CONTROL OF *POLYMYXA BETAE* BY *BACILLUS AMYLOLIQUEFACIENS* LIPOPEPTIDES

Nicolas Desoignies[1], Florence Schramme[1], Marc Ongena[2] and Anne Legrève[1]

[1] Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Phytopathology, L7.05.03 1348 Louvain-la-Neuve, Belgium
[2] Centre Wallon de Biologie Industrielle (CWBI), Unité de bio-industries, University of Liege/ Gembloux Agro-Bio Tech, Passage des déportés 2, B-5030 Gembloux, Belgium

Summary

Rhizomania is one of the most important diseases of sugar beet. It is caused by *Beet necrotic yellow vein virus* (BNYVV), which is transmitted by the protist *Polymyxa betae*. Until now, only a few resistance genes against BNYVV have been used in the control of the disease. Within the context of integrated pest management, the effect of molecules stimulating the host defence mechanism in *P. betae* infection was explored. Systemic acquired resistance (SAR) was induced by spraying the leaves with a synthetic analog of salicylic acid three times before inoculation with *P. betae* zoospores. Also before inoculation, induced systemic resistance (ISR) was elicited twice, with a 1-week interval, by watering the sugar beet roots with a *Bacillus amyloliquefaciens* lipopeptides solution. One week after inoculation, the plants were harvested and *P. betae* was quantified in the roots using a quantitative PCR assay. The ISR was controlled by assessing the expression of the *NPR1* gene and three PR genes. Both systemic resistances were induced in corresponding treated plants (SAR-salicylic acid analog; ISR-lipopeptides). Although no reduction in *P. betae* infection was observed in SAR-induced plants, *P. betae* infection decreased significantly for the two concentrations of zoospores from 10 times (5000 zoospores) to 16 times (500 zoospores) when the plants were treated with the bacterial lipopeptides. These results accord with the fact that *P. betae* induced the SAR. It therefore seems to be insensitive to the defences induced by SAR but not to those by ISR. This decrease in infection, due to ISR, might affect viral transmission and hence rhizomania pressure in fields. From an integrated pest management perspective, the decrease in disease pressure due to bacterial lipopeptides could help to preserve genetic resistance to the virus longer and provide a new way to manage *P. betae* inoculum potential in soils.

Introduction

The control of rhizomania in the field is still based mainly on sugar beet tolerance genes against *Beet necrotic yellow vein virus.* In the context of integrated pest management, the possibility of using partial genetic resistance from wild *Beta* species against the vector *Polymyxa betae*, as suggested by Barr *et al.* (1995) and Mc Grann *et al.* (2009), is an attractive proposition. The control of this vector would help to reduce both virus and vector titers in sugar beet roots and hence in soils. Taking a similar approach, we tested the possibility of controlling *P. betae* infection by inducing the systemic resistance in the plants. Plants have two “immune systemic reaction” strategies (Van Loon *et al.*, 1998). The first occurs when a pathogen attacks the plant. Recognition of the pathogen leads to a response in the tissues surrounding the pathogen, which can spread throughout the plant by means of a signal molecule, salicylic acid. This kind of response is called ‘systemic acquired resistance’ (SAR). The second type of response, quite similar, is induced by non-pathogenic organisms such as plant growth-promoting rhizobacteria. This resistance is mediated by jasmonate and ethylene, and is called ‘induced systemic resistance’ (ISR).

The events leading to these types of resistance can be mimicked by synthesis molecules, such as the analogues of salicylic acid. Recently, cyclic lipopeptides of rhizobacteria, such as non-pathogenic *Pseudomonas* sp. and *Bacillus* sp., have been shown to confer resistance against plant pathogens by inducing ISR (Tran *et al.*, 2007; Ongena *et al.*, 2007). In this study, the efficiency of the each type of immune response was assessed.

Materials and Methods

Two-week-old sugar beet plants were treated twice, at an interval of 1 week, with Acybenzolar-S-methyl (ASM), a functional analogue of salicylic acid. Other 3-week-old sugar beet plants were treated with cyclic lipopeptides from *Bacillus* sp. (30 mg/L), or with water as the control. The plants were then inoculated with two concentrations of *P. betae* zoospores (500 and 5000 zoospores) 1 week after the last treatment. At inoculation time, the roots and leaves of some treated and control plants were harvested. The expression of two genes, markers of the establishment of the immune response, was tested by RT-PCR in both the root and leaf samples in order to assess the induction of the systemic response.

One week after inoculation, the *P. betae* infection in the treated and control plants was quantified by a qPCR assay targeting the ITS region. This quantification was normalized with the quantification of sugar beet DNA, and compared using ANOVA-1.

