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Lipopolysaccharide of plant-associated bacteria and their role in innate immunity of *Arabidopsis thaliana*

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Abstract

Lipopolysaccharide (LPS), a glycolipid of unique molecular composition, is the main component of the outer membrane of Gram-negative bacteria. It protects the bacterial cell from external stresses and is involved in various interaction processes between the bacteria and their environment. LPS can be divided into three substructures - the lipid A moiety, the core oligosaccharide (core-OS) and the O-polysaccharide (OPS) - which fulfill different functions and thus contribute to the properties of the molecule. The role of LPS during plant-bacteria interactions, however, remains unclear and genetic information about LPS synthesis in plant-associated bacteria is largely missing. As part of this thesis, genomes of *Pseudomonas* spp. were analysed for genes involved in LPS synthesis using *Pseudomonas aeruginosa* as reference. While homologs of most of the lipid A and core-OS synthesis genes could be identified, OPS gene clusters were not conserved in most plant-associated *Pseudomonas* strains. However, single homologs of genes involved in OPS synthesis were found. Gene homologs of the glycosyltransferase WbpL which initiates OPS synthesis in *P. aeruginosa* are present in all analyzed strains. Deletion of the respective gene in the plant pathogens *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) and *Pseudomonas cichorii* ATCC10857/DSM50259 disrupts OPS synthesis and leads to the production of OPS-deficient LPS. These Δ*wbpL* knockout strains display a reduced motility and are impaired in their ability to infect host plants. This indicates that OPS contributes to host colonization processes and therefore is an important virulence factor of plant-pathogenic *Pseudomonas* species. Elucidation of the *Pst* core-OS revealed a relatively high degree of phosphorylation. Together with the conservation of core-OS kinases in the analyzed *Pseudomonas* genomes the results suggest that a highly phosphorylated core-OS might be a common feature of the genus *Pseudomonas*.

LPS is considered to be a microbe-associated molecular pattern (MAMP) which is perceived by the plant immune system. In *A. thaliana*, immune responses after the treatment with LPS preparations from *Pseudomonas* spp. and *Xanthomonas* spp. require the receptor-like kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE). Screens with various LPS preparations and structurally similar compounds were conducted to identify the minimal MAMP-active motif of LPS. The results show that not LPS, but free medium chain 3-hydroxy (mc-3-OH) fatty acids are sensed by *A. thaliana* in a LORE-dependent manner. The strongest immune responses were elicited by 3-hydroxy decanoic acid (3-OH-C10). Bacterial compounds which comprise 3-OH-C10-acyl moieties with blocked 3-OH- or COOH- group such as LPS, rhamnolipids, lipopeptides, and N-acyl-homoserine-lactones are not inducing LORE-dependent immune responses. However, free mc-3-OH fatty acids could be released during synthesis of these compound or in other metabolic processes. The data therefore suggests *A. thaliana* senses microbial metabolites rather than complex bacterial compounds to trigger LORE-dependent immune responses.

Zusammenfassung

Lipopolysaccharid (LPS) ist der Hauptbestandteil der äußeren Membran von gramnegativen Bakterien. Das Glykolipid kann in die drei Substrukturen Lipid A, Kernoligosaccharid (Kern-OS) und O-Polysaccharid (OPS) unterteilt werden, welche durch ihre unterschiedliche Zusammensetzung zu der Funktion von LPS beitragen. Es schützt die Bakterienzelle vor äußeren Einflüssen und ist wichtig für Wechselwirkungen zwischen den Bakterien und ihrer Umgebung. Die Rolle von LPS in der Interaktion zwischen Pflanzen und Bakterien ist weitgehend unerforscht. Zudem ist wenig über die LPS-Synthese in pflanzenassoziierten Bakterien bekannt. Im Rahmen dieser Arbeit wurden deshalb Genome von *Pseudomonas* spp. mit *Pseudomonas aeruginosa* als Referenz auf Gene der LPS-Synthese untersucht. Während Homologe der meisten Lipid-A- und Kern-OS-Synthesegene identifiziert werden konnten, waren die OPS-Gencluster von *P. aeruginosa* in den meisten pflanzenassoziierten *Pseudomonas*-Stämmen nicht konserviert. Es wurden jedoch einzelne Homologe von Genen gefunden, die möglicherweise an der OPS-Synthese beteiligt sind. Genhomologe der Glykosyltransferase WbpL, welche die OPS-Synthese in *P. aeruginosa* initiiert, sind in allen analysierten Stämmen vorhanden. Die Deletion des entprechenden Gens in den Pflanzenpathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) und *Pseudomonas cichorii* ATCC10857/DSM50259 unterbricht die OPS Synthese und führt zur Produktion von OPS-freiem LPS. Die Motilität dieser Δ*wbpL* Stämme ist stark reduziert und ihre Fähigkeit Wirtspflanzen zu infizieren ist eingeschränkt. Diese Ergebnisse deuten darauf hin, dass OPS essentiell für die Kolonalisierung des Wirtes ist und deshalb einen Virulenzfaktor pflanzenpathogener *Pseudomonas*-Spezies darstellt. Die Strukturanalyse des Kern-OS von *Pst* zeigt eine vergleichsweise hohe Phosphorylierung. Dieses Ergebnis und die putative Konservierung von Kern-OS Kinasen in den analysierten *Pseudomonas*-Genomen legen nahe, dass diese Besonderheit möglicherweise charakterisisch für das Genus ist.

LPS gilt als mikroben-assoziiertes molekulares Muster (MAMP), welches vom pflanzlichen Immunsystem erkannt werden kann. Die Behandlung von *Arabidopsis thaliana* mit LPS-Präparaten aus *Pseudomonas* spp. und *Xanthomonas* spp. löst Immunreaktionen aus welche abhängig von der rezeptorähnlichen Kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) sind. Im Rahmen dieser Arbeit wurden Experimente mit verschiedenen LPS-Präparaten und strukturell ähnlichen Verbindungen durchgeführt, um das minimale MAMP-aktive Motiv von LPS zu identifizieren. Die Ergebnisse zeigen jedoch, dass LORE nicht an der Erkennung von LPS, sondern von freien, mittellangen 3-Hydroxy (3-OH) Fettsäuren beteiligt ist. Die stärksten Immunreaktionen werden durch 3-Hydroxydekansäure (3-OH-C10) ausgelöst. Bakterielle Verbindungen wie LPS, Rhamnolipide, Lipopeptide und N-Acyl-Homoserinlactone enthalten 3-OH-C10-Acyl Einheiten mit blockierter 3-OH- oder COOH-Gruppe und werden dementsprechen nicht von LORE perzipiert. Diese Ergebnisse zeigen, dass *A. thaliana*, anstatt einer komplexen bakeriellen Verbindung, ein einfaches bakterielles Metabolit mittels LORE erkennt und eine Immunreaktion auslöst.

List of publications

The following peer-reviewed publications are included in this thesis:

I Kutschera, A., & Ranf, S. (2019). The multifaceted functions of lipopolysaccharide in plant-bacteria interactions. *Biochimie*, 159:93–98.

II Kutschera, A., Schombel, U., Wröbel, M., Gisch, N., Ranf, S. (2019). Loss of *wbpL* disrupts O-polysaccharide synthesis and impairs virulence of plant-associated *Pseudomonas* strains. *Molecular Plant Pathology*, 20:1535-1549.

III Beaton, A., Lood, C., Cunningham-Oakes, E., MacFadyen, A., Mullins, A. J., (...), Kutschera, A., (...), Tucker, N. P. (2018). Community-led comparative genomic and phenotypic analysis of the aquaculture pathogen *Pseudomonas baetica* a390T sequenced by Ion semiconductor and Nanopore technologies. *FEMS Microbiology Letters*, 365(9).

IV Kutschera, A.*, Dawid, C.*, Gisch, N., Schmid, C., Raasch, L., Gerster, T., Schäffer, M., Smakowska-Luzan, E., Belkhadir, Y., Vlot, A. C., Chandler, C. E., Schellenberger, R., Schwudke, D., Ernst, R. K., Dorey, S., Hückelhoven, R., Hofmann, T., Ranf, S. (*authors contributed equally). (2019) Bacterial medium chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants. *Science*, 364(6436):178–181.

V Kutschera, A., Schombel, U., Schwudke, D., Ranf, S., Gisch, N. (2021). Analysis of the Structure and Biosynthesis of the Lipopolysaccharide Core Oligosaccharide of *Pseudomonas syringae* pv. *tomato* DC3000. *International Journal of Molecular Sciences*, 22(6):3250

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Further peer-reviewed publications from the doctoral work not included in this thesis:

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Kutschera, A. & Lamb, J. J. (2018) Light Meter for Measuring Photosynthetically Active Radiation. *American Journal of Plant Sciences*, 9:2420-2428.

