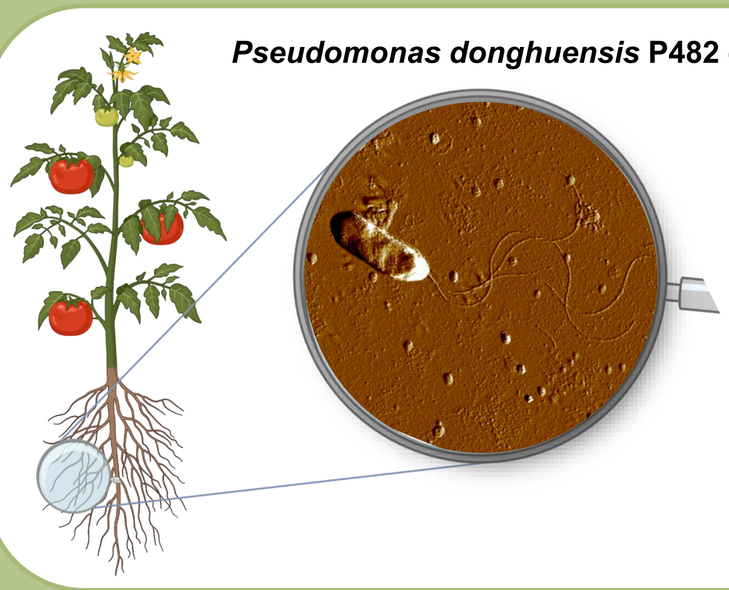
Pseudomonas donghuensis P482 – a beneficial plant-colonizing and biofilm-forming bacterium

Magdalena Rajewska, Sylwia Jafra

Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland; magdalena.rajewska@biotech.ug.edu.pl

Pseudomonas donghuensis P482 is a little-known isolate from tomato rhizosphere, which exhibits antimicrobial properties towards bacterial and fungal plant pathogens, and was shown to efficiently colonize various plant hosts. One of the mechanisms which increases the strain's environmental competence is its ability to form biofilms - complex, multicellular communities, both on biotic and abiotic surfaces. Our research demonstrated that the potential of P482 to form biofilms on abiotic surfaces are influenced by the carbon source available to the bacterium. The type of abiotic substratum, polystyrene or glass, also has impact on the ability of P482 to attach to the surface. Moreover, mutant strains of P482, in genes associated with cells' motility or chemotaxis, synthesis of polysaccharides, or encoding proteases or regulatory factors, defective in biofilm formation on glass, were still capable of colonizing rhizosphere of plant hosts, tomato and maize. This indicates that the ability of bacteria to colonize plant tissues does not necessarily go hand-in-hand with its biofilm formation capacity on abiotic surfaces. Our results bring broader perspective on the adaptation of plant-associated bacteria to various environments..

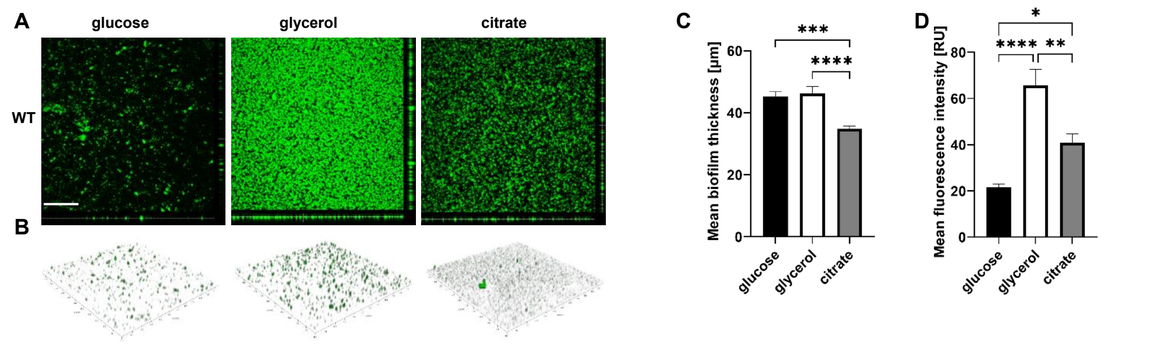
Pseudomonas donghuensis P482 exhibits antimicrobial activity



