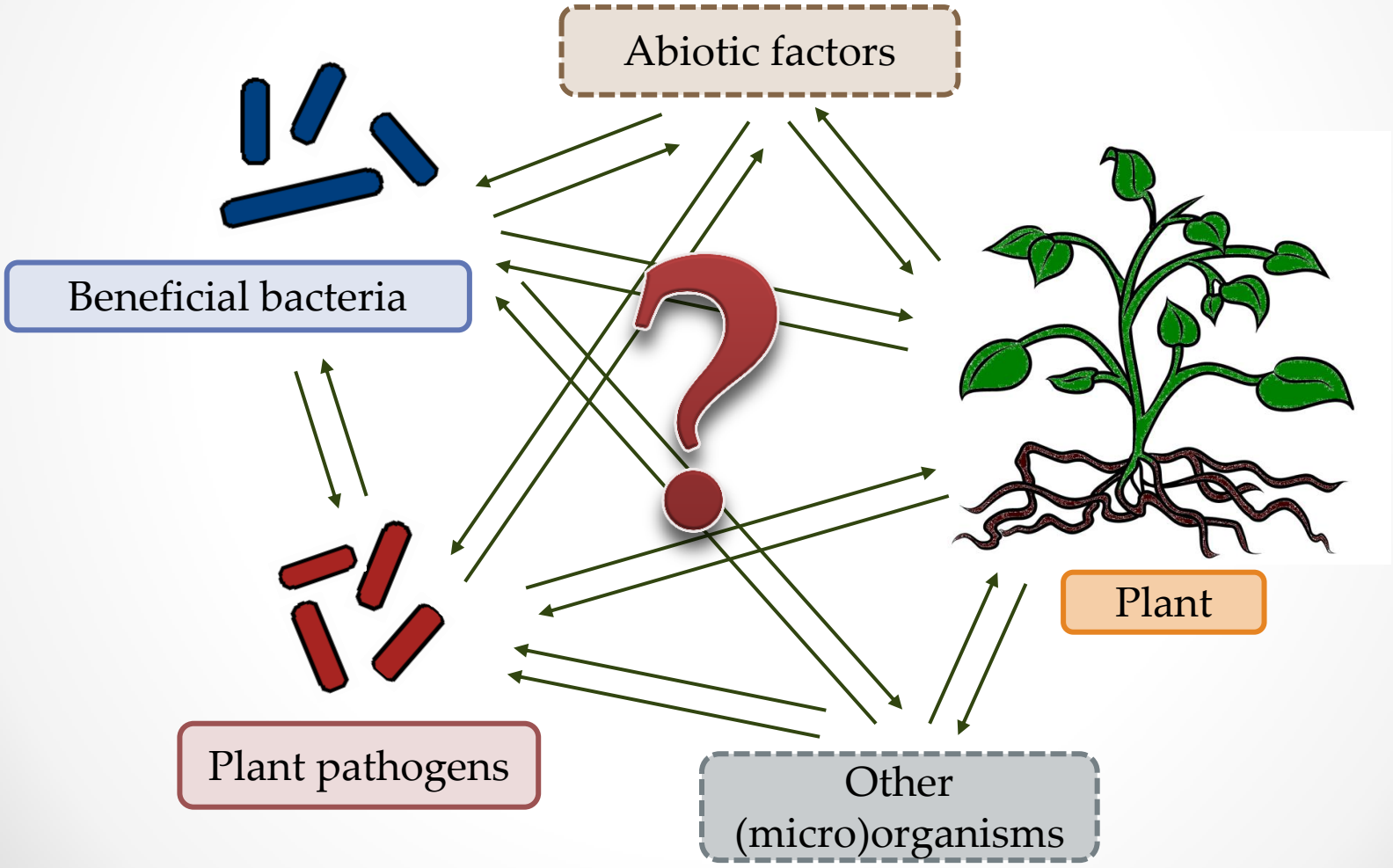
Intercollegiate Faculty of Biotechnology
University of Gdansk & Medical University of Gdansk