Abbreviations

3-OH-C10	3-hydroxy decanoic acid
acyl-ACP	acyl-acyl carrier protein
AHL	N-acyl homoserine lactones
BAI1	BRAIN ANGIOGENESIS INHIBITOR 1
BAK1	BRI1-ASSOCIATED RECEPTOR KINASE 1
BIK1	BOTRYTIS-INDUCED KINASE 1
CAMPs	cationic antimicrobial peptides and proteins
CD14	GLYCOPROTEIN CLUSTER OF DIFFERENTIATION 14
CDPK	calcium-dependent protein kinase
CERK1	CHITIN ELICITOR RECEPTOR KINASE 1
CFTR	CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR
Cm	carbamoyl
core-OS	core oligosaccharide
CPA	common polysaccharide antigen
DAMP	damage-associated molecular pattern
EF-Tu	ELONGATION FACTOR THERMO UNSTABLE
EFR	ELONGATION FACTOR THERMO UNSTABLE RECEPTOR
ETI	effector-triggered immunity
FLS2	FLAGELLIN-SENSING 2
Gal	D-galactose
Gal	N-acetyl-D-galactosamine
Glc	D-glucose
GlcN	D-glucosamine
GlcNA	N-acetyl-D-glucosamine
GPCR	G-protein-coupled receptor
HAA	(*R*)-3-hydroxyalkanoate
Hep	glycero-D-manno-heptose
HPLC	high-performance liquid chromatography
HR	hypersensitive response
IM	inner membrane
Kdo	3-deoxy-D-manno-oct-2-ulosonic acid
L-Ala	L-alanine
LBP	LPS-BINDING PROTEIN
LBR	LBP-RELATED PROTEINS
LORE	LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION
LP	lipopeptide
LPS	lipopolysaccharide
LRR	leucine-rich repeat
LYK4/LYK5	LysM-CONTAINING RECEPTOR-LIKE KINASE 4/5

LYM1/LYM3	LysM DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 1/3
LysM	lysin motif
MAMP	microbe-associated molecular pattern
MAPK	mitogen-activated protein kinases
mc	medium chain
MD2	EXTRACELLULAR MYELOID DIFFERENTIATION FACTOR-2
OM	outer membrane
OMV	outer membrane vesicle
OPS	O-polysaccharide
OSA	O-specific antigen
PAGE	polyacrylamide gel electrophoresis
Pci	*Pseudomonas cichorii* ATCC10857/DSM50259
PEtN	phosphoethanolamine
PG	peptidoglycan
PHA	polyhydroxyalkanoate
PR	*PATHOGENESIS-RELATED*
PRR	pattern recognition receptor
Pst	*P. syringae* pv. *tomato* DC3000
PTI	pattern-triggered immunity
R-genes	resistance genes
R-proteins	resistance proteins
RBOHD	RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D
Rha	L-rhamnose
RL	Rhamnolipid
RLK	receptor-like kinase
RLP	receptor-like proteins
ROS	reactive oxygen species
SDS	sodium dodecyl sulfate
TIR	Toll/Interleukin-1 receptor
TLR4	TOLL-LIKE RECEPTOR 4
UDP-GlcNAc	N-acetylglucosamine
undPP	undecaprenyl pyrophosphate

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1 Introduction

1.1 Preamble - Bacterial plant pathogens

Every year plant pathogens and pests cause significant economic damage and threaten global food security. Recent studies estimate yield losses between 17.2% and 30.0% in major crop species (Savary et al., 2019). Most importantly, the highest yield losses caused by pathogens and pests are observed in developing regions which are already affected by food shortage and possess limited resources. Foods produced from affected crops often are of reduced quality and can be harmful to health. In combination, the effects can lead to further economic and political instability in these regions (Savary et al., 2017, 2019). Moreover, newly introduced and re-emerging pathogens are a persisting challenge for an efficient agriculture. Sustainable crop protection is necessary to secure global supply with high-quality food now and in the future.

The survey of Savary et al. (2019) lists fungi, oomycetes, viruses and bacteria as major pathogens, which cause substantial damage to crops. Bacterial diseases of major crops are less frequent and thus lead to less economic damage compared to fungal and viral diseases. However, in recent years bacterial pathogens emerged, which threatened local and global cultivation of specific crop plants with devastating consequences. An alarming example is the kiwi canker outbreak in New Zealand. *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwi canker, was detected for the first time in New Zealand in late 2010 and rapidly spread in the agricultural areas (Vanneste, 2017). The disease causes significant yield losses and death of kiwi vines which had a massive economic impact, because at this time kiwi fruit made up 42.9% of all exported agricultural products in New Zealand. In the following years over 85% of vines of the prevalent cultivar Hort16A were replaced with the more resistant cultivar Gold3. In combination with extensive outbreak control this lead to a decline of the disease outbreak and the recovery of kiwi production and export (SOPI, 2014, 2018).

In the top 10 list of plant pathogenic bacteria published by the scientific journal "Molecular Plant Pathology" the *Pseudomonas syringae* pathovars take the lead position (Mansfield et al., 2012). While one might doubt the necessity of such a list, it still reflects the tremendous research interest in the listed pathogens. *P. syringae* is discussed not to be a single species but a species complex. Its genetic diversity is reflected by a subdivision into 13 distinct phylogenetic groups comprising numerous highly adapted pathovars (Baltrus et al., 2017; Xin et al., 2018). Among them are economical relevant pathogens such as the pathovars *actinidiae*, *phaseolicola* and *tomato* which can cause substantial yield losses in infected crops (Baltrus et al., 2017). Many of these *P. syringae* pathovars are considered as important model-organisms for studies of plant-bacteria interactions with their respective hosts. *P. syringae* pv. *tomato* DC3000 (*Pst*) in particular is a widely used laboratory strain due to its ability to infect the cruciferous model plant *Arabidopsis thaliana*. Elucidation of the *P. syringae* pathogenesis in the light of plant immunity will help to resolve the

interactions between bacteria and plants on the molecular level. This knowledge can contribute to the development of sustainable agronomical solutions for preventing and controlling bacterial disease outbreaks in the field.

This thesis focuses on the role of lipopolysaccharide (LPS) in the complex interplay between bacteria and plants. This glycolipid is the major component of the cell wall in Gram-negative bacteria and an important virulence factor in mammalian pathogens. In the following the role of LPS in the pathogenesis of plant-associated bacteria is discussed. Novel insights into the metabolism and synthesis of lipopolysaccharide of plant-associated *Pseudomonas* strains as well as in its putative functions during plant colonization are reported. Finally, immune perception of LPS in plants is elucidated and discussed in the light of the recent findings described in this thesis. They show not LPS itself, but bacterial metabolites which are associated with LPS are recognized in *A. thaliana*. These findings not only advance the understanding of plant immunity against bacterial pathogens, but might contribute to the development of sustainable plant protection measures in the future.

1.2 Plant immunity

Plants possess a multilayered immune system, which protects them against pathogenic organisms. It comprises constitutive barriers as well as inducible defense mechanisms (Jones and Dangl, 2006). Physical and chemical barriers such as the plant cuticle, antibacterial enzymes or secondary metabolites confer a general resistance against biotic stresses such as pathogenic microbes (Thordal-Christensen, 2003). Adapted pathogens eventually breach these barriers and are able to invade plant tissues. When their presence is perceived, innate immunity is triggered and specific responses are launched to fend off the invading pathogens (Nürnberger et al., 2004).

1.2.1 Overview of plant innate immunity

Plant innate immunity can be categorized into pattern-triggered and effector-triggered immunity (PTI and ETI) (Jones and Dangl, 2006). PTI is mediated by pattern recognition receptors (PRRs) located on the surface of plant cells. They sense molecular patterns which are either associated with microbes (microbe-associated molecular pattern, MAMP) or tissue damage (damage-associated molecular pattern, DAMP). This allows the perception of microbes by either direct recognition of bacterial cell components or indirectly by the detection of possible damage caused by pathogens. When a PRR recognizes its corresponding pattern, a signal cascade is triggered leading to activation of defense responses (Boller and Felix, 2009; Macho and Zipfel, 2014; Yu et al., 2017) (Fig. 1.1). However, adapted pathogens secrete effector proteins into the plant cell which suppress PTI or target specific cell components to facilitate an infection (effector-triggered susceptibility). In turn, plants evolved resistance genes (R-genes) which confer resistance against specific pathogens. They code either for R-proteins which directly interact with effectors, monitor

