The moss microbiome as a biotechnological resource: a metagenomic approach

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acib innovations from nature

Introduction

Mosses of the genus *Sphagnum* are a widespread vegetation form found in bog ecosystems (Fig. 1). Intrinsic characteristics of *Sphagnum* mosses are:

- Growth under extreme abiotic conditions (low average temperature, high acidity, repetitive desiccation, oxidative stress).[1]
- High cation-exchange capacity (acidification of the surrounding environment).[2]
- Highly diverse yet species-specific microbiome (leaf-associated microorganisms) which plays a crucial role in nutrient supply and pathogen defense.[3,4]

Fig. 1: *Sphagnum magellanicum* (Austrian Alpine peat bog)

In a previous study, we elucidated the functional microbial diversity in the *Sphagnum magellanicum* microbiome employing an Illumina-based metagenomic approach (Fig. 2).[5]

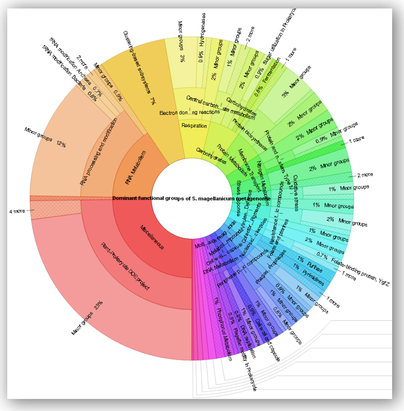

Fig. 2: Moss metagenome: functional groups distribution[5]

In silico analysis of the moss metagenome revealed high abundance of different enzyme classes: cupins, old-yellow-enzymes, xylose reductases, chitinases, polyketide synthases (PKS), non-ribosomal peptide synthethases (NRPS), decarboxylases, peptidases, phosphatases and lipases. Here we explored the potential of the *Sphagnum* moss microbiome for biotechnological and biomedical applications.

Methodology

A screening platform for proteins of valuable biotechnological potential was established (Fig. 3):

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

Fig. 3: Screening platform for identification of metagenomic sequences of interest in an *E. coli* fosmid clone library

Selected target enzymes for sequence-based screening: NRPS, PKS, decarboxylases
activity-based screening: lipases, phosphatases, peptidases

Novel lipolytic enzymes

90,000 fosmid clones screened using tributyrin agar plates (Fig. 4). 83 positive clones were identified.

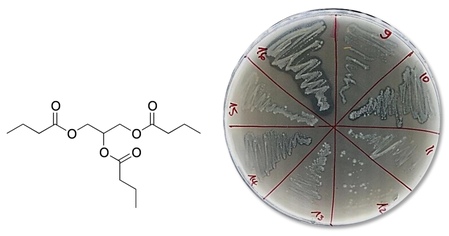

Fig. 4: Tributyrin agar plate assay

10 clones were selected after rescreening with *p*-nitrophenyl butyrate (*p*NPB, Fig. 5, Tab. 1).

Tab. 1: Specific activities with *p*NPB of best 5 novel metagenome lipases/esterases.

Clone	Specific activity (U g_{Lysate}^{-1})
F5	47.5
B3	12.7
G4	9.8
C5	7.4
B12	6.7

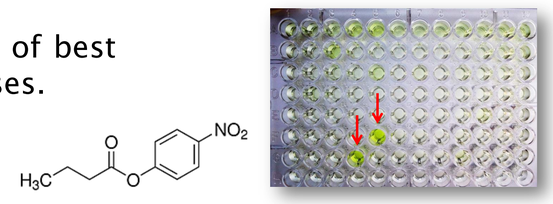

Fig. 5: *p*NPB assay in MTP

Subcloning and sequencing of the 5 most active clones (F5, B3, G4, C5) was applied.

BLASTx analysis revealed homology to lipases/esterases from different sources (Tab. 2).

Tab. 2: BLASTx analysis of the retrieved metagenomic sequences

Clone	Closest hit [source] (%Identity)
F5	1,4-Butanediol diacrylate esterase [*Bradyrhizobium* sp.] (74%)
B3	ß-lactamase [*Afipia* sp. P52-10] (61%)
G4	fatty acyltransferase-like protein [uncultured bacterium] (36%)
C5	esterase/lipase [*Caulobacter vibrioides*] (62%)
B12	1,4-butanediol diacrylate esterase [*Rhodopseudomonas palustris*] (54%)

Novel decarboxylases

Search for conserved motif (UbiD) of decarboxylases in the moss-metagenome database (*de novo* assembly):

Primer design based on alignment of best contigs (Fig. 6A).

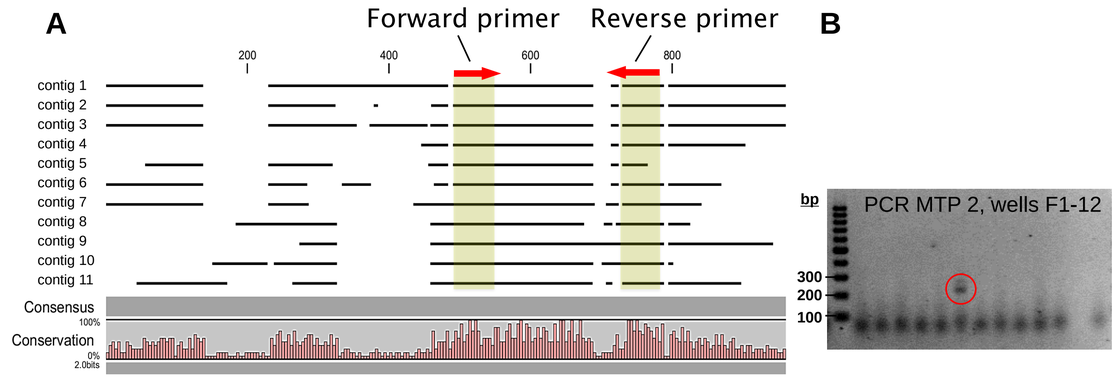

Fig. 6: (A) Conserved domain search and alignment (Cluster control) and (B) PCR-screening

Screening of 9,500 fosmid clones by PCR-amplification (Fig. 6B). 15 positive clones were identified.

Sequencing and BLASTx analysis of the selected clone 33-F33 revealed that the closest neighbour (74% identity) is a polyprenyl-4-hydroxybenzoate decarboxylase [*Ralstonia* sp. PBA].

Conclusion

Several clones containing putative proteins or gene sequences of interest were identified in the moss metagenome (exemplarily shown for lipases and decarboxylases).
The moss microbiome harbors great potential as a useful bio-resource for biotechnological and biomedical applications.

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References:

[1] Daniels, Eddy. Handbook of Europaen Sphagna. Natural Environment Research Council. Cambrian News: Aberystwyth, UK, 1985;
[2] Soudzilovskaia, *et al. Ecology* 2010, 91: 2716-2726;
[3] Bragina, *et al. ISME J* 2012, 6: 802-813;
[4] Opelt, *et al. Environ. Microbiol.* 2007, 91: 2795-2809;
[5] Bragina, *et al. Mol. Ecol.* 2014, doi: 10.1111/mec.12885.

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