Antagonistic Activity of *Pseudomonas* sp. P482 Towards Plant Pathogenic Bacteria and Fungus

Dorota Krzyżanowska, Adam Ossowicki
Sylwia Jafra

Interactions in the soil environment



Topics of interest

Plant pathogenic bacteria

Biological control

Biologically active secondary metabolites

Plant pathogenic bacteria

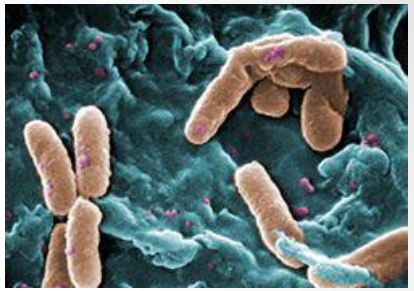
- *Pectobacterium* spp.
- *Dickeya* spp.

Soft rot, black leg and plant wilt diseases

Biological control of plant pathogens

- Application of microorganisms or the their products (secondary metabolites) for plant pest (bacterial or fungal pathogens) management and plant fitness enhancement.

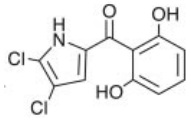
Pseudomonas spp.



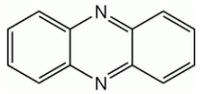
- Gram negative bacteria
- Very diverse group (core genome c. 60%)
- Present virtually everywhere (soil, water, plant tissue)
- Human and plant pathogens
- Plant Growth Promoting Bacteria (PGPB)
- Used in bioremediation and biological control of fungal pathogens

Pseudomonas secondary metabolites (with antimicrobial activity)