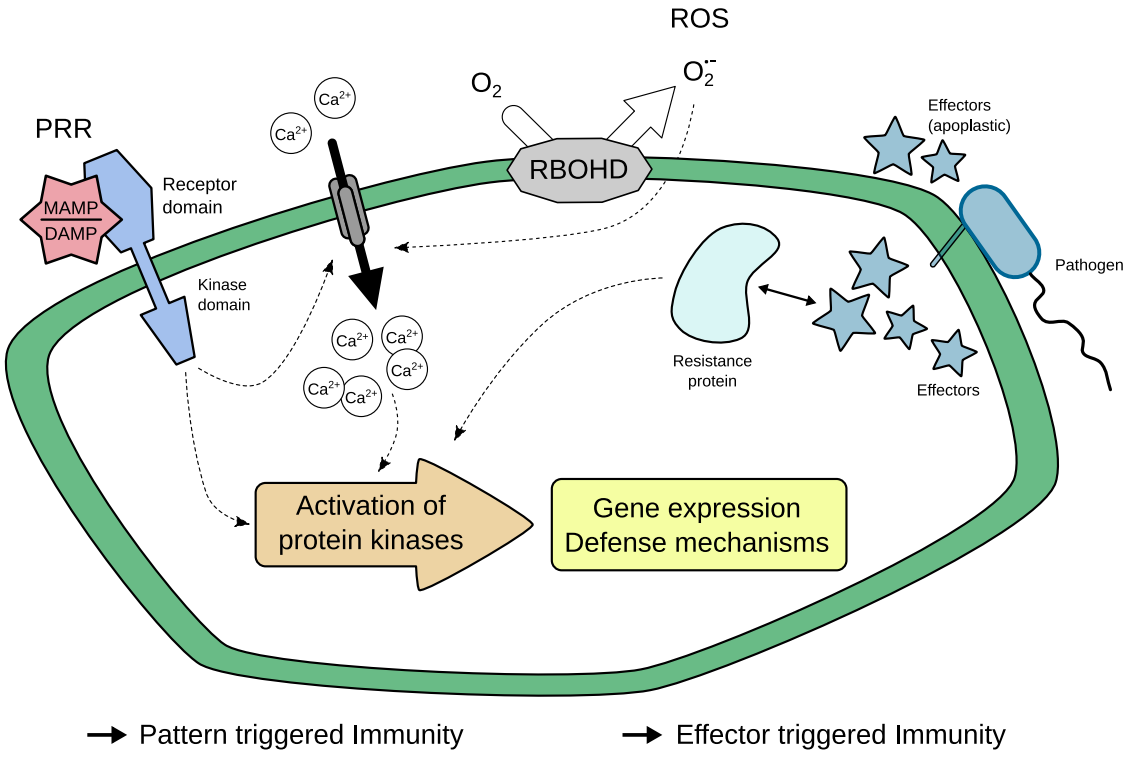

Figure 1.1: Pattern-triggered and effector triggered immune responses of plants. MAMPs or DAMPs are perceived by PRRs and induce PTI. An ion influx is triggered which leads to an elevation of the cytosolic Ca^{2+} concentration. The first active defense response, the production and release of ROS by RBOHD, additionally stimulates $[Ca^{2+}]_{cyt}$ increase as part of a feedback loop. Finally, further local and systemic immune responses and defence mechanisms are induced via a signalling cascade. Effectors released by adapted pathogens to facilitate an infection are recognized directly or indirectly by specific resistance proteins which induce immune immune responses via the activation of a signalling cascade (ETI).

the integrity of possible cellular effector targets (guard hypothesis) or degrade effectors such as toxins (Khan et al., 2016). If the presence of effectors is perceived by the plant, ETI is induced (Fig. 1.1). That way the pathogen is betrayed to the plant immune system by its own weapons. Hence, an effector gene for which a corresponding R-gene exists in the plant is called an avirulence gene (Jones and Dangl, 2006). The resulting immune recognition puts selection pressure on the pathogen and eventually leads to alterations of the respective effector so it is not recognized by the R-protein anymore. Alternatively, novel effectors can evolve, which again induce effector-triggered susceptibility and facilitate an successful infection. Plants, in turn, catch up with alteration of existing or evolution of novel R-proteins to sense the pathogen (gene-for-gene hypothesis). Many avirulence and R-gene pairs in the genomes are the silent witnesses of this constant evolutionary arms race between pathogens and their hosts (Boller and He, 2009).

ETI confers resistance against specific adapted pathogens, which constitutes the second level of plant innate immunity after PTI. The resulting hypersensitive response usually leads to programmed cell death at the infection site and in surrounding tissue to prevent further spread of the pathogen. PTI in contrast confers a broad resistance against most

pathogens (Jones and Dangl, 2006). The molecular mechanisms of PTI are extensively studied in order to generate knowledge which can be used to breed or engineer plants for resistance against a whole group of pathogens (Boutrot and Zipfel, 2017).

1.2.2 Pattern-triggered immunity

Perception of MAMPs by cell-membrane localized PRRs triggers intracellular signaling, which results in the activation of PTI. Most of the PRR described in plants can be classified either as receptor-like kinase (RLKs) or receptor-like proteins (RLPs) (Zipfel, 2014). They posses an ectopic domain which is responsible for ligand binding and takes part in oligomerization processes. The intracellular kinase domain of RLKs is commonly responsible for the signal transduction by activation of downstream signaling components through phosphorylation. RLPs lack an intracellular kinase domain and are therefore dependent on interactions with RLKs to initiate intracellular signaling (Macho and Zipfel, 2014). Recent studies indicate that although individual PRRs are responsible for perception of a particular MAMP, they act in multi-protein complexes to achieve an intracellular signal transduction (Yu et al., 2017).

One of the first measurable cellular responses to the perception of a MAMP is an increase of ion flux which leads to an elevation of the cytosolic calcium ion (Ca^{2+}) concentration. Subsequently, the NADPH oxidase RBOHD (RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D) is activated, which generates reactive oxygen species (ROS) (Yu et al., 2017) (Fig. 1.1). Due to their putative antimicrobial effects the resulting release of extracellular ROS is considered to be the first active defense response against intruding pathogens. Additionally, ROS initiate cross-linking reactions in the cell wall and thereby increase cell wall stability (Boller and Felix, 2009). Extracellular ROS activate specific ion channels and induce further accumulation of cytosolic Ca^{2+} (Fig. 1.1). This feedback loop leads to an amplification of the PTI signal in the affected cell and to a transmission to neighbouring cells (Dubiella et al., 2013). Downstream signaling after MAMP perception is mediated by receptor-like cytoplasmic kinases like BIK1 (BOTRYTIS-INDUCED KINASE 1), which further relay the signal and for example play a role in activation of RBOHD. Further downstream, an activation of mitogen-activated protein kinases (MAPK) and calcium-dependent protein kinases (CDPK) results in a change of gene expression patterns and metabolism (Fig. 1.1). Thus, hormone signaling, including jasmonic acid, salicylic acid, and ethylene signaling, is activated and additional local and systemic responses in the plant are induced. For example, this leads to a systemic upregulation of expression of genes related to MAMP recognition and signal transduction to increase the alertness in non-infected plant cells (Li et al., 2016). Additionally, the expression of pathogenesis-related genes is upregulated which results in the formation of antimicrobial agents such as phytoalexins, cationic peptides (e.g. thionins) or enzymes (e.g. lipases). The combination of these different reactions represents an active local and systemic defense response against microbial invaders (Sels et al., 2008; van Loon et al., 2006).

1.2.3 Elicitors and receptors of pattern-triggered immunity

MAMPs constitute non-self signatures, which are perceived by the plant immune system. They are characteristic for a class of microbes rather than species specific. Typical MAMPs are conserved and essential for the viability of pathogenic as well as non-pathogenic organisms. However, adapted pathogens evolved ways to mask the perceived structures to evade recognition or actively suppress PTI in order to infect the plant and cause disease (Aslam et al., 2009; Boller and Felix, 2009).

Previous studies identified various compounds, which elicit typical PTI responses in plants. Yet, for most of the compounds the recognized motif is unknown and/or corresponding PRRs could not yet be identified (Yu et al., 2017). The MAMP flg22 is a 22 amino acids large peptide from the highly conserved N-terminus of flagellin, a major component of bacterial flagella. It was the first MAMP to be described where a corresponding PRR could be identified in *A. thaliana*. The receptor FLAGELLIN-SENSING 2 (FLS2) binds flg22, and subsequently induces PTI (Chinchilla et al., 2006; Gómez-Gómez and Boller, 2000)(Fig. 1.2). Infection experiments showed FLS2 is important for resistance against bacterial pathogens in *A. thaliana* (Zipfel et al., 2004). FLS2 is classified as leucine-rich repeat (LRR) receptor kinase, because its ectodomain comprises a LRR motif which is known to mediate protein-protein interactions (Kobe and Deisenhofer, 1994). The ELONGATION FACTOR THERMO UNSTABLE (EF-Tu) RECEPTOR (EFR) represents another LRR receptor kinase sensing a bacterial MAMP. It binds an 18 amino acid segment of EF-Tu (elf18), a conserved element of protein biosynthesis in prokaryotes, which is highly abundant in the bacterial cytosol (Kunze et al., 2004). Both receptors, FLS2 and EF-Tu, require the LRR-RLK co-receptor BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1) to elicit full downstream signaling (Zipfel, 2014) (Fig. 1.2). Besides proteinaceous compounds, other microbial substances with different chemical properties elicit PTI in plants. For example, PRRs with a lysin motif (LysM) in the ectodomain are associated with the recognition of chitin and peptidoglycan (Buist et al., 2008; Yu et al., 2017). In *A.thaliana*, chitin oligosaccarides are bound by a receptor protein complex of CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) and the LysM-CONTAINING RECEPTOR-LIKE KINASE 4 or 5 (LYK4/LYK5). Recent studies suggest that LYK5 is the primary chitin receptor in *A. thaliana*, but formation of the receptor complex is required to induce downstream signaling (Cao et al., 2014; Liu et al., 2012). CERK1 also mediates the perception of bacterial peptidoglycan in complex with the RLPs LysM DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 1 and 3 (LYM1/LYM3) (Gust et al., 2007; Willmann et al., 2011) (Fig. 1.2).

