

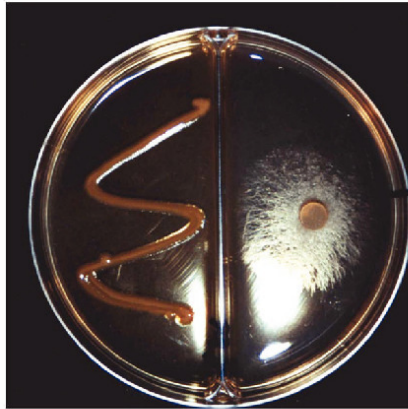


Suryo Wiyono (Autor)

**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**

Suryo Wiyono

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



Cuvillier Verlag Göttingen

<https://cuvillier.de/de/shop/publications/3073>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany

Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>

## LIST OF CONTENTS

	Title	Page
1.	<b>INTRODUCTION</b>	1
2.	<b>MATERIALS AND METHODS</b>	6
2.1.	<b>Location and time</b>	6
2.2.	<b>Materials</b>	
2.2.1.	<b>Chemicals</b>	6
2.2.2.	<b>Media</b>	7
2.2.3.	<b>Organisms</b>	11
2.3.	<b>Methods</b>	12
2.3.1.	<b>Production and testing of antifungal metabolites-overproducing mutants</b>	11
2.3.1.1.	Strain maintenance	11
2.3.1.2.	Production antifungal metabolite-overproducing mutants	12
2.3.1.3.	Screening for antifungal metabolites-overproducing mutants	13
2.3.1.4.	Characterisation of mutants <i>in vitro</i>	14
2.3.1.4.1.	Antifungal activity of culture filtrate produced by mutants	14
2.3.1.4.2.	Growth	14
2.3.1.4.3.	Bioassay of volatile antifungal metabolites	15
2.3.1.4.4.	HCN production	16
2.3.1.4.5.	IAA production	16
2.3.1.4.6.	Motility	17
2.3.1.5.	Root Colonisation	18
2.3.1.6.	Efficacy of antifungal metabolite- overproducing mutants <i>ad planta</i>	19
2.3.2.	<b>The role of indole-3-acetic acid (IAA) in the antagonistic activity of <i>Ps. fluorescens</i> B5</b>	20
2.3.2.1.	Relation between IAA production of mutants <i>in vitro</i> and its efficacy <i>ad planta</i>	20
2.3.2.2.	Antifungal activity of IAA and other indole substances against <i>P. ultimum in vitro</i>	20
2.3.2.3.	Effect of IAA and other indole substances and its combination with Pf B5 in suppressing <i>P. ultimum ad planta</i>	21
2.3.2.4.	Bioassay of the effect of mutants on the root growth of sugar beet seedlings <i>in vitro</i>	21

2.3.2.5.	Effect of IAA and <i>Ps. fluorescens</i> B5 on the growth of sugar beet seedlings	22
<b>2.3.3.</b>	<b>The role of sugar beet varieties in biological control of <i>P. ultimum</i> by using <i>Ps. fluorescens</i> B5</b>	<b>23</b>
2.3.3.1.	Antagonistic activity of <i>Ps. fluorescens</i> B5 in different sugar beet varieties <i>ad planta</i> under controlled environment	23
2.3.3.2.	Antagonistic activity of <i>Ps. fluorescens</i> B5 in different sugar beet varieties <i>ad planta</i> with two level of pathogen inoculums under controlled environment	23
2.3.3.3.	Root Colonisation	23
2.3.3.4.	Root Adherence	24
<b>2.3.4.</b>	<b>Optimisation of antagonistic activity of <i>Ps. fluorescens</i> B5 by improving formulation technique</b>	<b>24</b>
2.3.4.1.	Screening for appropriate pelleting materials	24
2.3.4.1.1.	Growth of Pf B5 in different materials	24
2.3.4.1.2.	Survival of <i>Ps. fluorescens</i> B 5 in different materials	25
2.3.4.1.3.	Antagonistic activity <i>in vitro</i> of <i>Ps. fluorescens</i> B5 after stored in different Materials	26
2.3.4.2.	Screening additives for formulation improvement of <i>Pseudomonas fluorescens</i> B5	26
2.3.4.2.1.	Screening for nitrogen sources and trace elements as formulation additives <i>in vitro</i>	26
2.3.4.2.2.	Screening for nitrogen sources and trace elements as formulation additives <i>ad planta</i>	27
2.3.5.	Statistical analysis	28
<b>3.</b>	<b>RESULTS</b>	<b>29</b>
<b>3.1.</b>	<b>The use of antifungal-metabolites overproducing mutants to enhance antagonistic activity of <i>Ps. fluorescens</i> B5</b>	<b>29</b>
3.1.1	Production and screening of antifungal-metabolites overproducing mutants	29
3.1.2	Characterisation of antifungal metabolites-overproducing mutants	31
3.1.2.1.	Antifungal activity of culture filtrate <i>in vitro</i>	31
3.1.2.2.	Growth	34
3.1.2.3.	Bioassay of volatile antifungal substances.	35
3.1.2.4	HCN production	37
3.1.2.5	IAA production	37
3.1.2.6	Motility	39
3.1.3	Root Colonisation	41

