In Search of Bioactive Molecules: Sourcing Plant Metagenomes

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Introduction

Bioactive molecules from microbial origin are of interest to biotechnology, the agriculture sector and medicine alike. With rising environmental awareness molecules exhibiting bioactive functions such as volatile organic compounds (VOCs) are attracting more and more attention. As a naturally occurring microbial mechanism to rival and outcompete other microbes, VOCs represent a sustainable remedy to combat human and plant pathogens [1]. Furthermore, the increasing threat of (multi-)resistances among bacteria is urging the discovery of novel antibiotics [2]. Microbes are currently being investigated to this end. However, despite of novel cultivation techniques, a large proportion of microbes remain not cultivable, leaving a tremendous source of microbial metabolites untouched; an obstacle that can be bridged by metagenomics.

Bioprospecting for VOCs & antibiotic/ -resistances

Project Plan

Generation of metagenomic libraries

- Lichen & *Sphagnum* moss microbiome as source for metagenomic DNA (Fig. 1)
- 40 kb DNA fragments
- *Escherichia coli* and *Pseudomonas putida* as library hosts (Fig. 2)

Functional screening for volatile organic compounds

- Adaption of Two-Clamps VOCs Assay [3] to high-throughput screenings
- Human and plant pathogenes *Verticillium* sp., *Rhizoctonia* sp., *Candida* sp. as test organisms

Functional screening for antibiotic & resistance genes

- Plating assays (Fig. 3A) and minimal inhibitory concentration assays
- Overlay assays using *Pseudomonas* sp., and *Candida* sp. as test organisms

Metabolic profiling of metagenomic VOCs

- Cultivation of candidate clones in head-space vials
- Solid-phase microextraction to collect and enrich VOCs
- Analysis via gas chromatography mass spectrometry

Subcloning and re-screening

- Plasmid isolation of candidate clones
- Analysis of DNA inserts (Fig. 3B) Restriction enzyme mapping
- Subcloning and re- screening, establishing shotgun libraries

Identification of encoding genes/pathways

- Sequencing of DNA inserts from candidate clones and computer based anaylsis of DNA sequences
- Product isolation and structure elucidation of novel compounds

Progress & Results

Generation of metagenomic libraries: Metagenomic DNA associated to the lichen *Lobaria pulmonaria* (sample site Johnsbach, Austria) was extracted for the generation of fosmid clone libraries (Fig. 1&2).

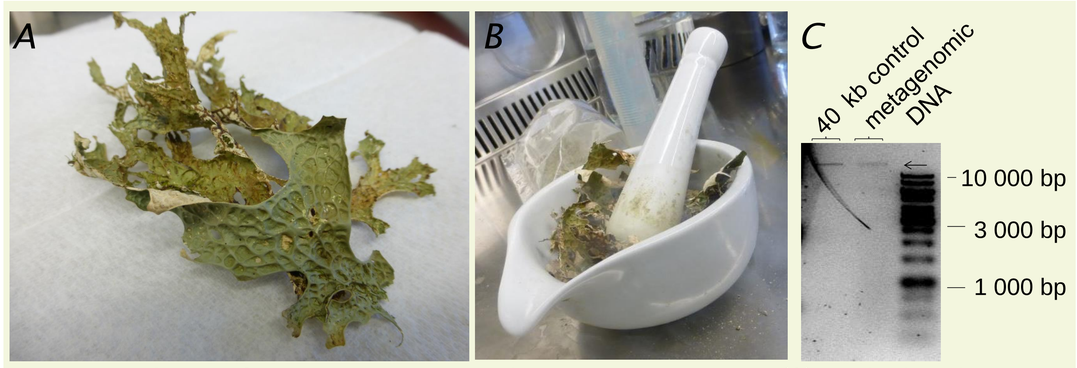

Figure 1: Isolation of metagenomic DNA. Lichen *Lobaria pulmonaria (A)* was broken down using liquid nitrogen to enrich microbes associated with it *(B)*. The metagenomic DNA was isolated using the Meta-G-Nome™ DNA Isolation Kit (Epicentre) leading to 40 kb large DNA fragments *(C)*.

