The *in vitro* and *in planta* antagonism of *Pseudomonas* sp. strain P482 against bacterial phytopathogens of the genera *Pectobacterium* and *Dickeya*

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Pseudomonas sp. P482, a Gram-negative bacterium originating from the rhizosphere of tomato, is able to inhibit the growth of various species of *Pectobacterium* and *Dickeya* in a plate assay. *Pectobacterium* and *Dickeya* are plant-pathogenic bacteria causing soft rot disease of many vegetables and ornamental plants. Despite serious economic losses caused by *Pectiobacterium* and *Dickeya*, the means to fight these pathogens are limited.

To reveal the mechanisms involved in the antagonism between P482 and the soft rot pathogens, we have applied a genome mining approach in combination with site-specific mutagenesis. The genome sequence of *Pseudomonas* sp. P482 has been commercially obtained and automatically annotated. Chosen features of the genome have been analyzed and summarized. In particular, the genomic sequence has been subjected to data mining in search for secondary metabolite biosynthetic clusters. The search has been performed both manually, with the blastp tool, and automatically, with the antiSMASH 2.0 software. Five genes have been selected based on the *in silico* study, each representing one biosynthetic cluster predicted by the antiSMASH 2.0 as a result of a default search against its internal database. Knock out of these genes has shown that they are not responsible for the antimicrobial activity of P482 against the soft rot bacteria. A more unrestricted antiSMASH 2.0 search based on Pfam domain probabilities revealed 18 more clusters designated as "hypothetical". A transposon mutant of P482 studied in our laboratory, not inhibiting the growth of *Dickeya* spp. *in vitro*, carries an insertion in one of these "hypothetical" clusters. Thus, genome mining for secondary metabolite clusters is a powerful approach although when novel mechanism are at stake, as it seems to be in case of the P482, it should be used in combination with other methods

Next we have investigated whether the P482 strain can be applied as a biological control agent against soft rot. Tissue protection assays, involving co-inoculation of plant tissue with mixtures of bacterial strains, have been performed on potato tubers and the leaves of chicory heads. The test on potato tuber tissue has shown that, although P482 inhibits the growth of both *Dickeya* and *Pectobacterium* in a plate assay, the biocontrol effect is pathogen-specific: the P482 can reduce soft rot caused by most *Pectobacterium* species but not by *Dickeya* spp. Surprisingly, the P482 is very effective against *Dickeya* on chicory. In an attempt to reveal the cause of this plant tissue-specific effect against *Dickeya* we have found that, when grown in plant juices, the P482 does alkalize the potato juice but acidifies the chicory juice. The organic acid produced in chicory juice has been identified by NMR as gluconic acid and a mutant P482 strain unable to produce the compound has been constructed. Preliminary results indicated that gluconic acid is contributing to the biocontrol potential of P482 on chicory but is not solely responsible for it. Interestingly, the P482 transposon mutant unable to inhibit the growth of *Dickeya* spp. *in vitro* is not compromised in its ability to protect chicory leaves from maceration. This implies that a different mechanism is responsible for the antagonism of P482 against *Dickeya* spp. in the *in vitro* and the *in planta* conditions.

Additionally, the ability of *Pseudomonas* sp. P482 to colonize the roots of soil-grown potato plants has been investigated. The P482 can be described a moderate potato root colonizer. Thus, potato plant can be used as a host plant for studying the interactions of the P482 with the soft rot pathogens.