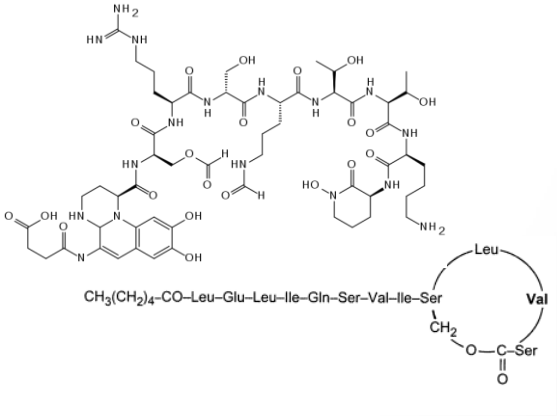
- Antibiotics
  - eg. pyoluteorin



- Toxins
  - eg. phenazine



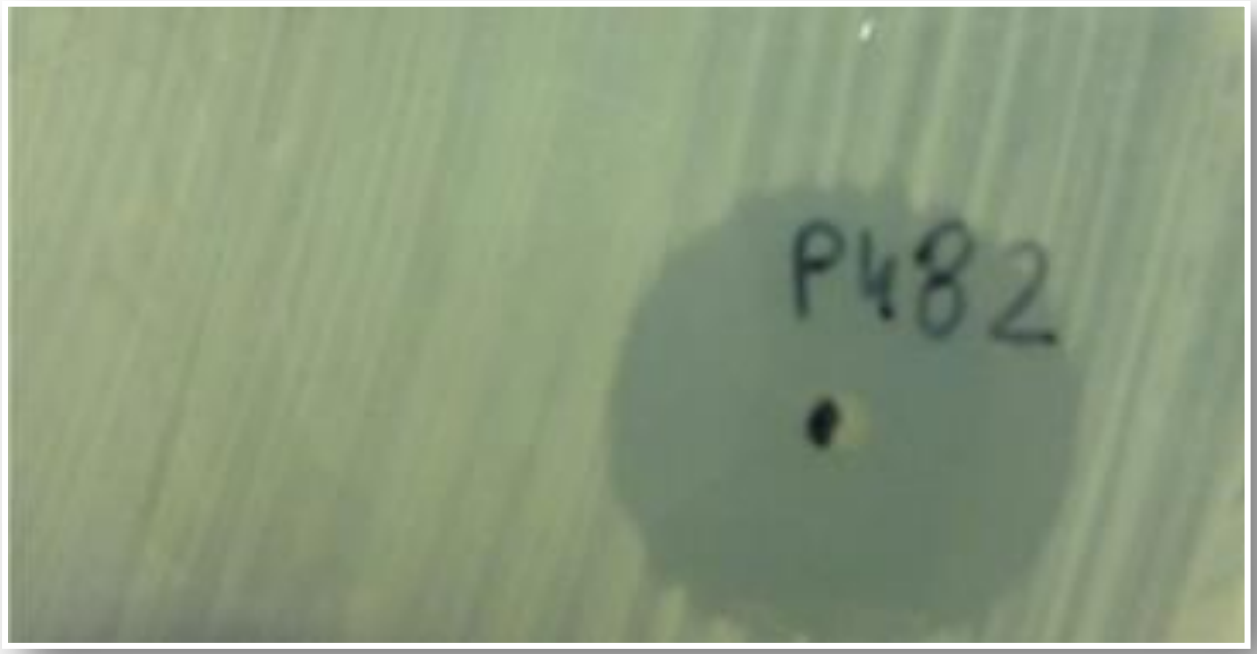
- Siderophores
  - eg. pyoverdin



- Biosurfactants
  - eg. putisolvin
- Volatile compounds
  - eg. hydrogen cyanide HCN