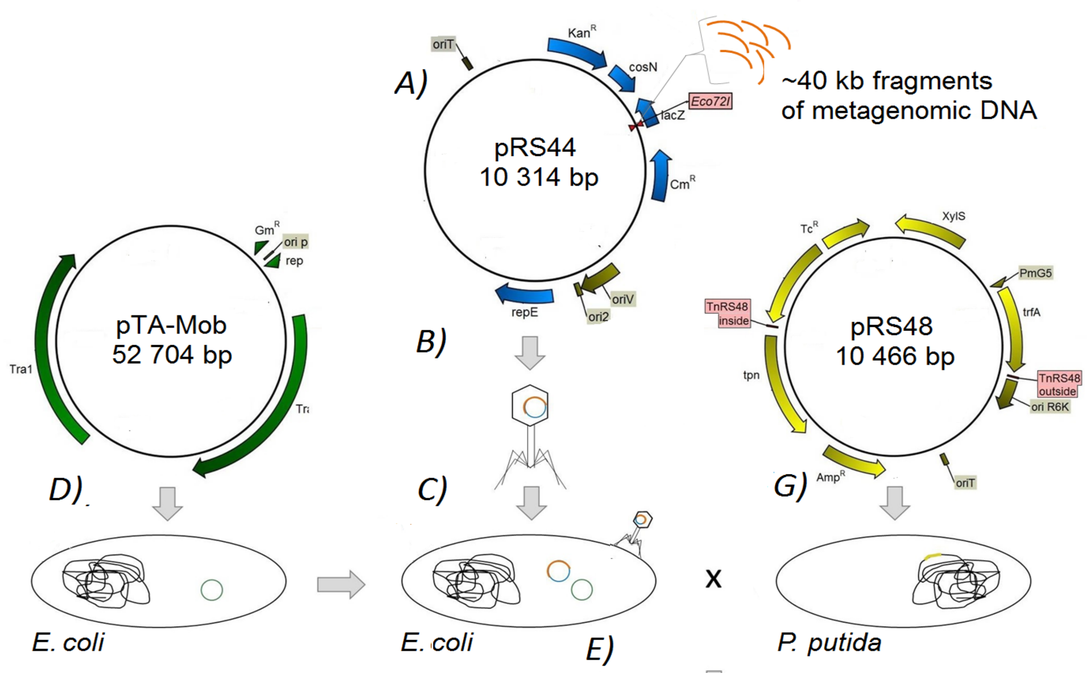

Figure 2: Construction of metagenomic libraries in an alternative host [4, 5]. DNA fragments are integrated into pRS44 via Eco72I *(A)*, packed into T4 phages *(B)* and transduced into *E. coli (C)*. pTA-Mob transformed into *E. coli (D)* harbours genes for genetic transfer (Tra1&2) and mobilises pRS44 into *P. putida (E)*. Replication of pRS44 in *P. putida* occurs through induced expression of the trfA gene present on a transposon *(F)* as incorporated into the genome via the suicide plasmid pRS48 *(G)*.

Objectives

Screening of metagenomic libraries and subsequent analysis aim to identify new bioactive compounds, VOCs and antibiotics, that are promising towards the application in medicine and agriculture. Further, the strategy will facilitate identification and localisation of genes involved in synthesis as well as unravelling underlying pathways.

Identifying VOCs and antibiotic/-resistances & their genes and biosynthesis pathways

2 novel ampicillin resistances ?

Functional screening of the metagenomic libraries: A *Sphagnum* moss library was screened for antibiotic resistance genes leading to the identification of 2 clones that show activity against Ampicillin (Fig. 3).

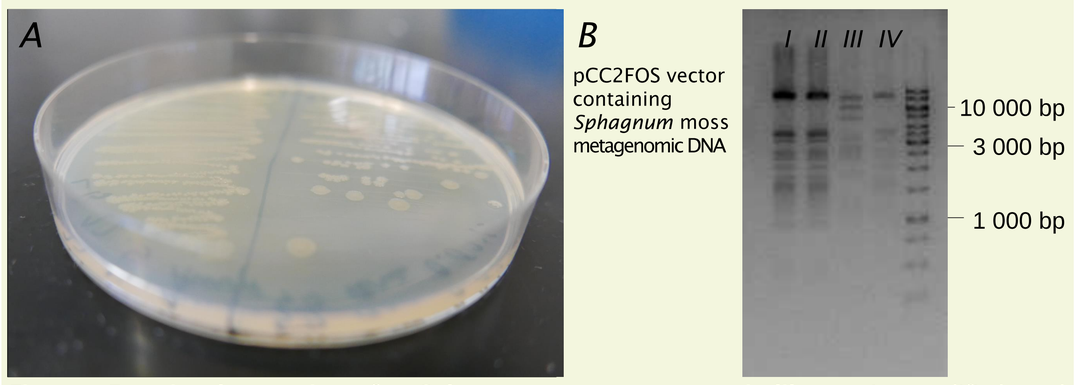

Figure : Functional screening of a *Sphagnum* moss metagenomic library. An amplified stock solution was plated to a 4 times coverage onto LB plates containing 50 µg/mL Ampicillin and incubated at 37° C over night *(A)*. Restriction digest of isolated pCC2FOS plasmids of Ampicillin resistant *Sphagnum* moss library clones using Eco RI and Bam HI *(B)*.

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