Investigation of the influence of glucose, glycerol and iron ions on the synthesis of antimicrobials and formation of biofilm by *Pseudomonas donghuensis* P482

PhD THESIS ABSTRACT

mgr Marta Matuszewska
supervisor: dr hab. Sylwia Jafra, prof. UG
auxiliary supervisor: dr Magdalena Rajewska

Pseudomonas donghuensis P482 is a tomato rhizosphere isolate. It is being investigated due to its exceptional abilities to inhibit growth of bacterial and fungal plant pathogens and to colonise plants' roots. The literature data suggest that biosynthesis of antimicrobials and biofilm formation (necessary for efficient plant colonisation) in bacteria are significantly influenced by the metabolised nutrients that take part in crucial biochemical pathways and regulate gene expression. Carbon and iron sources were shown to be highly important for these processes in *Pseudomonas* spp. Two commonly investigated carbon sources (glucose and glycerol) as well as iron(II) and iron(III) were used in this study to examine their influence on *P. donghuensis* P482 antimicrobial activity, biosynthesis of antimicrobials and expression of the genes responsible for this processes. Additionally, the influence of the given nutrients on abiotic biofilm formation by P482 was analysed.

The P482 mutants with inactivated genes in three regions selected for their involvement in antimicrobial activity (7-hydroxytropolone (7-HT) biosynthesis cluster, two genes responsible for pyoverdine (PVD) biosynthesis, and "cluster 17", responsible for the biosynthesis of an unknown antimicrobial), and two regulatory genes: *fur* responsible for iron metabolism and *gacA*, a transcription regulator of the global regulatory system Gac-Rsm. The tests of direct antagonism of the aforementioned mutants towards three plant pathogenic bacteria (*Dickeya solani* IFB0102, *Pectobacterium brasiliense* Pcb LMG21371 and *Pseudomonas syringae* pv. *syringae* Pss762*)* on minimal media with the given carbon and iron sources indicate no significant involvement of 7-HT biosynthesis cluster in the minimal medium with glycerol as a sole carbon source. Furthermore, "cluster 17" was showed to play an important role for the P482 antibacterial activity in glycerol. Regardless of the carbon source, the PVD biosynthesis genes are significantly involved in P482 antimicrobial activity and the supplementation with iron(II) ions causes the reduction of P482 antimicrobial activity dependent on these genes.

The extracts containing secondary metabolites obtained from the postculture filtrates of P482 wild type (wt) and mutants cultured in rich and minimal media with different carbon sources were analysed using HPLC-MS. The differences in the metabolome of P482 wt and mutants under given nutritional conditions were identified.

The expression of the genes of the aforementioned regions of P482 genome was tested in minimal medium with glucose/glycerol with or without the iron(II)/iron(III) supplementation using RT-qPCR method. The analyses were preceded with a detailed selection and validation of reference genes

and three genes, namely *gyrB*, *rpoD* and *mrdA* were found to be the most stably expressed. The expression of P482 7-HT biosynthesis genes was observed to be significantly downregulated in glycerol medium in comparison with glucose medium. Iron(II) supplementation was showed to downregulate the expression od 7-HT and PVD biosynthesis genes.

The analysis of the dependence of P482 biofilm formation ability on carbon and iron sources in the medium showed that the supplementation with iron(II) ions has a mild stimulatory effect on biofilm formation when glycerol is a sole carbon source, but a moderately negative effect in the glucose medium.

Analiza zależności formowania biofilmu abiotycznego przez P482 od źródeł węgla i żelaza obecnych w podłożu hodowlanym sugeruje, że suplementacja jonami żelaza(II) ma stymulujący wpływ na formowanie biofilmu przez P482 w podłożu z glicerolem, jednakże wpływ ten jest negatywny w podłożu z glukozą.

In summary, the study presents a high dependence of P482 antimicrobial activity, secondary metabolism and selected gene expression on the tested carbon and iron sources. The results obtained in the study contribute to the understanding of the influence of nutrients on P482 biocontrol mechanisms and its potential application.