Chitin and peptidoglycan are both essential cell wall components unique to fungi and bacteria respectively. While peptidoglycan accounts for 30-70% of the mass from the cell wall of Gram-positive bacteria this proportion is below 10% in Gram-negative bacteria. The Gram-negative genera *Pseudomonads* or *Xanthomonads*, which include prominent phytopathogenic species (Seltmann and Holst, 2010). In the Gram-negative cell wall the

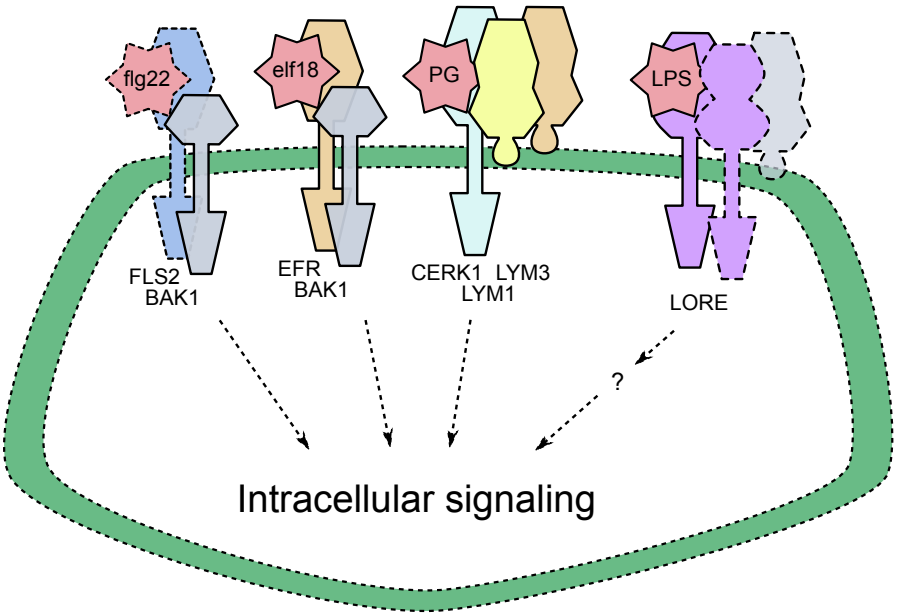

Figure 1.2: Exemplary PTI receptors and their (putative) co-receptors in *A. thaliana*. FLS2 recognizes a 22 amino acid peptide of bacterial flagellin (flg22). EFR binds an 18 amino acid section of bacterial EF-Tu. FLS2 and EFR are classified as LRR-RLKs and require the co-receptor BAK1 to elicit intracellular signaling. A complex of CERK1, LYM1 and LYM3 mediates the perception of peptidoglycan (PG) fragments. The bulb-type lectin S-domain-1 receptor-like kinase LORE is required for elicitation of immune responses upon treatment with lipopolysaccharide preparations. While it appears to homodimerize, it is still unclear if a co-receptor is necessary for signal transduction.

peptidoglycan layer is covered by the outer membrane (OM) and is not exposed to the environment if the cell is intact. (Fig. 1.3). However, LPS, the major component of the Gram-negative OM, also induces diverse immune responses in plants and is considered a MAMP (Erbs and Newman, 2012). While many studies on the role of LPS in plant immunity exist, the molecular mechanism of the putative LPS perception remains unclear (Kutschera and Ranf, 2019). Recently, the receptor kinase LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION, also referred to as S-DOMAIN-1 29) was identified to be required for the induction of PTI responses in *A. thaliana* upon treatment with LPS preparations (Fig. 1.2). LORE comprises a bulb-type lectin S-domain which has not been described in other plant PRRs before (Ranf et al., 2015). Further research is required to elucidate possible LPS perception mechanisms and reveal whether a specific molecular substructure of LPS is recognized by LORE.

1.3 Lipopolysaccharide

1.3.1 Importance of lipopolysaccharide

The cell wall of Gram-negative bacteria is divided into the inner membrane (IM) which surrounds the cytoplasm, a thin peptidoglycan layer, and the OM (Fig. 1.3). It forms a restrictive permeability barrier, which protects the bacteria from environmental stresses

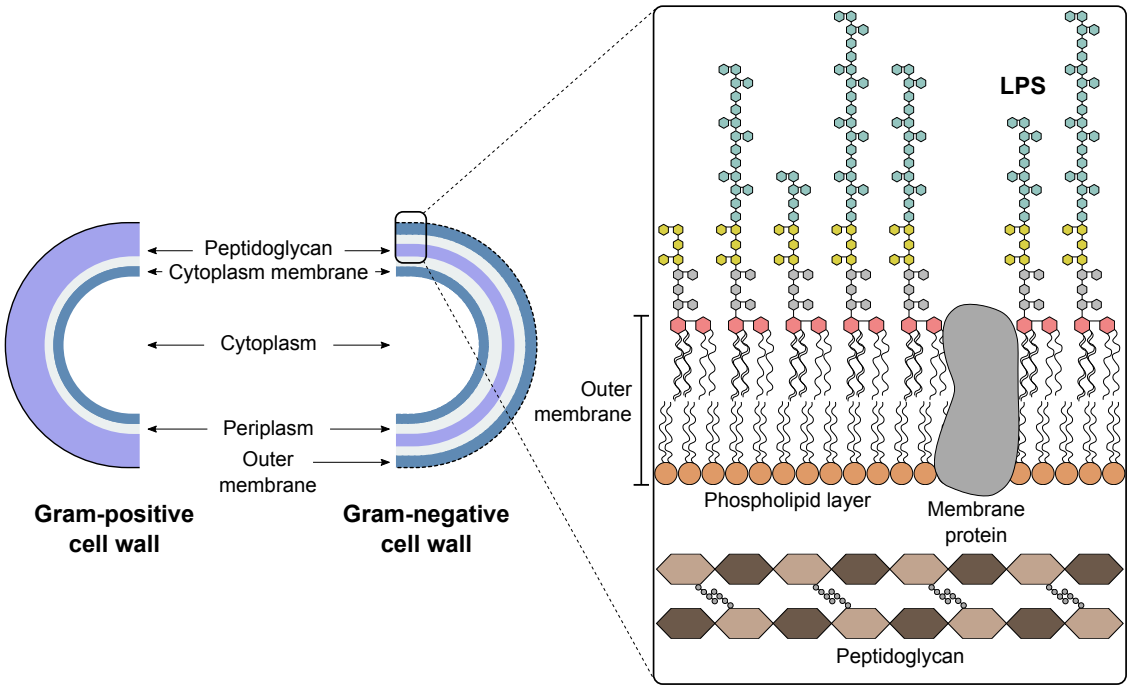

Figure 1.3: Schematic of the structure of bacterial cell walls with a closeup on the distal part of a Gram-negative cell wall. While Gram-positive bacteria are protected by a thick peptidoglycan layer, the cell wall of Gram-negative bacteria comprises a thin peptidoglycan layer, which is additionally enveloped by a second membrane. The outer leaflet of this outer membrane mainly consists of lipopolysaccharide molecules in most Gram-negative bacteria. Figure design inspired by Needham and Trent (2013).

and shields off antimicrobial substances while allowing material exchange and nutrient uptake (Bertani and Ruiz, 2018; Raetz and Whitfield, 2002). These properties are mainly mediated by LPS, a unique glycolipid which exclusively occurs in the OM of Gram-negative bacteria. LPS molecules are the major component of the outer leaflet of the OM and account for up to 75% of the cell surface in *Escherichia coli* (Alexander and Rietschel, 2001). Due to its unique chemical composition and outermost localisation, LPS influences interactions between the bacterial cells and their environment such as surface adhesion processes and biofilm formation (Raetz and Whitfield, 2002).

LPS was considered to be essential for the viability of Gram-negative bacteria, however, in recent years some species without LPS have been discovered. They include for example Myxobacterium *Sorangium cellulosum* So ce56, *Fibrobacter succinogenes* S85 and *Sphingomonas* spp., which produce and incorporate glycosphingolipids instead of LPS (Keck et al., 2011; Vinogradov et al., 2001; White et al., 1996). Especially the discovery of LPS-deficient mutant strains of usually LPS producing bacteria such as *Neisseria meningitidis* questioned the essentiality of LPS for bacterial viability (Steeghs et al., 1998; Zhang et al., 2013). Nevertheless, most Gram-negative bacteria are not able to compensate a loss of LPS and even subtle changes in the LPS structure often greatly influence bacterial lifestyle and virulence (Trent et al., 2006).

The overall structure of LPS is conserved in Gram-negative bacteria and comprises three chemically distinct subdomains with different biological properties: Lipid A, core oligosaccharide (core-OS) and O-polysaccharide (OPS). The composition of each substructure contributes to the unique properties of LPS and is closely linked to the characteristics of a bacterial species (Raetz and Whitfield, 2002). *E. coli* is one of best studied organisms in regard to LPS structure and biosynthesis. Many of the identified mechanisms and pathways have been identified in *E. coli* first and later shown to be conserved in other bacterial species as well (Alexander and Rietschel, 2001; Raetz and Whitfield, 2002). Due to its medical relevance, LPS structure and biosynthesis has been well elucidated in *Pseudomonas aeruginosa* in recent years (King et al., 2009). The general structure and biosynthesis of *E. coli* and *P. aeruginosa* LPS is summarized and compared in the following.

1.3.2 Kdo_2-lipid A structure and biosynthesis in the Raetz pathway

The lipophilic lipid A moiety anchors LPS molecules into the OM. Two glucosamine saccharides linked via a β, 1→6 glycosidic bond form the backbone of lipid A. They are usually acylated with four primary fatty acids either via an amide bond with the primary amine groups of the di-glucosamine at position 2 and 2' or via an ester bond with the hydroxy group at position 3 and 3', respectively. The primary fatty acids can be further esterified via additional (*R*)-hydroxy groups with up to three secondary fatty acids in total. The general structure of lipid A is conserved but acylation and acyl chain length often vary between bacterial families. For instance, enterobacterial lipid A is often hexa-acylated with longer acyl chains (C12/C14) in an asymmetric fashion while pseudomonads mostly produce penta-acylated and/or symmetrically hexa-acylated lipid A with shorter acyl chains (C10/C12) (Knirel et al., 2006; Lam et al., 2011). The di-glucosamine backbone is usually further substituted with phosphates at position 1 and 4' but they can be replaced by other phosphate derivatives such as pyrophosphate, phosphoethanolamine, di-phosphoethanolamine or phosphate linked arabinosamine residues. Lipid A is covalently attached to the core-OS via an 6→2 glycosidic bond with a LPS specific 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) residue (Fig. 1.4).