3.1.4	Antagonistic activity of mutants <i>ad planta</i>	42
3.1.5.	Relationship of <i>in vitro</i> physiological traits and their efficacy <i>ad planta</i> of tested mutants	43
<b>3.2.</b>	<b>The role of indole -3- acetic acid (IAA) in the biocontrol activity of <i>Ps. fluorescens</i> B5 against damping- off of sugar beet seedlings caused by <i>P. ultimum</i></b>	46
3.2.1.	Correlation between IAA production <i>in vitro</i> and antagonistic activity <i>ad planta</i> of tested mutants	46
3.2.2.	Antifungal activity of IAA <i>in vitro</i>	46
3.2.3.	Antifungal activity of IAA <i>ad planta</i>	48
3.2.4.	Effect of IAA on the seedling's emergence and growth	50
3.2.5.	Effect of antifungal metabolites-overproducing mutants the seedling's emergence and growth	52
3.2.6.	Correlation of some physiological features of mutants <i>in vitro</i> and the growth of treated seedlings <i>ad planta</i>	54
<b>3.3.</b>	<b>The role of sugar beet varieties in biological control of <i>P. ultimum</i> by using <i>Ps. fluorescens</i> B5</b>	55
3.3.1.	Efficacy of <i>Ps. fluorescens</i> B 5 against <i>Pythium ultimum</i> <i>ad planta</i> in different sugar beet varieties under controlled environment	55
3.3.2.	Efficacy of <i>Ps. fluorescens</i> B 5 against <i>Pythium ultimum</i> <i>ad planta</i> in different sugar beet varieties with two levels of pathogen inoculum and controlled environment	58
3.3.3.	Root Colonisation	59
3.3.4.	Root Adherence	61
3.3.5.	Relation of antagonistic activity <i>ad planta</i> of <i>Pseudomonas fluorescens</i> B 5, colonisation and adherence on different sugar beet varieties	61
<b>3.4.</b>	<b>Optimisation of antagonistic activity of <i>Ps. fluorescens</i> B5 by improving formulation technique</b>	62
3.4.1.	Effect of pelleting materials on the growth, adhesion survival and antagonistic activity of Pf B5	62

3.4.1.1.	Growth of <i>Ps. fluorescens</i> B5 in different materials	62
3.4.1.2	Adhesion of <i>Pseudomonas fluorescens</i> B5 in different pelleting materials	62
3.4.1.2.	Survival <i>Ps. fluorescens</i> B5 in different materials	65
3.4.1.3.	Antagonistic activity of <i>Ps. fluorescens</i> B5 <i>in vitro</i> after storage in different materials	65
3.4.2.	Effect of nitrogen compounds and trace elements as additives in the formulation of <i>Ps. fluorescens</i> B5	66
3.4.2.1.	Effect of an addition of nitrogen compounds and trace elements on the <i>in vitro</i> production of antifungal substances by <i>Ps. fluorescens</i> B5	66
3.4.2.2.	Effect of an addition of nitrogen compounds and trace elements on the growth of <i>Ps. fluorescens</i> B5 <i>in vitro</i>	68
3.4.2.3.	Effect of trace elements and nitrogen compounds as formulation additives on the antagonistic activity of <i>Ps. fluorescens</i> B5 <i>ad planta</i>	70
3.4.2.4	Direct effect of trace elements incorporated into pelleted seeds as formulation additives on emergence and growth of sugar beet seedlings	72
<b>4.</b>	<b>DISCUSSIONS</b>	<b>75</b>
4.1.	The use of antifungal metabolites- overproducing mutants to enhance antagonistic activity of <i>Ps. fluorescens</i> B5	76
4.2.	The role of IAA in biocontrol activity of <i>Ps. fluorescens</i> B5 against <i>Pythium ultimum</i> of sugar beet	81
4.3.	The role of sugar beet varieties in the antagonistic activity of <i>Ps. fluorescens</i> B5 against <i>P. ultimum</i>	83
4.4.	Optimisation of biocontrol efficacy of <i>Ps. fluorescens</i> B5 by improving formulation technique	86
4.4.1	Effect of pelleting materials on the growth, survival and antagonistic activity of <i>Ps. fluorescens</i> B5	87
4.4.2.	Effect of formulation additives on the antagonistic activity of <i>Ps. fluorescens</i> B5	89
<b>5.</b>	<b>OUTLOOK</b>	<b>95</b>
<b>6.</b>	<b>SUMMARY</b>	<b>96</b>
<b>7.</b>	<b>REFERENCES</b>	<b>99</b>
	<b>AUTHOR BIOGRAPHY</b>	