



Universiteit
Leiden
The Netherlands

Towards a structural understanding of plant-microbiota interactions using cryo-EM techniques

Liedtke, J.

Citation

Liedtke, J. (2025, December 4). *Towards a structural understanding of plant-microbiota interactions using cryo-EM techniques*. Retrieved from <https://hdl.handle.net/1887/4284406>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4284406>

Note: To cite this publication please use the final published version (if applicable).

**Towards a Structural Understanding
of Plant–Microbiota Interactions
using cryo-EM Techniques**

Janine Liedtke

PhD. Thesis, Leiden University, 2025

Towards a Structural Understanding of Plant–Microbiota Interactions using cryo-EM Techniques

ISBN 978-94-6496-461-5

Printed by: Gildeprint, gildeprint.nl

Cover design: Lucia Berti, orangethetiger.com

Layout: Janine Liedtke

Copyright © 2025 by J. Liedtke. All rights reserved.

An electronic version of this dissertation is available at
<http://openaccess.leidenuniv.nl>.

Towards a Structural Understanding of Plant–Microbiota Interactions using cryo-EM Techniques

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op donderdag 4 december 2025
klokke 10:00 uur

door

Janine Liedtke

geboren te Berlin, Duitsland.

Promotores

Prof. dr. A. Briegel

Prof. dr. G. P. van Wezel

Promotiecommissie:

Prof. dr. A. H. Meijer

Prof. dr. D. Claessen

Dr. J. Willemse

Prof. dr. C. M. J. Pieterse

Dr. S. C. Howes

Utrecht University

Utrecht University

... für Papa

*Denn die einen sind im Dunkeln
Und die anderen sind im Licht.
Und man siehet die im Lichte
Die im Dunkeln sieht man nicht.*

Brecht

CONTENTS

1	General Introduction and Thesis Outline	1
2	How advances in cryo-electron tomography have contributed to our current view of bacterial cell biology	7
2.1	Introduction	8
2.2	Cryo-ET automation exemplified insight into bacterial cytoskeleton	9
2.3	CEMOVIS as a method to visualize cell envelope layers	12
2.4	Advances in sample thinning for cryo-ET	13
2.5	Cryo-CLEM is a method that provides the distinction of compartments of a structure	15
2.6	Subtomogram averaging	17
2.7	The future of cryo-ET	20
3	Morphological plasticity of <i>Chitinophaga pinensis</i> and its role in plant-microbiome interactions	23
3.1	Introduction	24
3.2	Material & Methods	25
3.2.1	Strains and culturing conditions	25
3.2.2	Quorum sensing assay	26
3.2.3	Motility assay	26
3.2.4	Fluorescence microscopy	27
3.2.5	Cryo-electron microscopy	27
3.2.6	Cryo-electron tomography	27
3.3	Results	28
3.3.1	<i>Chitinophaga pinensis</i> shows two distinct cell morphologies	28
3.3.2	Resistance profiles of cell morphologies to environmental and chemical stressors	28
3.3.3	Development of a transformation protocol and expression of GFP .	29
3.3.4	Transcriptomic analysis of 20 h and 40 h cultures	29
3.3.5	Morphological variation under different culture conditions.	30
3.3.6	<i>C. pinensis</i> spherical cells are dispersed by <i>B. subtilis</i>	31
3.4	Discussion	35
3.4.1	Conclusion	37
3.5	Supplementary Figures	39
3.6	Supplementary Methods	44
3.6.1	Physical stressor assay	44
3.6.2	Chemical stressor assay	44
3.6.3	Stress-reaction test	45
3.6.4	Transcriptomics and data analysis	45

3.6.5	Expression of green fluorescent protein (GFP)	46
4	Optimizing cryo-EM Sample Preparation for High-Resolution Imaging of the Ultrastructure of AMF Hyphae	47
4.1	Introduction	48
4.2	Material & Methods	49
4.2.1	Sample and sample preparation	49
4.2.2	Fluorescence staining of AMF hyphae network and spores	50
4.2.3	Vitrification procedure	51
4.2.4	Milling of AMF waffle grids & lamella preparation	52
4.2.5	Milling of AMF planchettes & cryo-volume imaging	53
4.2.6	Serial FIB-SEM Volume Imaging & Volume Lift-Out	53
4.2.7	Lamellae Preparation from Volume Samples	54
4.2.8	cryo-EM data acquisition & processing	54
4.2.9	cryo-volume SEM data processing	54
4.3	Results	55
4.3.1	Sampling techniques for AMF hyphal network and spores	55
4.3.2	Improved detectability through fluorescence staining	57
4.3.3	Vitrification methods for AMF samples	57
4.3.4	Challenges during milling and serial-SEM imaging	58
4.3.5	Challenges in AMF hyphal ultrastructure visualization	59
4.4	Discussion	64
5	Large-volume sample preparation of plant-tissue for cryo-electron microscopy	67
5.1	Introduction	68
5.2	Material & Method	69
5.2.1	Sample types	69
5.2.2	3D printed biopsy needles	70
5.2.3	Histological and fluorescence staining	71
5.2.4	High-pressure freezing	71
5.2.5	Preliminary Sample Processing: Volume Trimming	72
5.2.6	Milling of plant planchettes, volume lift-out & imaging	72
5.3	Results	73
5.3.1	Application of biopsy needles	73
5.3.2	Staining enhances traceability of plant tissue	74
5.3.3	Initial volume reduction	75
5.3.4	Volume sample preparation	75
5.4	Discussion	80
6	Distribution of temperate bacteriophages among endophytic Bacteroidota and their relevance	85
6.1	Introduction	86
6.2	Material & Methods	87
6.2.1	Bacterial strains and culture conditions	87
6.2.2	Prediction of prophage elements	87
6.2.3	Prophage induction assays	87

6.2.4	Screening for lytic phage activity.	88
6.2.5	Transmission electron microscopy	89
6.3	Results	89
6.3.1	Detection of active prophages regions among Bacteroidota strains .	89
6.3.2	Host range of extracts with lytic activity	91
6.3.3	TEM-based identification of phage-like structures	91
6.4	Discussion	93
7	Summary & General Discussion	97
	Samenvatting	107
	Zusammenfassung	111
	References	115
	Curriculum Vitæ	131
	List of Publications	133