Theme 1
Status and trends of global soil nutrient budget

Bacterial metabolites as a biocomponents dedicated to the improvement of the biological/chemical quality of soil

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INTRODUCTION

The soil microbiota fitness plays a key role in the plants-growth promoting. One way to improve the condition of the soil microbiome is the appropriate soil fertilization, which is still insufficient due to the low efficiency of chemical fertilizers. For this reason, new products stimulating the soil microbiota are highly sought in agriculture.

Figure 1: Logotype of SyderoFERT biofertilizers

The main aim of studies is to develop the low input and sustainable biocomponents – siderophores, stimulating the soil microbiota - branded as SyderoFERT. The biocomponents will be used as supplements improving the quality of liquid fertilizers available on the market. The final effect of the project will be development of novel multifunctional biocomponents and biofertilizers.

METHODS

- Siderophore production - cultivation of microorganisms in medium without iron (GASN) for 7 days.
- Concentration of produced siderophores - spectrophotometric CAS assay.
- BIOLOG Phenotype Microarrays (C,N,S,P) - screening of strain metabolic preferences and for optimization of medium composition.
- Soil biological quality - measurement of microorganisms quantity (CFU/ml), 16S rDNA sequencing.
- Soil chemical quality - extraction of bioavailable iron and phosphorus with Mehlich3 extractant.
- Germination test - soaking of the seeds in biocomponents and germination in dark conditions.
- Final biofertilizers composition was tested for stability by measuring biofertilizer pH, EC, siderophore concentrations, dry mass and volatile solids.

STRATEGY

- **First stage:** Screening for efficient siderophore producing bacteria. Goal of the first stage of the strategy was selection of bacterial strain, which exhibit efficient siderophores production.
- **Second stage:** Increasing production rate of metabolites by bacteria by optimization of biotechnological processes. Most crucial tasks are appropriate medium composition and culture conditions selection (search for efficiency and low cost of production.)
- **Third stage:** Assessment of the positive impact of metabolites on soil quality and plants. The influence on biological (microbiome quantity and diversity), chemical (nutrients bioavailability) soil quality and plants (germination tests).
- **Fourth stage:** Obtaining final composition of Green_SyderoFERT, by combining metabolites with mineral fertilizers used in agriculture. Selected composition include full plant nutritional demand and stable final product.