In *E. coli*, nine enzymes orchestrate the Kdo_2-lipid A biosynthesis in the Raetz pathway, which is highly conserved in Gram-negative bacteria (Opiyo et al., 2010; Raetz et al., 2007). The different reactions of the biosynthesis take place in the cytosol and at the cytoplasmic face of the IM. The first step is the transfer of an acyl chain from an acyl-acyl carrier protein (acyl-ACP) to the 3-hydroxy group of an uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) (Fig. 1.5). This transfer is catalyzed by the acyltransferase LpxA, which is highly selective for acyl chains of a specific length. A precise hydrocarbon ruler is responsible for the transfer of a 3-hydroxy tetradecanoic acid to UDP-GlcNAc position 3 in *E. coli* (Whitfield and Trent, 2014). Structural analyses revealed that this specificity is mediated by the size of a hydrophobic cleft which accommodates the acyl chain. The preference for a certain acyl chain length is modulated by a particular amino acid residue

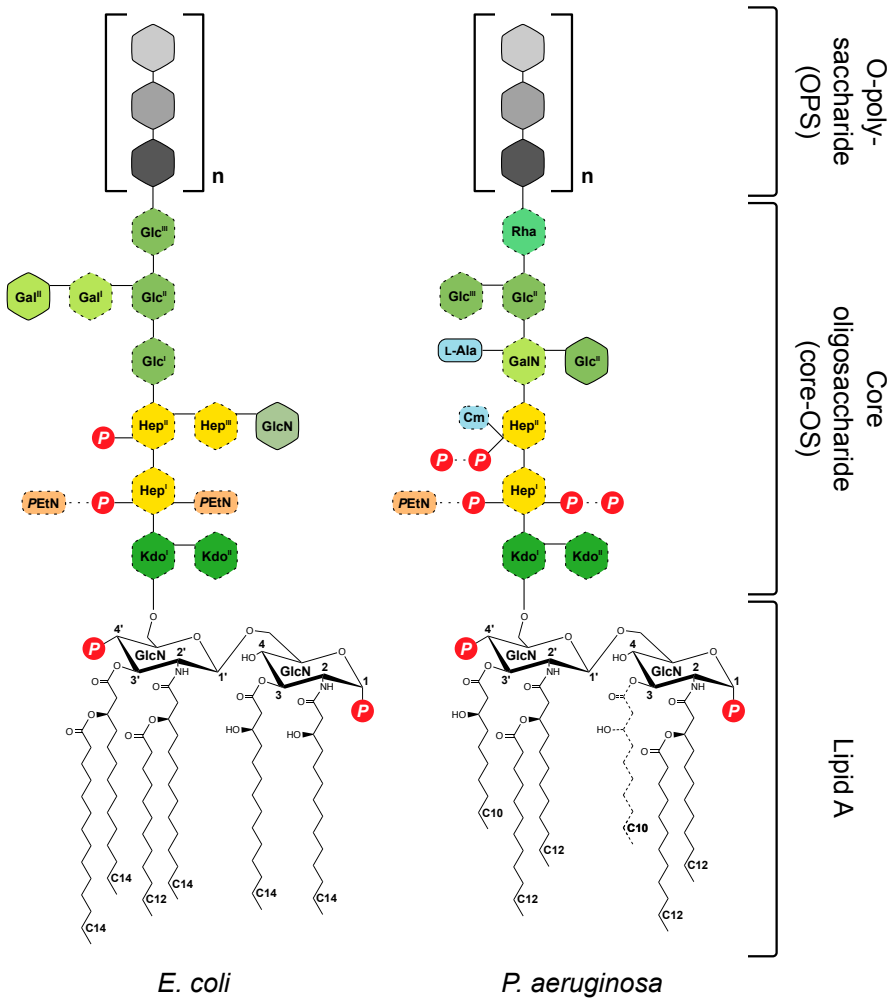

Figure 1.4: Schematic of the common lipid A and core-OS structures from *E. coli* (R1 core-OS) and *P. aeruginosa* (glycoform 2 core-OS). The OPS is either a homopolymer or a heteropolymer of repetitive units of several monosaccharides. OPS-deficient LPS can occur in parallel to LPS with OPS. Dashed lines indicate non-stoichiometric substitutions. Abbreviations: PEtN: phosphoethanolamine, Cm: carbamoyl residue, L-Ala: L-alanine, P: Phosphate, Kdo: 3-deoxy-D-manno-oct-2-ulosonic acid, Hep: D-glycero-D-manno-heptoses, Gal: D-galactose, GalN: D-galactoseamine, Glc: D-glucose, GlcN: D-glucosamine, Rha: L-rhamnose (figure from publication I).

in the acyl chain binding pocket of LpxA, which is, in case of *E. coli*, the second glycin residue (G173) of a VG**G**CS-motif. A methionine residue (M169) in the respective LpxA motif in *P. aeruginosa* decreases the size of the hydrophobic cleft and results in the specific transfer of 3-hydroxy decanoic acid. Accordingly, reciprocal mutation of the motif leads to a reversed selectivity for 3-hydroxy decanoic acid in *E.coli* (G173M) and 3-hydroxy tetradecanoic acid in *P. aeruginosa* (M169G) (King et al., 2009; Wyckoff et al., 1998).

Following the acyl chain transfer by LpxA, the deacetylation of the UDP-3-acyl-GlcNAc amino group is mediated by the deacetylase LpxC. This reaction is irreversible and is therefore considered to be the first committed step in the Kdo_2-lipid A biosynthesis. In the next step an acyl chain is transferred from an acyl-ACP to the deacetylated amino

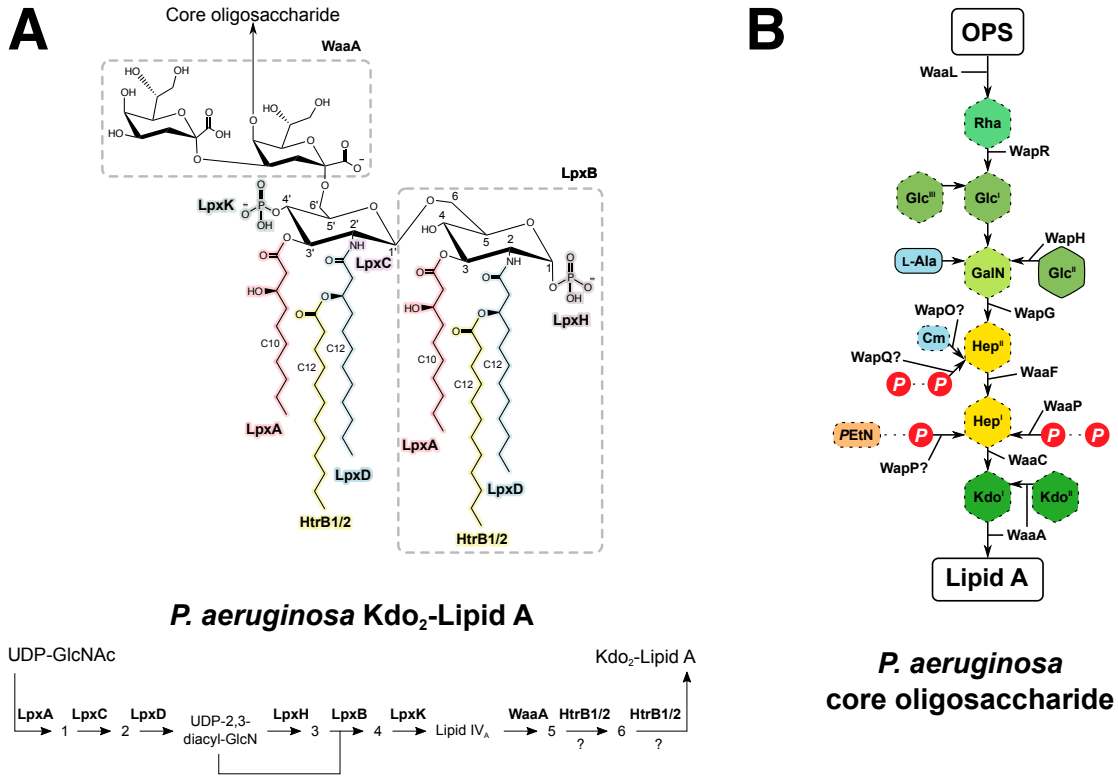

Figure 1.5: Schematic of the structure and biosynthesis of lipid A (**A**) and core-OS glycoform 2 (**B**) in *P. aeruginosa*. All involved enzymes which are known to date are shown and their respective catalytic step is marked. Non-stoichiometric substitutions in the core-OS are depicted with dashed lines. UDP-GlcNAc: uridine diphosphate N-acetylglucosamine, PEtN: phosphoethanolamine, Cm: carbamoyl residue, L-Ala: L-alanine, P: Phosphate, Kdo: 3-deoxy-D-manno-oct-2-ulosonic acid, Hep: D-glycero-D-manno-heptoses, GalN: N-acetyl-D-galactoseamine, Glc: D-glucose, Rha: L-rhamnose. Structures and biosynthetic enzymes according to Hittle et al. (2015); King et al. (2009); Knirel et al. (2006); Whitfield and Trent (2014).

group by LpxD (Fig. 1.5) (Whitfield and Trent, 2014). This acyltransferase is selective for a specific acyl chain length but to a lower extent as compared to LpxA. Accordingly, *E. coli* LpxD transfers either 3-hydroxy tetradecanoic or 3-hydroxy hexadecanoic acid in a ratio of 3:1. On the basis of the crystal structure and mutation experiments a methionine residue (M290) was identified which influence the observed acyl chain selectivity (Bartling and Raetz, 2009). In *P. aeruginosa* on the other hand, LpxD usually transfers a 3-hydroxy dodecanoic acid (King et al., 2009). A similar hydrocarbon ruler mechanism certainly exists in LpxD of other species as well, but no general motif has been described yet.