In order to control the absence of direct action by the lipopeptides on the zoospores, the zoospores were put in contact with lipopeptide concentrations from 0 mg/l (control) to 120mg/l. for 3 hours and their viability was evaluated by quantitation of the ATP using a luminescent assay.

Results and Discussion

The assessment of gene expression implicated in the systemic resistance responses showed that the genes were expressed or overexpressed in treated plants, whereas in the controls they were expressed as negatives in RT-PCR or poorly positive. Each treatment (ASM or lipopeptides) induced a systemic response in the whole plant.

No significant difference in *P. betae* infection between the ASM-treated and untreated plants was detected for either of the tested concentrations of zoospores. In contrast, there was a significant decrease in *P. betae* infection in the lipopeptide-treated roots: this decrease reached 93% or 88%, depending on the level of zoospore concentration (Fig. 1). No difference in ATP quantitation between *P. betae* zoospores in lipopeptide solution or Hoagland solution was observed. The likelihood of direct action by the lipopeptides on the zoospores membranes can be discounted. These results indicate that the systemic response induced by the bacterial-produced lipopeptides greatly reduced the infection rates of sugar beet by *P. betae.*

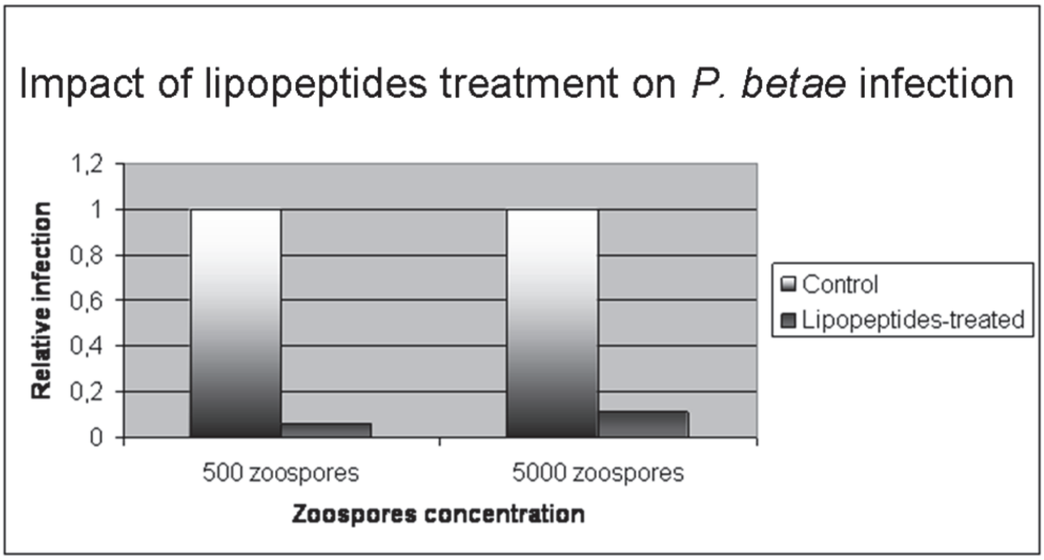

Figure 1. Comparison of *P. betae* infection in untreated plants (control) and lipopeptide-treated plants, 1 week after inoculation with two concentrations of zoospores

From these results it can be said that the systemic resistance induced by lipopeptides is active against *P. betae* under controlled conditions. In the context of integrated pest management, these results offer possible new ways of controlling rhizomania disease based on action against the virus vector, followed by a decrease in inoculum potential of the disease.

Acknowledgements

Nicolas Desoignies is a fellow of the Fonds de formation à la recherche en industrie et agriculture (FRIA). M. Ongena is Research Associate at the National Funds for Scientific Research

References

Barr, K.J., Asher, M.J., and Lewis, B.G. (1995). Resistance to *Polymyxa betae* in wild Beta species. *Plant Pathol.* 44, 301-307

McGrann, G.R.D., Townsend, B.J. , Antoniw, J.F., Asher, M.J.C., and Mutasa-Göttgens, E.S. (2009). Barley elicits a similar early basal defence response during host and non-host interactions with *Polymyxa* root parasites. *Eur. J. Plant Pathol.* 123, 5-15.

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Aripigny, J-L., and Thonart, P. (2007). Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol.* 9, 1084-1090

Tran, H., Ficke, A., Asiimwe, T., Höfte, M., and Raaijmakers, J.M. (2007). Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens. New Phytol.* 175, 731-742.

Van Loon, L.C., Bakker, P.A., and Pieterse, C.M. (1998). Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* 36, 453-483.