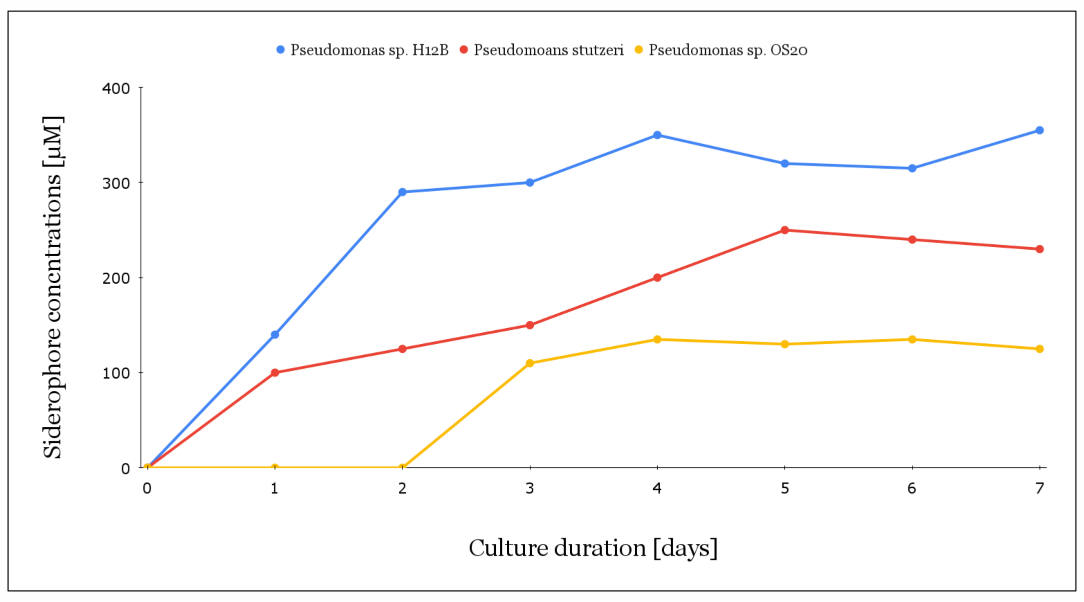

Figure 2: Kinetics of siderophore production by various tested microorganisms

RESULTS

- Performed experiments allowed selection of strain efficiently producing siderophores: *Pseudomonas* sp. H12B, which exhibited high siderophore production (~300 µM, Figure 1) and is a psychrotolerant organism (low-energy siderophore production).

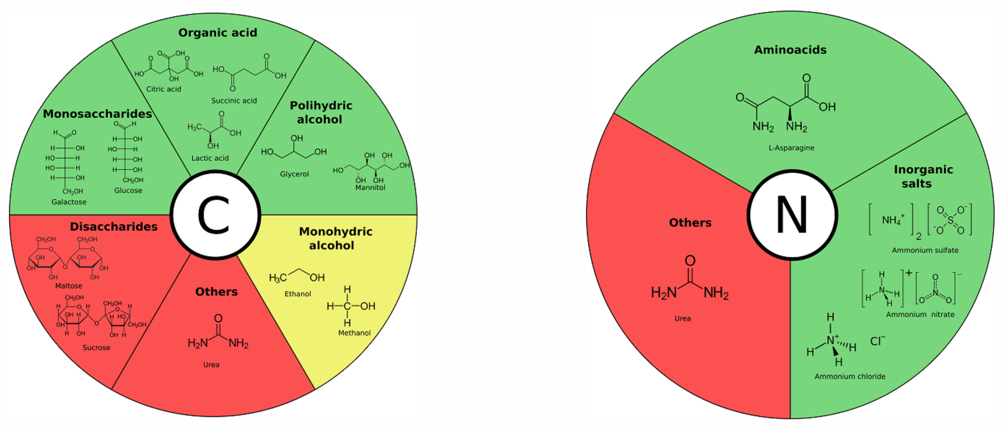

Figure 3: Versatile metabolism of *Pseudomonas* sp. H12B. Left circle represents utilization of carbon sources and right circle represents utilization of nitrogen sources. Bacteria growth is most efficient on green substrates, modarate on yellow and is inhibited on red.

- New media for siderophore were designed, based on cost-efficient substrates, e.g. glycerol, citric acid, ammonium sulfate. Siderophore yield on these media was even higher, than with traditional GASN medium (~400 µM). Depending on the composition, pH of siderophore was either acidic or alkaline. Reduction of costs was also achieved, due to low optimal production temperature (10 °C).
- The soil quality was positively affected by metabolites. Microorganisms quantity and diversity were improved. Bioavailability of crucial nutrients (iron and phosphorus) for plants was also elevated (10-30%).
- Use of biocomponents containing siderophores improved rate and speed of beetroot and pea seeds germination (Table 1)
- Combining metabolites with mineral fertilizers such as ammonium phosphate or urea ammonium nitrate solution resulted in stable products, suitable for further tests with plants

Table 1: Results of germination tests for beetroot and pea. In row „siderophores" are results for seeds pre-soaked in metabolites solution prior germination and in row „Control" are results for seeds pre-soaked in Knopp medium. Each column T1-T6 represents next day of trial and numer of germinated seeds (from 100 total)

BEETROOT						
Treatment	T1	T2	T3	T4	T5	T6
Siderophores	6	56	77	82	82	83
Control	5	44	64	70	72	75
PEA						
Treatment		T2	T3	T4	T5	T6
Siderophores	4	24	92	100	100	100
Control	0	12	62	95	99	100

CONCLUSIONS

The presented strategy helped develop new biocomponents based on bacterial siderophores. The process was optimized to achieve high efficiency of siderophore production. The study found that the biocomponents improved the soil quality. The soil microbiome and bioavailability of nutrients were stimulated. The production cost was reduced thanks to the use of a psychrotolerant strain and optimized cultivation conditions.

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