In the following step, uridine monophosphate is cleaved off from a fraction of UDP-2,3-diacyl-GlcN by hydrolysis of the phosphate bond through LpxH. The resulting 2,3-diacyl-1-phosphate-GlcN is then, catalyzed by LpxB, linked to unhydrolized UDP-2,3-diacyl-GlcN via a β, 1'→6 glycosidic bond. The subsequent phosphorylation at the 4' position by LpxK leads to the formation of tetraacyl disaccharide 1,4'-diphosphate, commonly referred to as lipid IV_A (Fig. 1.5) (Whitfield and Trent, 2014).

The last four steps in the Raetz pathway are performed by membrane proteins at the cytosolic face of the IM. In *E. coli*, the transfer of two α, 1'→6 linked Kdo to the 6' position of lipid IV_A (α, 1→6') is required before secondary acyl chains are added to the LPS precursor. Therefore, this addition of the first core-OS sugars, mediated by the glycosyltransferase WaaA, is considered to be part of the lipid A biosynthesis (Whitfield and Trent, 2014). The following substitution of 3-hydroxy groups of the primary acyl chains with a dodecanoyl or tetradecanoyl in *E. coli* is catalyzed by the acyltransferases LpxL or LpxM, respectively. Both enzymes possibly originate from a gene duplication event and are not homologous to LpxA or LpxD. Furthermore, LpxM appears to be only conserved in a small phylogenetic group of Gammaproteobacteria including *Escherichia*, *Yersinia* and *Vibrio* species (Opiyo et al., 2010). Generally, most Gram-negative bacteria synthesize a complete hexa-acylated lipid A, but modifications such as the alteration of the acylation pattern can occur post synthesis (Needham and Trent, 2013).

In *P. aeruginosa*, Kdo_2 transfer to lipid IV_A is not required for the secondary acylation due to a different substrate specificity of the corresponding acyltransferases. Furthermore, *P. aeruginosa* lacks a LpxM homolog but possesses two LpxL homologs (HtrB1/PA0011 and HtrB2/PA3242). They transfer two dodecanoyl acyl chains to the primary acyl chains at position 2 and 2' of the di-glucosamine backbone. They are then presumably 2-hydroxylated in a non-stoichiometric fashion by two homologs of the *S. typhimurium* oxygenase LpxO (PA4512/PA1936) (Gibbons et al., 2008; Hittle et al., 2015; King et al., 2009) (Fig. 1.4). In general, secondary lipid A acylations differ considerably in acyl chain length, saturation, hydroxylation as well as location and therefore contribute significantly to the structural heterogeneity observed in lipid A from different bacteria (Whitfield and Trent, 2014).

1.3.3 Structure and biosynthesis of the core oligosaccharide

The core-OS is covalently linked to the di-glucosamine backbone of lipid A via a α, 1→6' glycosidic bond. It can be subdivided into the inner and outer core region, which are often defined by their saccharide composition. The first saccharides of the structurally conserved inner core, Kdo, are transferred during the Raetz pathway (see section 1.3.2). Kdo is a characteristic and essential component of the core-OS and is therefore often used to detect and quantify LPS (Lee and Tsai, 1999). The inner core usually contains L- or D-glycero-D-manno-heptoses (L,D-Hep) too, which carry anionic substituents such as phosphate, di-phosphate or di-phosphoethanolamine (Fig. 1.4). The composition of the outer core is less conserved but mainly comprises hexoses like D-glucose (D-Glc), D-galactose (D-Gal), D-glucosamine (D-GlcN), N-acetyl-D-glucosamine (D-GlcNAc) or N-acetyl-D-galactosamine (D-GalN). Furthermore, the core-OS is often extended by non-stoichiometric modification including the addition of carbamoyl and alanine substituents or O-acetylation (Frirdich and Whitfield, 2005; Raetz and Whitfield, 2002) (Fig. 1.4).

The core-OS is build up by the subsequent addition of monosaccharides to the Kdo of the Kdo_2-lipid A. The saccharide transfer is mediated by various different glycosyltransferases of

the Waa-family, which are mostly peripheral membrane proteins located at the cytoplasmic face of the IM (Whitfield and Trent, 2014). Most of the enzymes of the core-OS biosynthesis in *E. coli* have been identified in recent years. Three L,D-Hep of the inner core are successively transferred by the heptosyltransferases WaaC, WaaF, and WaaG. Additionally, L,D-Hep^{I} and L,D-Hep^{II} are phosphorylated by the core-OS kinases WaaP and WaaY, respectively (Frirdich and Whitfield, 2005). The outer core of *E. coli* comprises three D-Glc, a lateral D-Gal and a terminal L,D-Hep which serve as acceptor for the OPS ligation. Their addition is catalyzed by the glycosyltransferases WaaG, WaaO, WaaR, WaaB, and WaaU, respectively (Frirdich and Whitfield, 2005). The inner core of *P. aeruginosa* contains only two L,D-Hep, which are transferred by WaaC and WaaF orthologs (King et al., 2009; Knirel et al., 2006). They are substituted with multiple phosphates or di-phosphates by WaaP, WapP and possibly additional core-OS kinases (Fig. 1.4). The *P. aeruginosa* core-OS is therefore considered to be one of the highest phosphorylated core-OS structures discovered to date. The heptoses are followed by D-GalN, two D-Glc and a lateral D-Glc residue. *P. aeruginosa* produces two core-OS glycoforms, which differ in the linkage of a L-rhamnose (L-Rha) residue (Knirel et al., 2006). The core-OS glycoform 1 is formed by the transfer of L-Rha to the lateral D-Glc^{II} by MigA, which is subsequently joined with a fourth D-Glc residue. Alternatively, if L-Rha is transferred to D-GalN it can serve as acceptor for the ligation of OPS (Fig. 1.4). This glycoform 2 is also referred to as capped core-OS of *P. aeruginosa* (Knirel et al., 1995).

1.3.4 Types of O-polysaccharide and their biosynthesis

LPS usually comprises an OPS as most distal part of the molecule. Very few naturally occurring bacterial strains have been described to date which produce OPS-deficient LPS. While different OPS variants exist, all are covalently bound to the core-OS and are very heterogeneous in molecular size. Most Gram-negative bacteria synthesize an O-specific antigen (OSA), a heteropolysaccharide with a varying number of oligosaccharide repeat units consisting of 3-8 monosaccharides. The composition of the OSA is highly diverse and variations between bacteria of one species commonly determine their serological and antigenic specificity. The extreme differentiation of OSA reflects in the over 185 variants, which have been identified in *E. coli* so far (DebRoy et al., 2016; Hong and Reeves, 2014). Another OPS variant is the capsular polysaccharide, a highly hydrated heteropolymeric or homopolymeric molecule with up to 95% water content (Hong and Reeves, 2014). Besides these two general OPS types further forms exist, which are specific to particular bacteria families or species. The heteropolymeric enterobacterial common antigen is such a surface antigen added to the core-OS in enteric bacteria (Gozdziewicz et al., 2014; Kuhn et al., 1988). Similarly, LPS of *P. aeruginosa* serotypes can also comprise a D-Rha homopolymer, known as common polysaccharide antigen (CPA). In general, LPS species with a capsular polysaccharide, enterobacterial common antigen or CPA occur in parallel or instead of LPS with OSA.

