General introduction

It is estimated that the world's population will increase by 2.3 billion (or 34%) by 2050. Moreover, the average food consumption is also expected to rise from 2000 to 3070 kcal per person per day. To meet these demands, agricultural production has to be increased by 60% over the next 40 years (FAO, 2012). This can be achieved by the expansion of the amount of farmland and/or by increasing agricultural productivity. Expanding agricultural land is difficult because this possibility is limited by a number of important constraints such as competing with urban growth and scarcity of fresh water. Therefore, improvement of agricultural productivity will be the key approach for reducing the global food insecurity over the coming decades.

It is possible to increase agricultural productivity by stimulating plant yield and by protecting crops from phytopathogens. Commercial fertilizers and pesticides, which are commonly used for these practices, are dominated by synthetic products. However, because of growing concern about the negative impact of chemical fertilizers and pesticides on human and environmental health, farmers are encouraged to use more environmentally friendly alternatives [Directive 2009/128/EC, Regulation (EC) 1107/2009]. Biofertilizers and biopesticides may become the preferred substitutions for some conventional synthetic products. Since such biopreparations are based on non-pathogenic life microorganisms, they can substantially contribute to the sustainable production of environmentally friendly and low chemical residue products.

At present, the majority of the registered bacterial products in Europe is based on species of *Bacillus* and *Pseudomonas* (EU Pesticides Database, 2012). Members of both genera are predominant in soil and plant microenvironments, presumably due to their high growth rate and simple nutritional requirements. These species are widely known for their versatile metabolic activity and diverse beneficial effects on plant vigor and health. Moreover, their beneficial action can be expressed on a large range of plants which places these bacteria among the best candidates for the development of biopreparations. However, despite these positive characteristics, bacterial products can show some inconsistency between trials (Montesinos, 2003). This is assumed to be due to the short persistence of bacterial cells in the rhizosphere/soil environment and their susceptibility to unfavorable environmental conditions.

One possible way to overcome these drawbacks is to develop biopreparations based on beneficial *endophytic* bacteria. Since bacterial endophytes colonize the plant

interior, which is a stable and protected environment, their interaction with a plant can grow into a longer relationship. In addition to housing endophytic bacteria, plants provide them with nutrients and, in turn, some endophytes recompense their hosts by stimulating plant growth and suppressing phytopathogens. If, after production, such beneficial strains can be re-introduced into an endophytic stage, a sustainable and effective crop production system can be achieved.

Aims of the thesis

This Ph.D. thesis focuses on the isolation and characterization of novel beneficial endophytic bacteria with plant growth promotion and biocontrol abilities. Aims were as follows:

1. To isolate endophytic bacteria from different plants of agricultural and horticultural importance
2. To characterize potential plant-beneficial traits of the isolated endophytes
3. To test the most promising isolates for their ability to promote plant growth and to control plant disease, and
4. To characterize the endophytic lifestyle of selected strains.

Outline of the thesis

Chapter 1 contains a brief introduction to the main aims of the thesis. **Chapters 2**, **3** and **4** give a detailed overview of the three most relevant topics treated in this thesis. **Chapter 2** provides an introduction to endophytic bacteria with specific emphasis on how they enter a plant, live inside and contribute to plant health. Mechanisms of plant growth promotion and biocontrol which were found for endophytes in *in planta* studies are discussed in detail. This chapter ends with the evaluation of available genomic, metagenomic and postgenomic tools to get a deeper insight into plant-endophyte beneficial interactions. **Chapters 3** and **4** describe our knowledge of known mechanisms of plant growth promotion and biocontrol, respectively. In **Chapter 3**, examples are given of microbes which provide a plant with essential nutrients, secrete phytohormones and other plant growth promoting substances and increase plant resistance to abiotic stresses. **Chapter 4** describes biocontrol bacteria and their secondary metabolites involved in various biocontrol mechanisms.

In **Chapter 5**, the isolation of endophytic bacteria from different plants of agricultural and horticultural importance is described. The isolated endophytes were subsequently characterized with regard to their plant-beneficial traits and ability to

promote plant growth and control plant diseases. This resulted in the selection of a novel beneficial strain, namely *Bacillus subtilis* HC8 from giant hogweed. This strain is able to produce a wide range of bioactive compounds, a trait which probably contributes to the beneficial effect mediated by HC8. The secondary metabolites produced by *B. subtilis* HC8 include cyclic lipopeptides (c-LPs) which were further characterized in **Chapter 6** using liquid chromatography mass spectrometry (LC-MS) followed by *in vitro* bioactivity tests. Endophytic bacteria with biocontrol properties were also isolated and characterized as described in **Chapter 7**. Those isolated strains, which were identified as members of the *Pseudomonas* genus, were compared with rhizospheric pseudomonads with respect to their abilities to utilize various carbon sources. This resulted in identifying the carbon source L-arabinose as a nutrient which might be important for the endophytic lifestyle of *Pseudomonas* species.

Chapter 8 is a general discussion on the results obtained in this thesis in comparison with the literature. Moreover, additional information is provided on plant growth promotion, biocontrol and the endophytic lifestyle of some strains. Concluding remarks and future prospects complete this chapter. **In Chapter 9**, a summary is given of the major findings of the thesis, in both English and Dutch.

References

Directive 2009/128/EC. See http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:EN:PDF

European Union Pesticides Database. 2010. See (http://ec.europa.eu/food/plant/protection/evaluation/database_act_subs_en.htm). Accessed 25 August 2012.

Food and Agriculture Organization. 2012. See (http://www.keepeek.com/Digital-Asset-Management/oecd/agriculture-and-food/oecd-fao-agricultural-outlook-2012_agr_outlook-2012-en).

Montesinos E. 2003. Development, registration and commercialization of microbial pesticides for plant protection. Int. Microbiol. 6: 245–252.

Regulation (EC) 1107/2009. See http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF

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