Pathogens growth inhibition

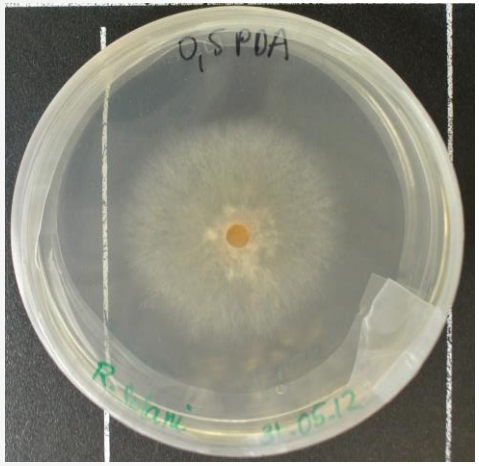
Antibacterial activity



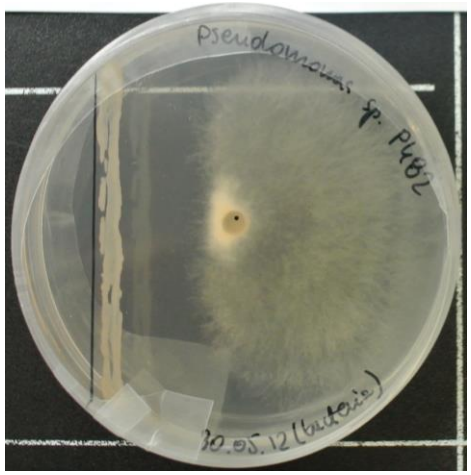
Krzyżanowska et al., 2012

Pathogens growth inhibition

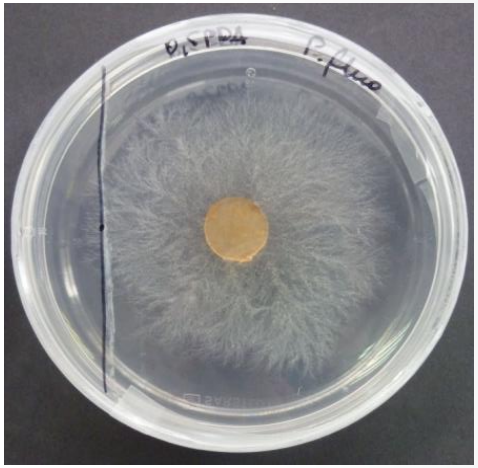
Antifungal activity



Rhizoctonia solani

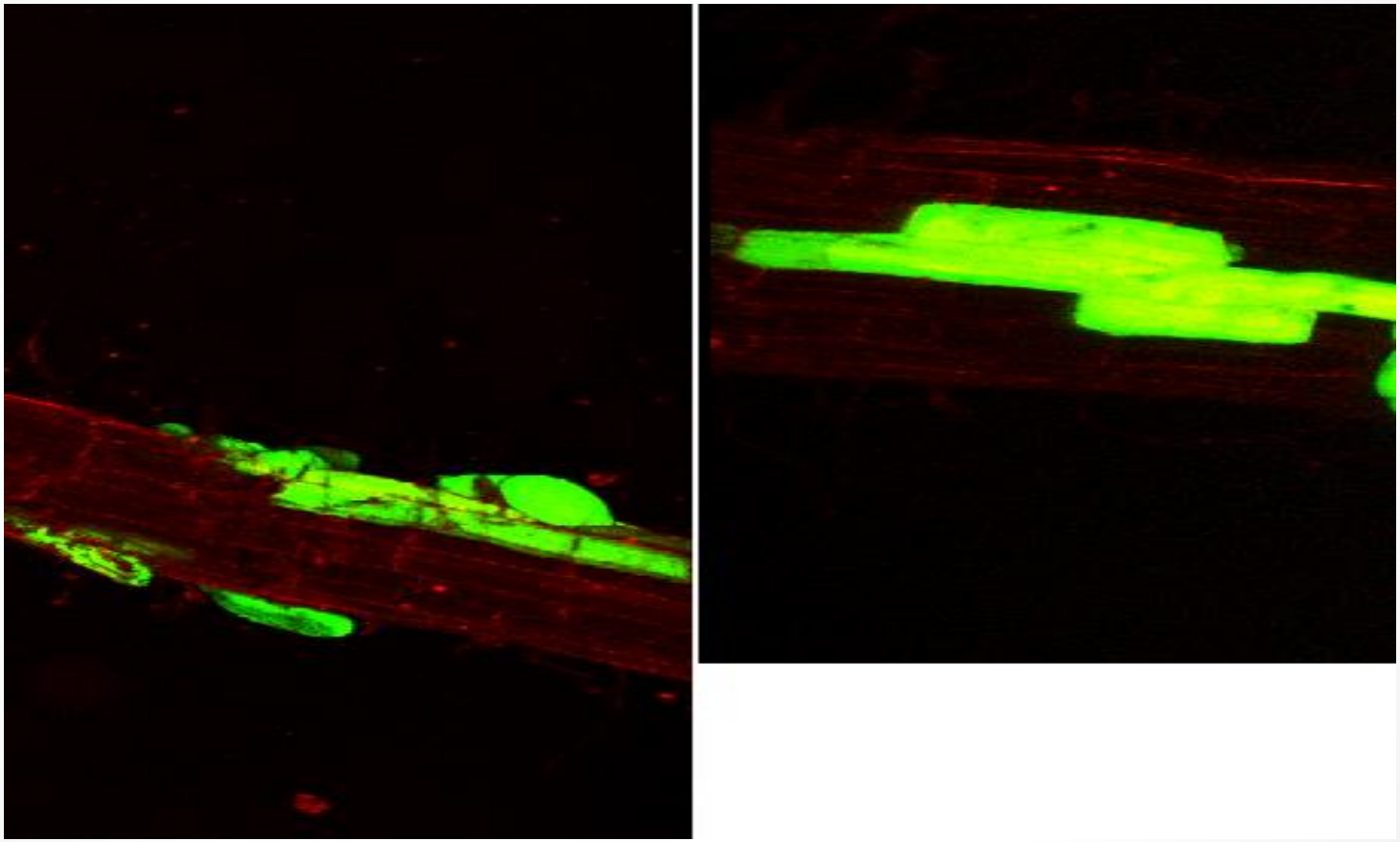


Pseudomonas sp.P482 vs. *Rhizoctonia solani*



P. fluorescens CCM 2115 vs. *R.solani*

Potato root colonisation



Krzyżanowska et al., 2012

Pseudomonas sp. P482

- is a tomato rhizosphere isolate
- inhibits growth of plant pathogenic bacteria and fungus
- is able to protect plant tissue from the maceration caused by *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya solani*
- is able to colonize potato rhizosphere
- does not produce well described antimicrobial factors such as: pyoluteorin, phenazine, putisolvin or 2,4-diacetylphloroglucinol (2,4-DAPG)

The aim of the project is

to reveal the mechanisms of *Pseudomonas* sp. P482 underling its antimicrobial activity