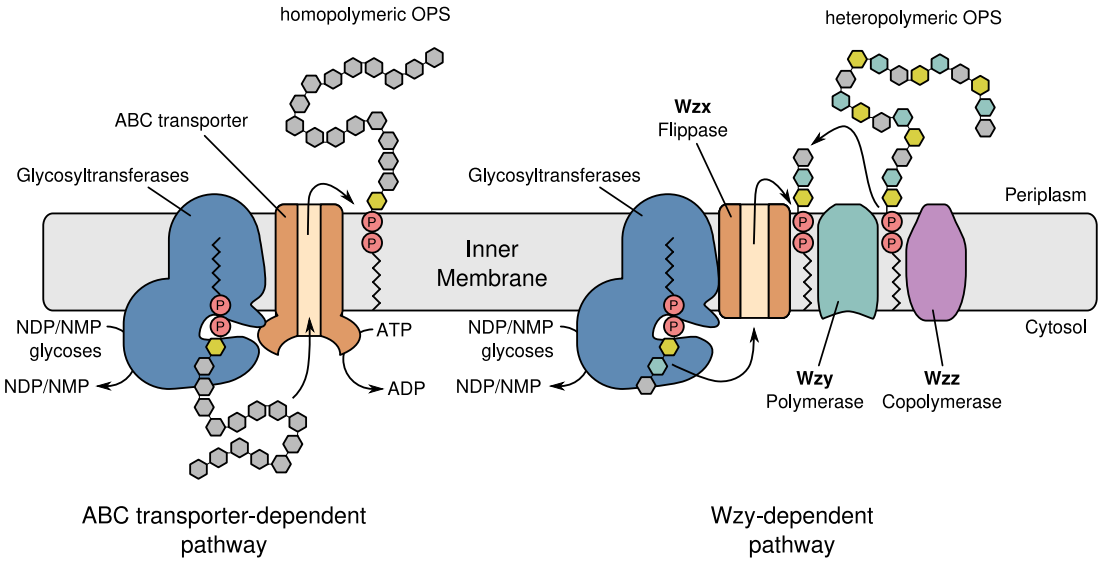

Figure 1.6: Schematic illustration of two OPS biosynthesis mechanisms. OPS synthesized via the ABC transporter-dependent pathway is exclusively build up on the cytoplasmic face of the IM. An initial saccharide is transferred to an undecaprenyl pyrophosphate anchor. Further saccharides are added by different glycosyltransferases and usually leads to the formation of a homopolymeric polysaccharide. It is subsequently transferred to the periplasmic face of the inner membrane through an ABC-transporter. In the Wzy-dependent pathway, at the cytosolic face of the IM single repeat units are synthesized on an undecaprenyl pyrophosphate anchor by the action of different glycosyltransferases. The subunits are then transported to the periplasmic face of the IM by the flippase Wzx. The polysaccharide formation is catalyzed by polymerase Wzy which adds repeat units to the nascent polysaccharide. The polysaccharide length is regulated by the copolymerase Wzz. OPS synthesized via the Wzy-dependent pathway is usually heteropolymeric. Figure design inspired by Greenfield and Whitfield (2012).

Despite the remarkable heterogeneity of OPS structures, there are only three synthesis pathways described: the Wzx/Wzy-dependent, ABC transporter-dependent and synthase-dependent OPS biosynthesis. Most heteropolymeric OSA structures are synthesized via the Wzx/Wzy-dependent pathway (Raetz and Whitfield, 2002). The oligosaccharide repeating units of the OPS are assembled at the cytoplasmic face of the IM through the successive transfer of saccharides to an undecaprenyl pyrophosphate (undPP) carrier by the action of various glycosyltransferases. After the completion of the assembly, the oligosaccharide subunit is transported to the periplasmic face by the flippase Wzx and subsequently joined with the nascent OPS by the polysaccharide polymerase Wzy. This process is co-regulated by the polysaccharide polymerase Wzz, which influences the final length of the polysaccharide (Islam and Lam, 2014; Kalynych et al., 2011). The respective genes of the enzymes involved in this pathway are usually located in a gene cluster at a conserved locus. Resembling the extensive structural variations, the content of this gene cluster often differs considerably between strains and serovars of one bacterial species. Comparative analysis of the respective OSA gene clusters in *E. coli* and *P. aeruginosa* indicates the general conservation of key enzymes such as Wzx and Wzy. Furthermore, additional glycosyltransferases located in the cluster determine the structural variation of the resulting polysaccharide (DebRoy et al., 2016; Lam et al., 2011). The Wzx/Wzy-

dependent mechanism is generally associated with the synthesis of heteropolymeric and branched OPS. Homopolymeric OPS structures such as the CPA of *P. aeruginosa*, on the contrary, are synthesized by the ABC transporter-dependent mechanism (Greenfield and Whitfield, 2012). CPA is synthesized on an undPP carrier located in the cytoplasmic leaflet of the IM by the continual addition of saccharide residues to the non-reducing terminus of the nascent polysaccharide. Upon completion, the CPA is finally transported to the periplasmic phase of the IM by a specific ABC transporter system (Greenfield and Whitfield, 2012). In *P. aeruginosa*, the corresponding genes of the glycosyltransferases and ABC transporter subunits involved in CPA synthesis are organized in a genecluster. This cluster is highly conserved in all *P. aeruginosa* serovars as well as some closely related strains such as *P. fluorescens* Pf0-1 (Lam et al., 2011). Synthase-dependent OPS synthesis has only been described in the *Salmonella enterica* serovar Borezze and is not well-understood so far. Presumably, the combined glycosyltransferase and transport activity of a synthase, possibly in complex with glycosyltransferases, mediates OPS assembly and transfer to the periplasm (Greenfield and Whitfield, 2012; Keenleyside and Whitfield, 1996).

1.3.5 Lipopolysaccharide assembly and transport to the outer membrane

The final step of LPS assembly, the ligation of lipid A-core-OS with OPS, takes places at the periplasmic face of the IM. Prior to this, the lipid A-core-OS is transported to the periplasm by the flippase MsbA. MsbA belongs to the ABC-transporter protein superfamily and is considered to play a crucial role in LPS quality control. Its high selectivity for hexa-acylated phosphorylated lipid A-core-OS, prevents the transport of incomplete or altered molecules. Accordingly, lipid A-core-OS precursors accumulate in the cytosol if their synthesis is artificially disturbed (Voss and Stephen Trent, 2018; Whitfield and Trent, 2014). After the transport of lipid A-core-OS to the periplasm, the OPS is transferred from the undPP carrier to the core-OS by the OPS-ligase WaaL (Abeyrathne et al., 2005; Whitfield and Trent, 2014). The complete LPS molecule is subsequently transferred to the outer leaflet of the OM. The corresponding transport complex, a protein bridge spanning from the IM to the OM, has been recently identified and characterized in *E. coli* (Owens et al., 2019). The IM located complex of LptBFG and LptC extracts fully assembled LPS molecules from the IM and transfers it to the periplasmic LptA. LPS is subsequently transported to the OM translocon LptDE, which finally inserts the molecule into the outer leaflet of the OM (Sherman et al., 2018; Whitfield and Trent, 2014).

1.3.6 Structure to function relationship of lipopolysaccharide

LPS is a versatile molecule involved in many vital processes. The combination of the chemically distinct substructures lipid A, core-OS and OPS makes LPS a unique bacterial molecule with special properties. It confers structural integrity to the bacterial cell and forms a restrictive permeability barrier, while simultaneously being involved in interaction as well as immune evasion processes (Fig. 1.8). The lipid A is integrated in the outer leaflet

of the OM. The length and saturation of its acyl chains influences the thickness and packing of the lipid bilayer. The lipid A structure thus determines mechanical properties such as membrane fluidity and density (Kim et al., 2016). This influences the permeability and rigidity of the OM and correlates with temperatures in the natural environment of bacteria (Erridge et al., 2002; Needham and Trent, 2013). Negatively charged residues in the lipid A and core-OS mediate ionic interactions with divalent cations. The phosphate groups of the lipid A backbone and inner core-OS contribute to the formation of cross-links between LPS molecules, which are crucial for the structural and functional integrity as well as rigidity of the cell wall. The inner core-OS of *Pseudomonas* spp. is one of the most highly phosphorylated substructures of LPS molecules analyzed to date. This feature presumably contributes to the general resistance of these bacteria and might influence the versatility and adaption capacity, which are considered to be characteristic for this genus (Knirel et al., 2006; Raetz and Whitfield, 2002; Silby et al., 2011). The inner core-OS phosphates of *P. aeruginosa* are essential for its viability and intrinsic drug resistance (Walsh et al., 2000). Disruption of core-OS phosphorylation leads to the synthesis of truncated LPS molecules, which do not pass the quality control by MsbA and thus disturbs LPS transport to the OM (Delucia et al., 2011).

The cross-linking of LPS in the OM is essential for the viability of Gram-negative bacteria. Many antimicrobial agents target the negatively charged residues in the lipid A-core-OS region to destabilize the bacterial cell wall (Fig. 1.8). Polymyxin antibiotics as well as cationic antimicrobial peptides and proteins (CAMPs) mask the negative core-OS charges or replace the divalent cations and thereby intercept the ionic interaction between the LPS molecules (Alexander and Rietschel, 2001; Ranf, 2016). The core-OS linked OPS forms a steric shield surrounding the bacterial cell and therefore confers immanent resistance against such antimicrobials (Fig. 1.8). The varying length of the hydrophilic polysaccharide directly influences the polarity of the bacterial cell surface. Thus, OPS influences cell-surface as well as cell-cell attachment processes such as biofilm formation (Dongari-Bagtzoglou, 2008; Ranf, 2016). Furthermore, OPS might contribute to the establishment of a protective extracellular matrix together with other extracellular polymeric substances (Flemming and Wingender, 2010) (Fig. 1.8). In summary, each of the substructures of LPS fulfills a specific function and makes LPS a unique and crucial membrane component in Gram-negative bacteria.