The *P. donghuensis* P482 was isolated from the rhizosphere of tomato plant in Gdynia, Poland, as a strain inhibiting the growth of bacterial plant pathogens (including those of *Dickeya* and *Pectobacterium* genera). It was characterized morphologically and phenotypically (left - image of the flagellated cell obtained with atomic force microscopy, AFM). It also inhibits growth of fungal pathogens of plants (e.g. *Fusarium* sp., *Rhizoctonia* sp.). The antimicrobial activity of P482 is determined by production of plausibly several defined (i.e. 7-hydroxytropolone) and yet undefined compound(s).

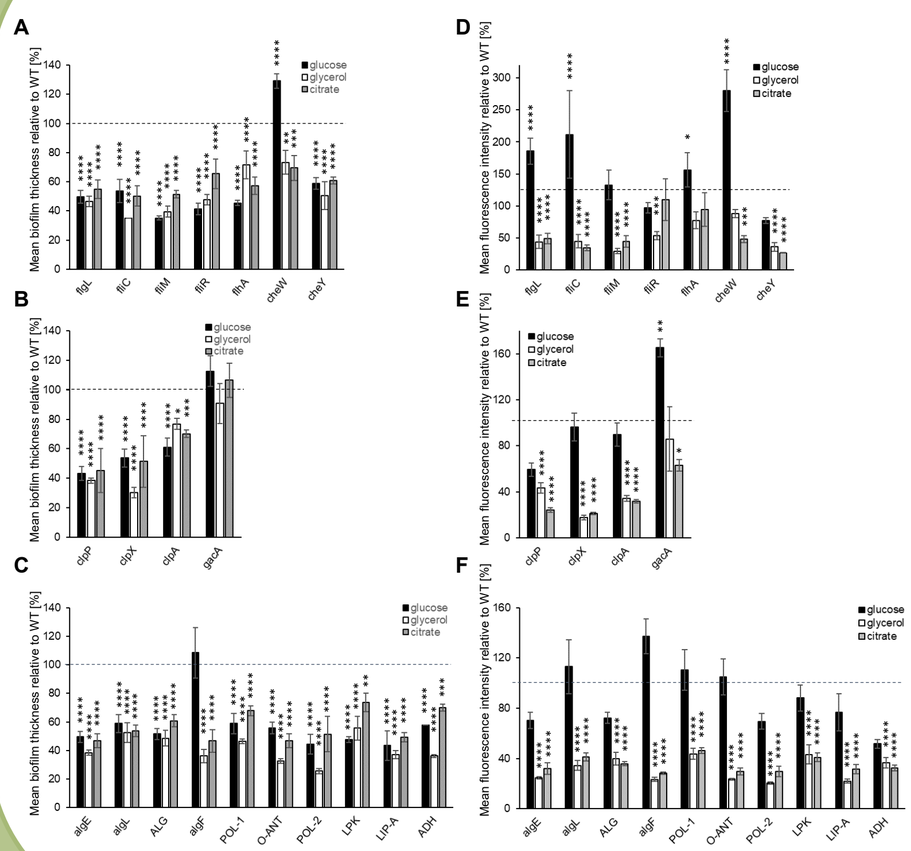
Function	Mutant's name	Gene annotation
motility/chemotaxis	*flgL*, *fliC*, *fliM*, *fliR*, *flhA*, *cheW*, *cheY*	*flgL* – flagellar hook, *fliC* – flagellin, *fliM* – flagellar motor switch, *fliR* – flagellar biosynthesis protein, *flhA* - flagellar biosynthesis protein, *cheW*, *cheY* – chemotaxis proteins
protease/regulatory	*clpP*, *clpX, clpA*, *gacA*	*clpP*, *clpX, clpA* – proteases, *gacA* – response regulator in the GacS/GacA system
adhesion/biofilm matrix	*algE*, *algL*, ALG, *algF*, POL-1, O-ANT, POL-2, LPS, LIP-A, ADH	*algE* – alginate production protein, *algL* – alginate lyase, alginate O-acetyl transferase, *algF* – alginate O-acetyl transferase, polysaccharide biosynthesis protein, O-antigen ligase family protein, polysaccharide biosynthesis/export family protein, lipopolysacharide kinase family protein, lipid-A-disaccharide synthase, adhesin

