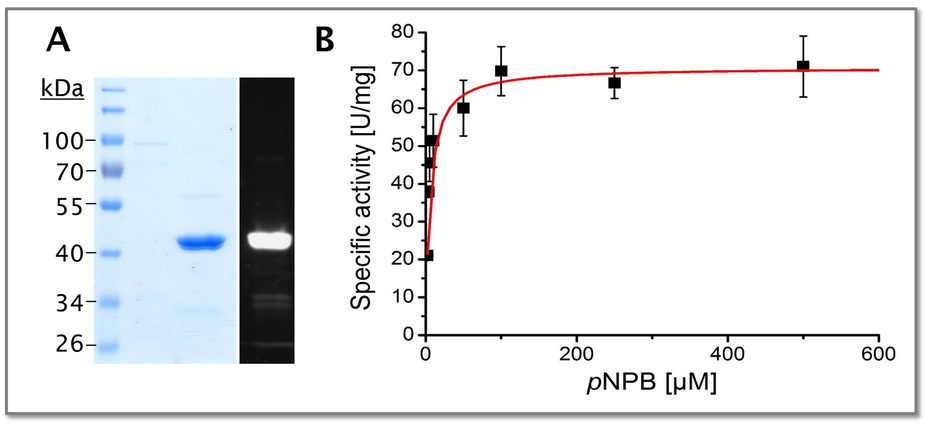

Fig. 3: Purified metagenome esterase. A) SDS-PAGE (left: Coomassie staining; right: fluorescence labeling with hydrolase probe); B) Kinetic characterization with *p*-nitrophenyl butyrate (*p*NPB).

Nonribosomal peptide synthetases

In silico data mining revealed clear differences in the bacterial diversity of NRPS and PKS genes.[4] Dominant groups in the *Sphagnum* metagenome are:
PKS → *Actinobacteria*
NRPS → *Proteobacteria*

Thirteen novel NRPS-related sequences were identified by PCR-amplification screening in the fosmid clone library. Phylogenetic analysis of selected sequences demonstrates clustering into three main groups (Fig. 4).

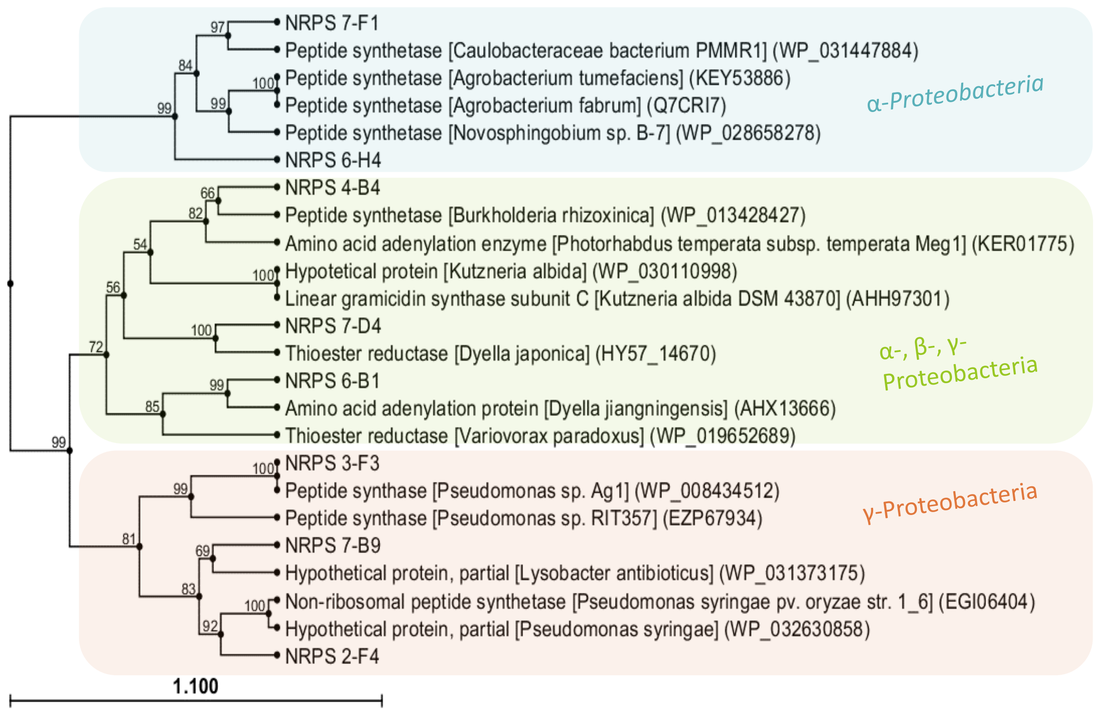

Fig. 4: Phylogenetic tree of eight novel metagenomic NRPS clones (reference sequences from NCBI protein data base; 1.1 substitutions per amino acid position).[4]

Our findings suggest the presence of gene clusters for production of bioactive compounds, especially of siderophores, toxins and antibiotics.

Conclusion

Several clones containing putative proteins or gene sequences of interest were identified in the moss metagenome. New polymer hydrolases and nonribosomal peptide synthetases are currently under evaluation as promising biocatalysts for biotechnological processes. Our recent findings demonstrate that the moss microbiome is a treasure chest for the discovery of novel bioactive molecules.

References:

[1] Opelt K *et al*. 2007. FEMS Microbiol. Ecol. 61:38–53.
[2] Bragina A *et al*. 2012. ISME J. 6:802-813.
[3] Bragina A *et al*. 2014. Mol. Ecol. 23:4498–4510.
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