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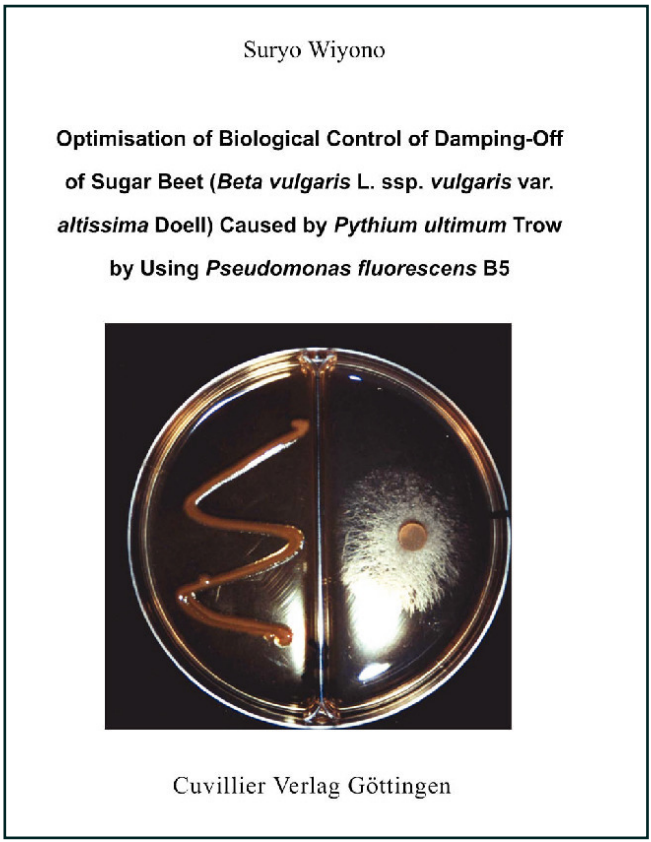
Optimisation of Biological Control of Damping-Off of Sugar Beet (Beta vulgaris L. ssp. vulgaris var. altissima Doell) Caused by Pythium ultimum Trow by Using Pseudomonas fluorescens B5



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I. INTRODUCTION

Sugar beet is one of the most important sugar-producing crops in the world, with a contribution of 40 % on world sugar production. Germany, with 415.000 ha and an average productivity of 54.68 ton/ha during the last decades is ranking on fourth position in sugar beet production in the world, next to USA, China and France (Wirtschaftliche Vereinigung Zucker-Verein der Zuckerindustrie, 2003).

Problems of pests and diseases substantially are limiting factors in sugar beet production. In Germany, damping-off is one of the most important soil borne diseases caused by a complex of pathogens and mainly by *Pythium ultimum* and *Aphanomyces cochlioides.* It is an important sugar beet disease in Germany since 1880'es (Koble, 1987). Damping-off contributes in substantial amount to yield losses due to fungal diseases, *e.g.* 16 % and 16.5 % in USA and worldwide respectively (James, 1982). Until now, disease management is mainly based on chemical control by applying fungicides which are incorporated into pelleted seeds. Other control strategies, such as the use of resistant varieties and other agronomical practices, do not provide effective control. However, decreasing of public acceptance of the use of chemicals and their environmental risks makes it important and relevant to develop novel biological control strategies by using microbial antagonist.

One of effective bacterial antagonists against *P. ultimum* is *Pseudomonas fluorescens* B5 (Pf B5) which had been studied intensively in the Institute of Plant Pathology and Plant Protection Georg-August University Germany (Heupel 1992, Maass, 1996; Schulz *et al.*, 1994; Schulz and Wolf, 2002). The antagonist showed a biocontrol efficacy of 44% against damping-off in sugar beet seedlings in controlled environment under high inoculum pressure of pathogen, and has also been proven effective in field experiments (Heupel, 1992).

Some important features associated with the antagonistic activity of *Ps. fluorescens* B5 have been characterized. Heupel (1992) described that under *in vitro* conditions, besides the production of cyanide (HCN) and the

ferric iron binding siderophores, hydrolytic enzymes such as glucanases, xylanases and amylases were detected in the culture filtrate of B5. Further investigations with transposon (Tn 5) mutants had been done to identify the antagonistic mechanisms of Pf B5 (Schulz *et al.*, 1994; Schulz and Wolf, 2002). The study showed that siderophore production plays a minor role in biocontrol in the system *Pythium ultimum* - sugar beet seedlings – *Ps. fluorescens B5*. Moreover, no strong correlation between adhesion to roots, motility and colonisation in the rhizosphere was observed. Even though all examined traits of Pf B5 *i.e.* production of antibiotics and siderophores, adhesion, motility and colonization ability contributed to the antagonistic activity of Pf B5, it was apparent that antibiosis is one of the most important mechanism of its biocontrol performance (Schulz and Wolf, 2002).

Application of mutants that overproduce antifungal metabolites is one alternative to improve the antagonistic activity of an antagonistic strain (Walsh *et al.,* 2001). For instance, biocontrol performance of *Ps. fluorescens* CHAO against *P. ultimum* in cotton seedlings could be improved by applying antibiotics overproducing mutants (Maurhofer *et al.*, 1992; Maurhofer *et al.,* 1995). Moreover, Delany *et al*. (2001) could found a similar effect using *Ps. fluorescens* F113 against *Pythium ultimum* in sugar beet by altering the production of 2,4-diacetylphloroglucinol. The possibility to enhance the biocontrol activity of *Ps. fluorescens* B5 by applying its antibiotic overproducing mutants was studied in this research.