Type of abiotic surface impacts P482 biofilm structure and biomass



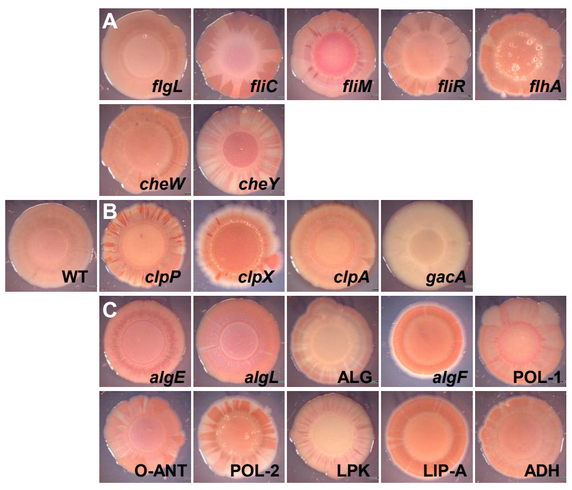
Biofilm formation by P482 WT strain on glass, in relation to carbon source. The biofilm formed on glass was most developed when glycerol was present in the medium. (A) Orthogonal and (B) 3D view of biofilm formed by GFP-tagged WT P482 strain on glass-bottom 24-well plates in M9 minimal medium with glucose, glycerol, or citrate. (C) Measurement of mean thickness of the biofilm formed by the GFP P482 strain. (D) Quantification of mean fluorescence intensity for the biofilm formed by the GFP P482. Bar in (A) = 200 µm.

Mutations in P482 genome affect the biofilm formation on glass



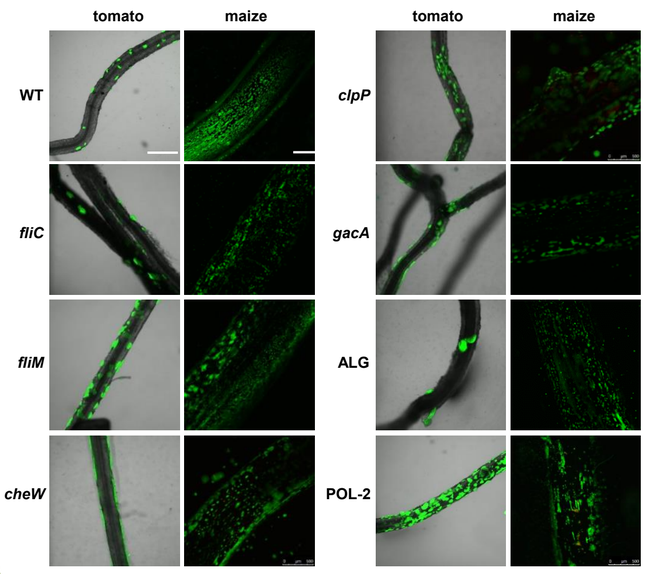
Measurements of biofilm thickness for P482 GFP-tagged mutants, relative to WT (A,B,C); quantification of mean fluorescence intensity for the biofilm formed by the GFP P482 mutants (D,E,F), in the M9 medium supplemented with the given carbon sources, for CLSM z-stack images collected for (A, D) knock-outs in motility and attachment-related genes, (B, E) knock-outs in proteases-encoding and *gacA* genes, and (C, F) knock-outs in matrix synthesis-related genes.

