Tapping Bacterial Resources – Accessing Secondary Metabolites of the Uncultivated

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Nature is the source for a vast diversity of metabolites in use today in various applications, which encompass only a small fraction of the existing repertoire. The huge diversity reflects the biological role of secondary metabolites, which are required as mediators in interactions between organisms and their environment, stressing their important roles as signals and toxins.

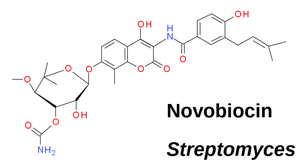
Natural products and their derivatives represent a large fraction of today's approved pharmaceutical drugs. More than 2/3 of antibiotic drugs are natural products or their semi-synthetic derivatives. Microorganisms, above all bacteria of the genus *Streptomyces* sp., are the main sources for novel lead discovery in antibiotic research.

Resistances to almost every antibiotic placed into clinical practice so far have occurred, which has led to serious threats for public health regarding multidrug resistances. Despite the urgent need for novel antibiotics to conquest existing and novel diseases, including re-occurring problematic diseases such as tuberculosis, the numbers of antibiotics in the pipeline of approval have been decreasing in the last years.

The reason for diminishing numbers of potential antibiotic candidates lies partly in the withdrawal of pharmaceutical companies from the cost-intensive antibiotic development, especially in the field of natural products. At the same time, the usage of combinatorial chemistry – providing less molecular complexity than exists in many natural products and without evolutionary pre-screen – has been rather unproductive. Another reason is that the most abundant naturally occurring and easy accessible antibiotics –the "low hanging fruits" – (e.g. actinomycin, chloramphenicol, streptomycin, and tetracycline) have been found already. This results in the often labor-intensive re-discovery of already well known antibiotics. Nevertheless, yet undiscovered natural products are far from being depleted. This has been exemplified for antibiotics produced by the well studied genus *Streptomyces* sp., of which an estimated fraction of only less than 5% has so far been accessed.

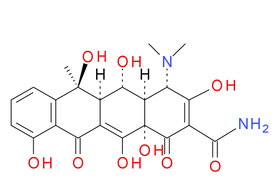
Here, we present strategies for discovering novel secondary metabolites of bacterial origin.

Streptomyces spp. as a Source of Secondary Metabolites



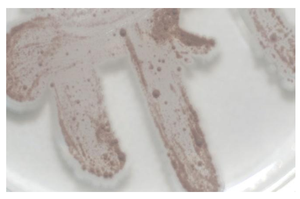
Novobiocin

Streptomyces niveus



Oxytetracycline

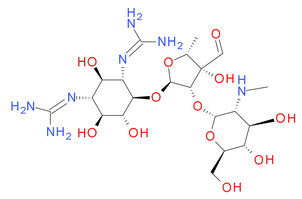
Streptomyces rimosus* subsp. *rimosus



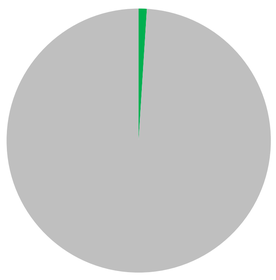
***Streptomyces* spp. (lower Austrian forest) with antibacterial activity:**

have high likelyhood to produce common antibiotics such as Streptomycin

Strategies for finding new compounds produced by bacteria required



Metagenomic Libraries to Reach Novel Chemical Space

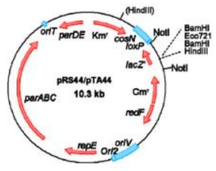


The majority of bacteria is not accessible by cultivation.

This represents a huge reservoir of genetic information encoding for unknown and unexploited secondary metabolites.

1 **Cultivable**

2 **Not Cultivable**



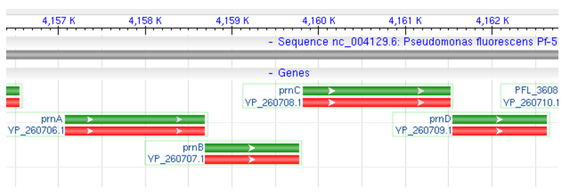
Shuttle vectors to express Libraries in *E. coli*, *Pseudomonas*, and *Streptomyces* to allow production of distinct metabolites.

Clusters of biosynthesis and resistance genes allow metagenome library screens.

Avoidance of re-isolation of well known metabolites by sequencing of defined clones

Community analysis In Austrian Pine forests: High percentage of *Streptomyces* and uncultivated *Actinobacteria* – a promising source for secondary metabolites

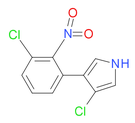
Validation of High-Throughput Library Screening Tools



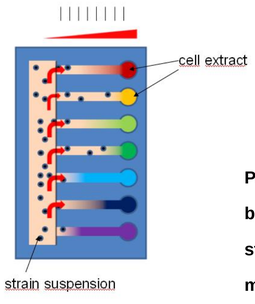
prnA – prnD: in Pseudomonas and Burkholderia spp., cluster encompass 6000bp – Burkholderia pyrrocinia as known pyrrolnitrin producer:

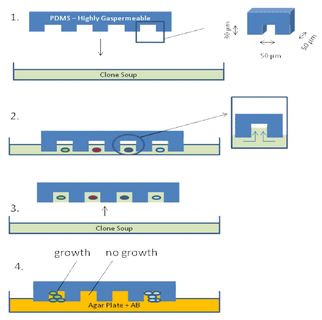
Pyrrolnitrin has antifungal and activity against gram-positive bacteria

Library as positive screening control in Proof-of-concept of micropatterning and microfluidic chip assays



Schematic layout of the cross directional microfluidic biochip used to generate concentration gradients within the horizontal microchannels.





Patterning of reporter cells. Cells are picked into microwells by capillary forces. After transfer to standard growing plate by stamping techniques, each cell forms a micro colony containing metagenome clones clearly separated from the neighboring microcolonies.

References

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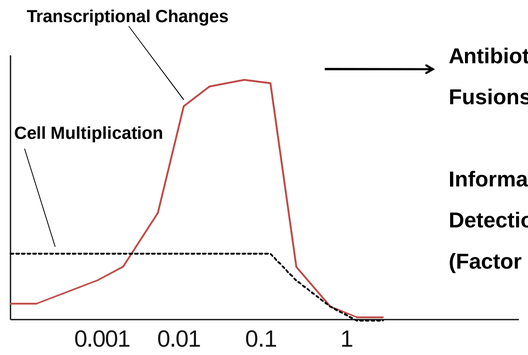
Sensing Antibiotics in Sublethal Concentrations



Antibiotic responsive reporters

Fusions to GFP, YFP, Luc, Lux casette

Information on function

Detection of small amounts

(Factor 10^2 – 10^3)

Promoters responding to classes of antibiotics disturbing particular targets in *Bacillus subtilis* 1S34