



Internship proposal - Master 1 – 2023 (April-July)

Determination of crude *Bacillus* sp. extracts effects to inhibition of pathogenic traits of plant pathogens

Location: Chair of Microbiology, Faculty of Biology, University of Belgrade

This work will be carried out under the supervision of Prof. dr Slaviša Stanković, Prof. dr Tanja Berić and Ivan Nikolić, PhD.

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Context: Chemical pesticides are highly effective in plant disease control, but their extensive use over the years has led to severe environmental pollution and an increase in pathogen resistance. Many studies have discovered *Bacillus* species as an environmentally friendly and efficient alternative. Today, more than half of the commercially available bacterial biocontrol agents are *Bacillus*-based products. *Bacillus* sp. not only produces a broad spectrum of bioactive molecules with antimicrobial activity against plant pathogens, but their immunostimulatory potential is well-described in various plant hosts. The best-known molecules are non-ribosomal synthesized cyclic lipopeptides with recognized biotechnological potential. The families of surfactin, iturin, and fengycin lipopeptides include a variety of molecules that often act synergistically and have a wide range of activities due to their physicochemical properties, such as permeabilization of cell membranes and inhibition of pathogen adherence on plant tissues. In addition, cyclic lipopeptides enable the colonization of plant tissues with beneficial *Bacillus* sp. strains necessary for biological control. Cyclic lipopeptides have demonstrated their potential to inhibit the growth as well as pathogenic traits (biofilm formation, motility, siderophore production, etc.) of economically important plant pathogens when applied directly as crude lipopeptide extracts (CLEs) or via inoculation of beneficial producer strains.

Objectives: The aim of this project is to test the inhibitory potential of lipopeptides extracted from *Bacillus* biocontrol strains against different plant pathogens (*Pseudomonas syringae*, *Xanthomonas campestris* and *Erwinia amylovora*). The specific objectives of the internship are:

- to evaluate minimal inhibitory concentration (MIC) of selected CLEs against plant pathogens
- to test CLEs activity to decrease pathogenic traits in different *in vitro* tests
- to detect a potential reduction of foliar disease symptoms *in planta* after application of CLEs

Work plan:

- *Bacillus* sp. crude extract preparation via ethyl acetate extraction of lipopeptides and MIC assay
- Battery of *in vitro* assays for testing different CLEs concentration to inhibition of pathogenic traits of plant pathogens (Motility assays - swimming, swarming and twitching motility inhibition, detection of changes in siderophore and extracellular lipase production and inhibition of biofilm formation).
- *In planta* biocontrol assay (Examination of effects of selected concentration of CLEs in plant disease symptom suppression)