P482 mutants are affected in EPS-Congo Red binding



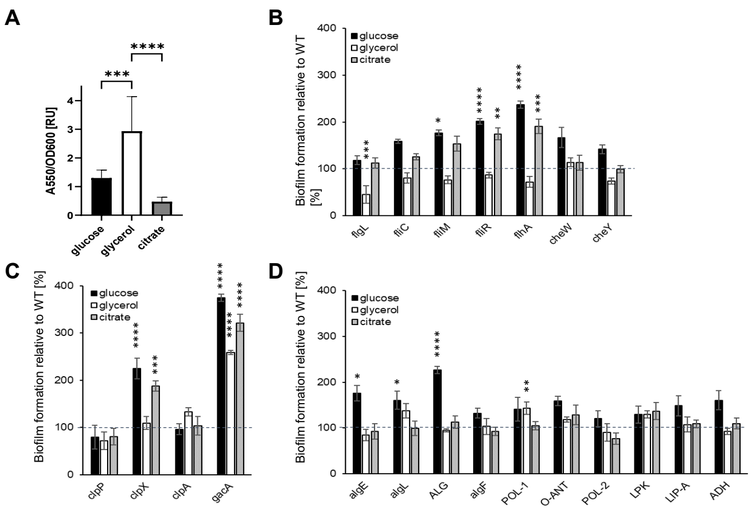
Images of Congo-red stained colonies of the P482 wild-type (WT) and mutant strains: (A) knock-outs in motility and attachment-related genes, (B) - knock-outs in proteases-encoding and *gacA* regulatory genes, (C) - knock-outs in matrix synthesis-related genes. The differences in the distribution of the dye in the colonies result from possible variations in levels of EPS production and dye binding in the P482 mutants (e.g. *gacA*, ALG, POL-2, LPK). The morphology of bacterial colonies of selected P482 mutants and their ability to bind the Congo-red dye was assessed on solid medium containing the dye. The images were obtained with a Leica MZ10 F stereomicroscope.

Colonization of plant host rhizosphere unaffected in P482 mutants



Example confocal fluorescence microscopy images of plant rhizosphere colonized by P482 GFP-tagged mutant strains. Panels on the left - roots of tomato (merged bright-field and GFP channels), colonized with the P482 strains, visualized 4 weeks post inoculation; panels on the right - P482-colonized maize roots (GFP channel), visualized 1 week post inoculation. Bars = 500 µm. None of the analyzed mutants was affected in plant root tissue colonization, despite observed deficiencies in biofilm formation ability on abiotic surface (glass).

Carbon source influences P482 biofilm formation on polystyrene



Biofilm formation by the P482 strains, WT (A) and mutants (B) - knock-outs in motility and attachment-related genes, (C) - in proteases-encoding and *gacA* regulatory genes, (D) - in matrix synthesis-related genes, was determined in crystal violet binding assay on polystyrene in M9 minimal medium supplemented with 22.2 mM glucose, 43.5 mM glycerol or 20 mM citrate. The assay was performed on 96-well microtiter plates, incubated at 28°C.

- The performed analyses showed that different carbon sources impact the ability of *P. donghuensis* P482 to form a biofilm, with glycerol promoting the process.
- The investigation of P482 mutants revealed that mutants incapable of forming biofilm on glass surface demonstrated a contrasting capability to fully colonize plant root tissues.
- Mutants were identified, unaffected in their biofilm formation, irrespective of the surface or carbon source used.
- These findings contribute to a deeper understanding of the subtle factors governing biofilm formation in P482, opening new opportunities to advance our comprehension of plant-microbe interactions.

Details available at: Rajewska et al., Int. J. Mol. Sci. 2024, 25(15), 8351; DOI: 10.3390/ijms25158351

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