Biochemical approach for identification of the antimicrobial factors

EXTRACTION

- Cell-free culture supernatant
- Organic solvents

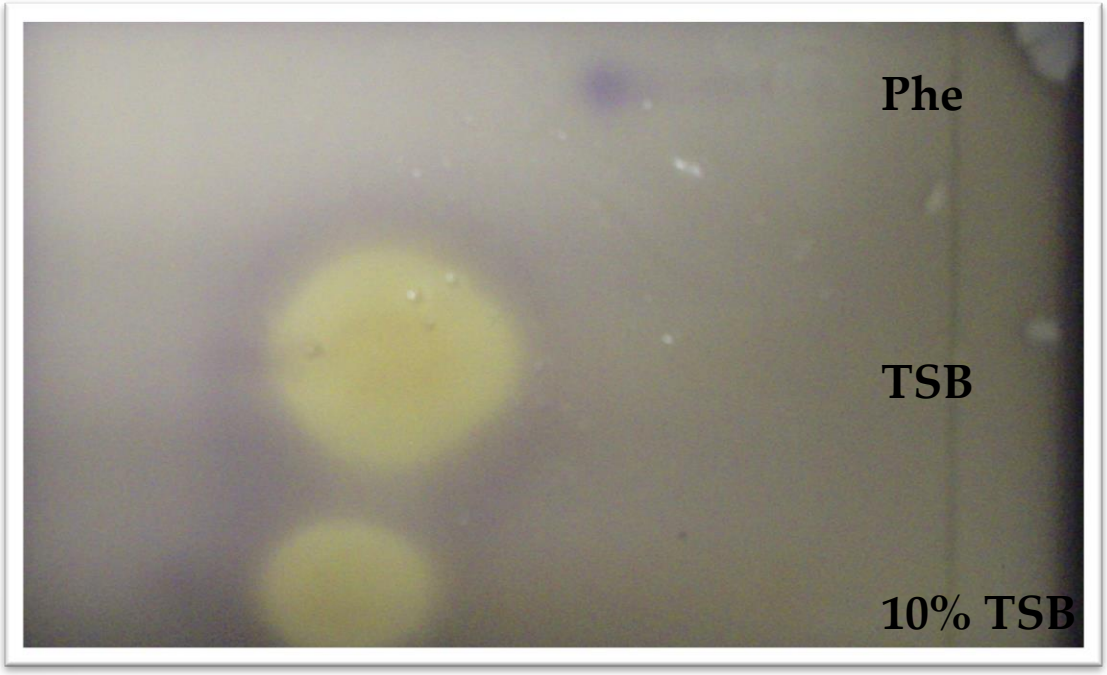
SEPARATION

- Thin layer chromatography (TLC) combined with ‘overlayer’ assay
- HPLC

IDENTIFICATION

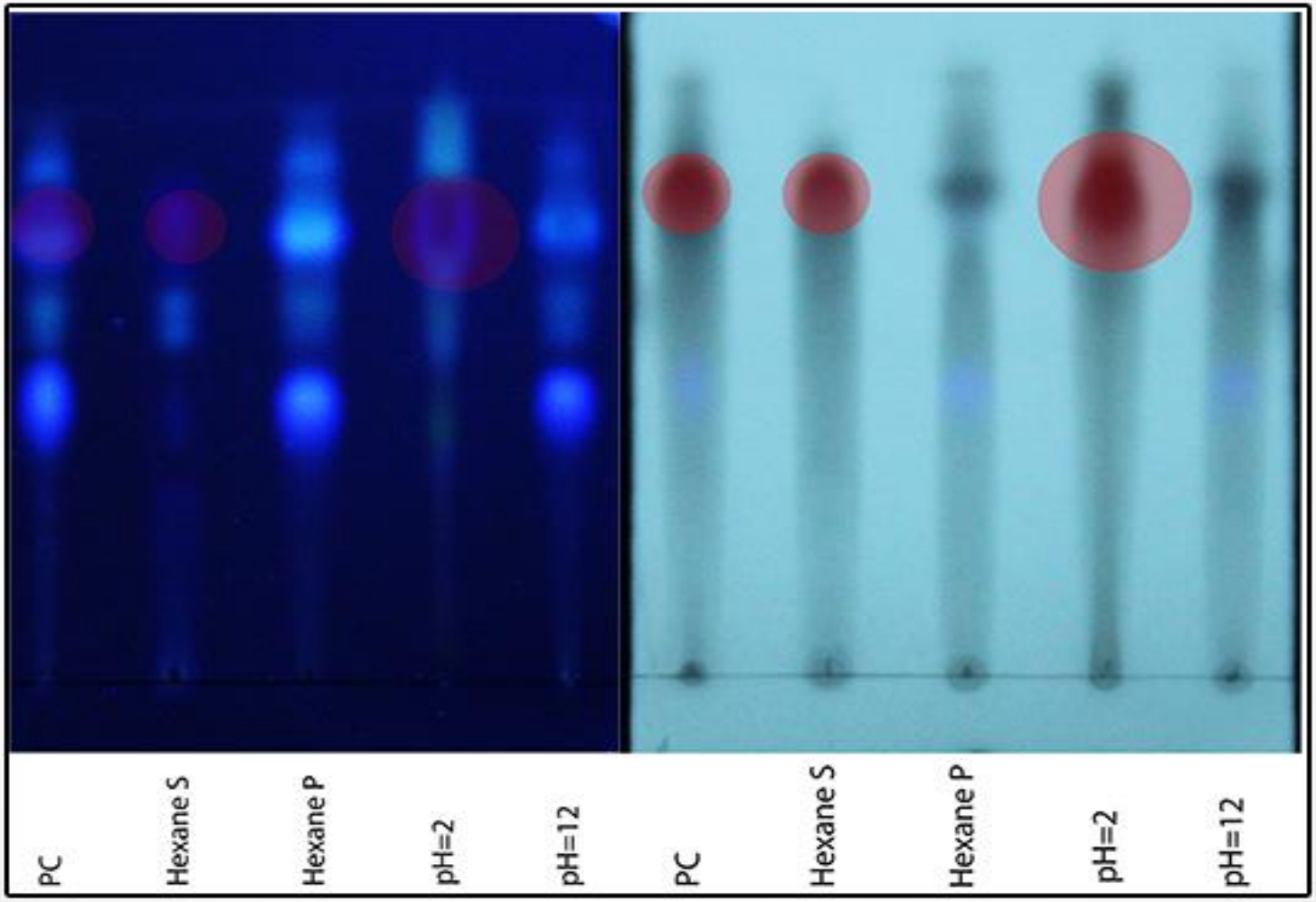
- Structure analyses
  - Mass spectrometry
  - NMR