It is well known that some biocontrol strains of the pseudomonads produce regulators of plant growth such as indole acetic acid (IAA) (Nautiyal *et al.*, 1997; Zhao, 2001; Bano and Mussarat, 2003), and therefore could act as plant growth-promoting rhizobacteria (PGPR) (Xie *et al.,* 1996; Barazani and Friedman, 1999; Asghar *et al.*, 2002). Studies on IAA production with fluorescent pseudomonads are mostly related to its role as growth regulator, excluding studies on its fungicidal activity.

Ps. fluorescens B5 produces IAA in a considerable amount *in vitro* and preliminary research showed that IAA inhibits also the growth of *P. ultimum in*

vitro. Indeed, the required concentration of IAA to suppress the disease *ad planta*, and the relation of IAA production *in vitro* and biocontrol activity *ad planta* are still unknown. Therefor the role of IAA in biocontrol and growth promotion by *Ps. fluorescens* B5 was investigated in this study.

Host properties are components often neglected in the development of biological control. In some studies, host varieties had no effect on the activity of antagonistic pseudomonads (Hebar *et al.,* 1998), while Smith and Goodman (1999) could find an affect of host plant genotypes on biocontrol efficiency. Regarding the application of *Ps. fluorescens* B5 in the system sugar beet – *Pythium ultimum*, the role of sugar beet varieties on biocontrol is poorly understood. But this aspect was found to be essential to optimise biocontrol activity of *Ps. fluorescens* B5 and hence was integrated into the studies.

An important step to establish biocontrol of plant disease with microbes is the development of appropriate formulation techniques. It plays a crucial role to guarantee success of the biocontrol strategy in the field. There are two approaches for the optimisation of an antagonist formulation *i.e.* the screening for appropriate carrier materials like wood–flour (Vidyasekaran, 1997; Imam Ali *et al.* 2001, Krishnamurthy and Gnanamanickam, 1998b), and formulation additives (Schmidt *et al.*, 2001). Wood flour-based materials seem to be suited for the formulation of fluorescent pseudomonads as seed pellet (Heupel, 1992; Tilcher, 2002). Since fluorescent pseudomonads do not produce spores, the development of appropriate carrier materials, which protect cells from desiccation and sustain long-lasting survival and antagonistic activity of microorganisms, is essential. For that reason, the study to find suitable pelleting materials was enforced.

Moreover, a further technique to improve an antagonist formulation is the use selective additives. Ideal formulation additives should provide the advantages for disease control in antagonist-pathogen-host plant system. This means, additives should improve the biocontrol efficacy of pseudomonads without enhancing the growth and activity of pathogens and

should not have detrimental effects on host plants. Studies on formulation additives of fluorescent pseudomonads are very limited. Some foregoing research indicated the importance of formulation additives for the use of *Bacillus mycoides* against *Cercospora beticola* under green house and field conditions by adding 1 % 1,3-ß-glucan (Kiewnick and Jacobsen, 1996). Moreover, addition of glycine into the seed pellet could improve rhizosphere colonization of *Ps. fluorescens* B5, and calcium gluconate increased the efficacy of B5 against *P. ultimum* in sugar beet seedlings (Schulz and Wolf, 1998).

With the exception of Schulz and Wolf (1998), who incorporated the addities directly into the seed pellet, nutrients were applied as fertilizer or as soil applications in most of the studies to improve activity of fluorescent pseudomonads. For instance, soil applications of nitrogen fertilizers containing mixtures of NO_3^- and NH_4^+ enhanced the capacity of *Pseudomonas fluorescens* strains to promote plant growth and to inhibit *Fusarium* growth on rye (Kurek and Jaroszuk-Sciseł, 2003). Glucose and zinc applied into the soil were reported to enhance the biocontrol ability of *Pseudomonas fluorescens* CHAO and *Pseudomonas aeruginosa* IE-6S+, both *in vitro* and *ad planta* against *Macrophomina phaseolina* in soybean (Shaukat and Siddiqui, 2003). Moreover, Hamid *et al.* (2003) stated that soil application of ammonium molybdate mediated the enhancement of biocontrol efficacy of *Ps. fluorescens* CHAO against the root knot nematode *Meloidogyne javanica* in soybean. On the contrary, information on the use of nitrogen compounds and trace elements as formulation additives to improve biocontrol performance of fluorescent pseudomonads is very limited or even not available. Therefore, in the last part of this work the possibility of the use of nitrogenous compounds and trace elements as formulation additives was examined.

Based on the above reasons, a series of experiments was done to achieve the following objectives:

1. To optimise biological control activity of *Ps. fluorescens* B5 against *Pythium ultimum* in sugar beet by means of mutants overproducing antifungal metabolites.
2. To study the role of indole-3-acetic acid (IAA) in the biological control activity of *Ps. fluorescens B5* and its growth promoting effect on sugar beet seedlings.
3. To study the role of sugar beet varieties in antagonistic activity of *Ps. fluorescens* B5 against *P. ultimum*.
4. To optimise biological control activity of *Ps. fluorescens* B5 by improving formulation techniques through a) appropriate pelleting materials which support long term survival and antagonistic activity of B5 and, b) formulation additives from nitrogen sources and trace elements which enhance biocontrol activity of *Ps. fluorescens* B5 against *P. ultimum*.