



Michael Meyer (Autor)

# Tomato Subtilase 3: Propeptide-Mediated Maturation and Function During Insect Resistance



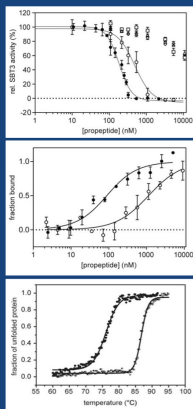
UNIVERSITÄT HOHENHEIM  
SCHRIFTENREIHE ZUR PHYSIOLOGIE UND  
BIOTECHNOLOGIE DER PFLANZEN



Michael Meyer

**Tomato Subtilase 3: Propeptide-Mediated Maturation  
and Function During Insect Resistance**

A. Schaller (Herausgeber) - Band 8



Cuvillier Verlag Göttingen  
Internationaler wissenschaftlicher Fachverlag

<https://cuvillier.de/de/shop/publications/7473>

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>



---

# CONTENT

LIST OF FIGURES .....	iv
LIST OF TABLES .....	iv
SUMMARY .....	v
ZUSAMMENFASSUNG.....	vii
1 INTRODUCTION.....	1
1.1 Wound Signaling in Plants .....	1
1.2 Cell-Wall Modification by Pectin Methylesterases .....	2
1.3 Subtilases in Defense Responses .....	4
1.4 Function and Maturation of Subtilases .....	5
1.5 Aims of the Present Work .....	8
2 RESULTS.....	11
2.1 Functional Characterization of Propeptides in Plant Subtilases as Intramolecular Chaperones and Inhibitors of the Mature Protease .....	11
2.1.1 Abstract .....	13
2.1.2 Introduction.....	14
2.1.3 Experimental Procedures .....	17
2.1.3.1 Cloning of Subtilase Propeptides .....	17
2.1.3.2 Expression and Purification of Recombinant Propeptides .....	17
2.1.3.3 Purification of SBT3 and Activity Assay.....	18
2.1.3.4 Propeptide Binding Assays.....	18
2.1.3.5 Circular Dichroism (CD) Spectroscopy .....	19
2.1.3.6 Fluorescence Spectroscopy .....	19
2.1.3.7 Thermal Stability of SBT, SBT3PP and the SBT3/SBT3PP complex	20
2.1.3.8 Identification of the Internal Propeptide Cleavage Site.....	20
2.1.3.9 Transient Protein Expression in <i>N. benthamiana</i> .....	21
2.1.3.10 Protein Extraction from <i>N. benthamiana</i> Leaves .....	21
2.1.3.11 Analysis of SBT3 Substrate Specificity.....	21



2.1.4	Results .....	23
2.1.4.1	Secondary Structure of the SBT3 Propeptide.....	23
2.1.4.2	Requirement of SBT3PP and Related Plant PPs for Folding and Secretion .....	28
2.1.4.3	Ability of SBT3PP and Related Plant Propeptides to Associate with SBT3 in an Auto-Inhibited Complex.....	32
2.1.4.4	Folding Assistance by SBT3PP and its Inhibitory Activity are not Necessarily Linked .....	35
2.1.4.5	Propeptide Cleavage and Release of Mature SBT3.....	37
2.1.5	Discussion .....	40
2.2	The Subtilisin-like Protease SBT3 Contributes to Insect Resistance in Tomato .....	43
2.2.1	Abstract .....	45
2.2.2	Introduction.....	46
2.2.3	Materials and Methods .....	49
2.2.3.1	Growth of Tomato Plants, Wounding and Insect Bioassays.....	49
2.2.3.2	Transgenic Tomato Plants.....	49
2.2.3.3	RNA Extraction and Quantitative Reverse Transcription (RT)-PCR Analysis .....	50
2.2.3.4	Northern and Southern Blot Analysis .....	51
2.2.3.5	Alkalinization Assay for Systemin Activity .....	52
2.2.3.6	Proteinase Inhibitor Assay.....	52
2.2.3.7	Stability of SBT3 in <i>M. sexta</i> Frass.....	53
2.2.3.8	Zymography .....	53
2.2.3.9	PME Activity Assay .....	53
2.2.3.10	In-gel Assay of PME Activity.....	54
2.2.3.11	Analysis of Cell Wall Composition .....	54
2.2.4	Results and Discussion .....	56
2.2.4.1	Expression of <i>SBT3</i> is Induced by Wounding and Insect Herbivory..	56
2.2.4.2	Insect Resistance is Reduced in Plants Silenced for <i>SBT3</i> Expression .....	59
2.2.4.3	Systemin Processing.....	60
2.2.4.4	Pectin Methyltransferase Activity and Cell Wall Composition.....	65
2.2.4.5	Post-ingestive Activity of SBT3.....	68



## Content

---

2.2.5	Conclusions .....	73
3	DISCUSSION .....	75
4	PUBLICATION BIBLIOGRAPHY .....	83
5	APPENDIX .....	101
5.1	Functional Characterization of Propeptides in Plant Subtilases as Intramolecular Chaperones and Inhibitors of the Mature Protease .....	101
5.1.1	Supplemental Material .....	101
5.2	The Subtilisin-like Protease SBT3 Contributes to Insect Resistance in Tomato .....	102
5.2.1	Supplemental Material .....	102
6	DANKSAGUNG .....	109
7	ERKLÄRUNG .....	111
8	LEBENS LAUF .....	113