Example of TLC combined with ‘overlayer’ assay



Extracts separeted on Silica RP-18 F_{254} plate:
10 % TSB – Ethyl acetate extract from Pseudomonas sp. P482 cultivated in 10% TSB medium
TSB – Ethyl acetate extract od Pseudomonas sp. P482 cultivated in TSB medium
Phe – Phenazine, used as standard

TLC combined with ‘overlayer’ assay



Genetic approach for identification of the antibacterial factors

SEQUENCING

- Genome sequencing
- Analysis *in silico*
  - Selection of the candidate genes

MUTAGENESIS

- Transposon mutagenesis
- Site-directed mutagenesis

IDENTIFICATION

- Gene(s) or operon identification
  - Confirmation by the activity tests
    - plant protection assay

In silico analyses

Genes encoding

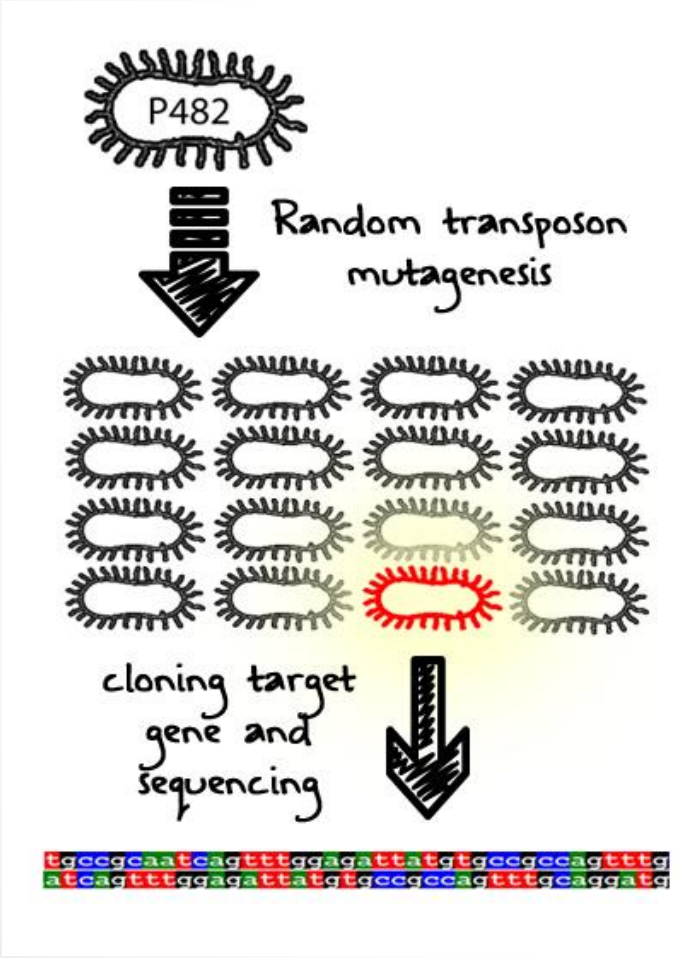
- Non-ribosomal peptide synthases (NRPS)
- Polyketide synthases
- Bacteriocins
- well described antibiotics of *Pseudomonas* sp.

Genes involved in quorum sensing

Regulatory genes

- *gacS/gacA* system
- *rpoS*

Transposon mutagenesis



- More than 3000 transposon mutant were generated
- One mutant was lacking antimicrobial activity
- The site of mutation is now undergoing sequencing.

Summary

- *Pseudomonas* sp. P482 exhibits antimicrobial activity
- P482 does not produce well described antimicrobial factors such as pyoluteorin, phenazine, putisolvin or 2,4-diacetylphloroglucinol (2,4-DAPG)
- The methodology for antimicrobial (antibacterial) factor extraction was established
- The efforts are undertaken to identify the genetic background of the antimicrobial activity
  - The *in silico* analysis of the genome has been started
  - Transposon mutant generation and selection is continued