

Genome mining of the rhizosphere bacterium *Pseudomonas* sp. SH-C52

Menno van der Voort¹, Yvonne Schmidt², Jeramie Watrous³, Rodrigo Mendes^{1,4}, Harald Gross², Pieter C. Dorrestein³ & Jos M. Raaijmakers¹

1 Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

2 Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany

3 Departments of Pharmacology and Chemistry and Biochemistry, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA, USA

4 Brazilian Agricultural Research Corporation, Embrapa Environment, Jaguariuna, Brazil

Soil ecosystems represent an enormous untapped resource for discovering novel microorganisms, traits and bioactive genes. Natural disease suppressive soils are particularly interesting as they have a relatively higher abundance of beneficial microorganisms that guard plants against infections by soil-borne pathogens. By using both culture-independent and culture-dependent approaches, we recently discovered a novel group of *Pseudomonas* species in the rhizosphere of sugar beet seedlings grown in a soil that is suppressive to the fungal pathogen *Rhizoctonia solani*. Representative strain *Pseudomonas* sp. SH-C52 was shown to inhibit hyphal growth of *R. solani* and various other fungal and oomycete pathogens. Sequencing of *Pseudomonas* sp. SH-C52 revealed a genome size of 6.7 Mb with approximately 4% of the genome dedicated to secondary metabolism. *In silico* analysis of the genome sequence showed the presence of at least 3 large nonribosomal peptide synthetase (NRPS) gene clusters. The first NRPS cluster was predicted to encode for a 9-amino acid chlorinated lipopeptide, designated thanamycin. The partial structure of thanamycin, resolved by nanoDESI mass spectrometry, was consistent with the predicted structure. The antifungal activity of thanamycin was confirmed by mutagenesis of the biosynthesis genes and by *in vitro* bioassays with the purified compound. Activity of *Pseudomonas* sp. SH-C52 against oomycetes was shown to be mainly due to a second NRPS-encoded peptide, which is predicted to consist of a 22-amino acid peptide moiety. The third peptide, designated brabantamide, is a 2-amino acid peptide linked to a C14-glycosylated fatty acid. It is governed by a 12-kb cluster of genes encoding a NRPS, a glycosyltransferase, a monooxygenase, a transcriptional regulator and an RND-type efflux protein. Activity assays showed that this dipeptide has broad-spectrum antibacterial activities. Lipopeptides typically affect membrane integrity and disturb ion fluxes. For the dipeptide brabantamide, however, this mode of action seems unlikely as it appears to act on defined cellular structures in the targets.

In conclusion, this study provides new insights into the diverse secondary metabolism of rhizosphere bacteria and their role in natural disease suppressive soils.