1.4 Immunogenicity of Lipopolysaccharide

LPS is not only crucial for the viability of Gram-negative bacteria but it is also an important virulence factor for pathogens. It can mask the bacterial surface to evade immune recognition and successfully colonize host tissue. The highly abundant surface molecule is recognized by components of the innate and adaptive immune system in mammals. Generally, lipid A is considered to be a very potent immune stimulant. Lipid A perception leads to immune responses such as pro-inflammatory mechanisms or pyroptotic

cell death. Excessive immune stimulation by LPS or lipid A can have pathological effects and can result in septic shock, a life-threatening medical condition of humans (Needham and Trent, 2013; Raetz and Whitfield, 2002). Historically LPS is therefore also referred to as endotoxin (Alexander and Rietschel, 2001; Kieser and Kagan, 2017). Treatment with LPS preparations also elicits typical PTI responses in plants and putative proteins and receptors involved in the perception processes have been identified in recent years (Kutschera and Ranf, 2019). Beyond that, LPS is targeted by specific antimicrobial agents in order to disrupt the bacterial cell wall and eventually to kill the bacteria (Ranf, 2016) (Fig. 1.8). In turn, bacteria evolved to constitutively and dynamically alter their LPS structure as part of adaption processes to specific hosts and to bypass immune sensing as well as defense responses (Needham and Trent, 2013).

1.4.1 Immune sensing of lipopolysaccharide in mammals

LPS is recognized as MAMP by the immune systems of organisms from the plant and animal kingdom (Kagan, 2017). The OPS is considered to be a highly immunogenic antigen, which is targeted as part of the opsonization process of the adaptive immune system in vertebrates. The resulting selection pressure potentially led to the extreme diversification of OPS structures (described in section 1.3.4) to evade immune recognition (Whitfield and Trent, 2014). The cell membrane located pattern recognition proteins BRAIN ANGIOGENESIS INHIBITOR 1 (BAI1) and CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) induce innate immune responses upon perception of core-OS of enterobacterial or *P. aeruginosa* LPS, respectively (Das et al., 2011; Schroeder et al., 2002). Lipid A is perceived by a complex formed by the extracellular domain of the TOLL-LIKE RECEPTOR 4 and the EXTRACELLULAR MYELOID DIFFERENTIATION FACTOR-2 (TLR4-MD2). However, lipid A is not accessible for a direct perception because it is anchored in the bacterial OM. Moreover, if LPS is released from the membrane, it rapidly aggregates in aqueous solutions and forms micelles, which conceal the lipid A inside. The LPS-BINDING PROTEIN (LBP) and the GLYCOPROTEIN CLUSTER OF DIFFERENTIATION 14 (CD14) mediate LPS extraction from bacterial cells and monomerizes LPS aggregates in the serum. Single LPS molecules are subsequently transferred to a hydrophobic cavity in the TLR4-MD2 dimer. The sixth acyl chain at position 2 of the lipid A is exposed on the surface of MD2 and facilitates hydrophobic interactions with specific conserved regions of TLR4 in a second TLR4-MD2 complex. This leads to the formation of a TLR4-MD2 tetramer, which induces innate immune signaling via the cytosolic Toll/Interleukin-1 receptor (TIR) domain (Kieser and Kagan, 2017; Park et al., 2009). Due to their cellular localization, immune recognition by BAI1, CFTR or the TLR4-MD2 complex is restricted to extracellular LPS. When Gram-negative bacteria are taken up by macrophages, the lysis of respective phagolysosomes leads to a release of LPS into the cytosol. Additionally, LPS is constantly shed off the bacterial membrane in outer membrane vesicles (OMVs) which can enter and transfer LPS into cells (Kieser and Kagan,

2017). Recent studies revealed that such intracellular LPS molecules are recognized by the caspases 4/5 in humans and caspase 11 in mice (Shi et al., 2014).

1.4.2 Lipopolysaccharide perception in plants

LPS from various bacteria was reported to elicit plant immunity. Typical responses observed upon treatment with LPS preparations include an oxidative burst, expression of *PATHOGENESIS-RELATED* (*PR*) genes and nitrogen monoxide generation (Dow et al., 2000). Recent publications suggest that the substructures of LPS possibly trigger different components of the plant immune system analogous to LPS perception in mammals. It was shown that synthetic oligo-rhamnans, a subunit of the L-Rha-rich OPS from plant-associated bacteria, induce *PR* gene expression and suppresses the hypersensitive response (HR) reaction in *A. thaliana* (Bedini et al., 2002). Treatment of *A. thaliana* with the ribose-glucose heteropolymer OPS and core-OS section of *Burkholderia cepacia* LPS induced a change of gene expression (Madala et al., 2011). Core-OS of *Xanthomonas campestris* pv. *campestris* appears to elicit an oxidative burst in *Nicotiana tabacum* and *PR*-gene expression in *A. thaliana* (Braun et al., 2005; Silipo et al., 2005). Lastly, studies also indicated that lipid A is sufficient to induce PTI in *A. thaliana* (Ranf et al., 2015). Most of the corresponding immune perception mechanisms and components for LPS sensing have not yet been identified. It is still unclear how single LPS molecules can reach putative PRRs located at the plant plasma membrane, which is surrounded by the cell wall matrix, especially since plants do not possess a serum equivalent. Similar to mammals, LPS molecules might be delivered to the plant plasma membrane via OMVs. Notably, LBP-RELATED PROTEINS (LBR) are present in plants. From the respective LBRs in *A. thaliana*, LBR1 and LBR2, at least LBR2 was shown to be secreted into the apoplast and both appeared to be capable of binding LPS from *E. coli* and *P. aeruginosa* in *in vitro* experiments (Iizasa et al., 2016). Further studies indicate, the expression of a subset of *PR* genes is upregulated in response to treatment with *Pseudomonas* LPS and this is dependent on LBR2. However, *lbr1/lbr2* mutants showed only a delayed *PR1* expression and a reduced ROS burst in response to the LPS treatments (Iizasa et al., 2017). LBRs could potentially be involved in the extraction and monomerization of LPS as well as the delivery of single LPS molecules to membrane localized receptors, but other, functional redundant systems might exist. Various putative LPS-interacting proteins which are located at or in the plasma membrane were identified via affinity purification and mass spectrometry analysis but their function has not yet been confirmed in *in vitro* or *in vivo* experiments (Vilakazi et al., 2017). Currently, there is no consistent model of how LPS may be processed and transported in the plant apoplast.

With the identification of LORE in *A. thaliana*, the first key component of LPS perception was discovered (Ranf et al., 2015). Close homologs of this lectin S-domain receptor kinase only exist in cruciferous plants. Recent studies report, CERK1 is involved in immune sensing of LPS in rice but CERK1 and other LysM proteins do not seem to be

required for LPS perception in *A. thaliana* (Desaki et al., 2018). *Pseudomonas* lipid A was shown to induce a late ROS burst in different plant species, which was only partially dependent on LORE in *A. thaliana* (Shang-Guan et al., 2018). Furthermore, LORE seems to be only involved in the sensing of LPS from pseudomonads and xanthomonads but not from enterobacteria such as *E. coli* and *S. enterica* (Ranf et al., 2015). Taken together, these findings indicate that LPS perception systems might exist, which are independent of LORE or CERK1 and which possibly posses a different sensing specificity. Beyond that, it remains unresolved whether LPS is directly perceived by LORE and CERK1 or if yet unknown receptors and co-factors are required (Desaki et al., 2018; Ranf et al., 2016). In summary, while it is evident that LPS is sensed as MAMP in plants, there are many open questions about the key components required for LPS sensing and the specificity of LPS perception systems in plants.

1.4.3 Lipopolysaccharide and LORE-dependent elicitation of plant immunity

LORE was identified in a forward-genetics screen for *A. thaliana* mutants with impaired PTI responses to LPS preparations of *P. aeruginosa*. Further analyses indicated that LORE is required for the sensing of LPS from various *Pseudomonas* and *Xanthomonas* species including the economically relevant plant pathogens *Pst* and *X. campestris*. With the identification of this key immune component it was possible to unambiguously evaluate the relevance of LPS recognition in plant immunity. *A. thaliana lore* mutants were more susceptible to *Pst* infection than the respective wild type plants. In contrast to the wild type, *lore* plants displayed no LPS-induced resistance against *Pst* after pre-treatment with *P. aeruginosa* LPS preparations (Ranf et al., 2015). *P. aeruginosa* lipid A is sufficient to induce LORE-dependent PTI, however, treatment with the corresponding OPS-deficient LPS elicited stronger early PTI responses. In the experiments of Ranf et al. (2015), *A. thaliana* plants did not respond to core-OS directly, but it could possibly influence LPS delivery or the perception process of LORE. Interestingly, in the same study enterobacterial LPS or Kdo_2-lipid A of *E. coli* did not elicit any LORE-dependent immune responses in *A. thaliana*. This constitutes an inverted specificity compared to the TLR4/MD2-mediated lipid A recognition system in humans, which is strongly induced by *E. coli* lipid A while *P. aeruginosa* lipid A is considered a weak elicitor (Alexander and Rietschel, 2001). *E. coli* and *P. aeruginosa* lipid A differ in the respective acylation pattern (see section 1.3.2). While *P. aeruginosa* produces mainly penta-acylated lipid A with shorter acyl chains (C10/C12), *E. coli* lipid A is usually asymmetrically hexa-acylated with longer acyl chains (C12/C14) (Raetz and Whitfield, 2002). In humans, the three dimensional shape of lipid A impacts recognition by TLR4/MD2. The three-dimensional shape is influenced by the grade and symmetry of the acylation as well as the acyl chain length (Netea et al., 2002). *P. aeruginosa* LPS treated with hydrazine contains lipid A with only the amide-bound acyl chains and does not trigger PTI responses in *A. thaliana* (Ranf et al., 2015). Similarly, phosphorylation and acylation patterns influence the immunogenicity of *X. campestris* pv. *campestris* LPS in *A. thaliana* (Silipo et al., 2008). Finally, these findings indicate

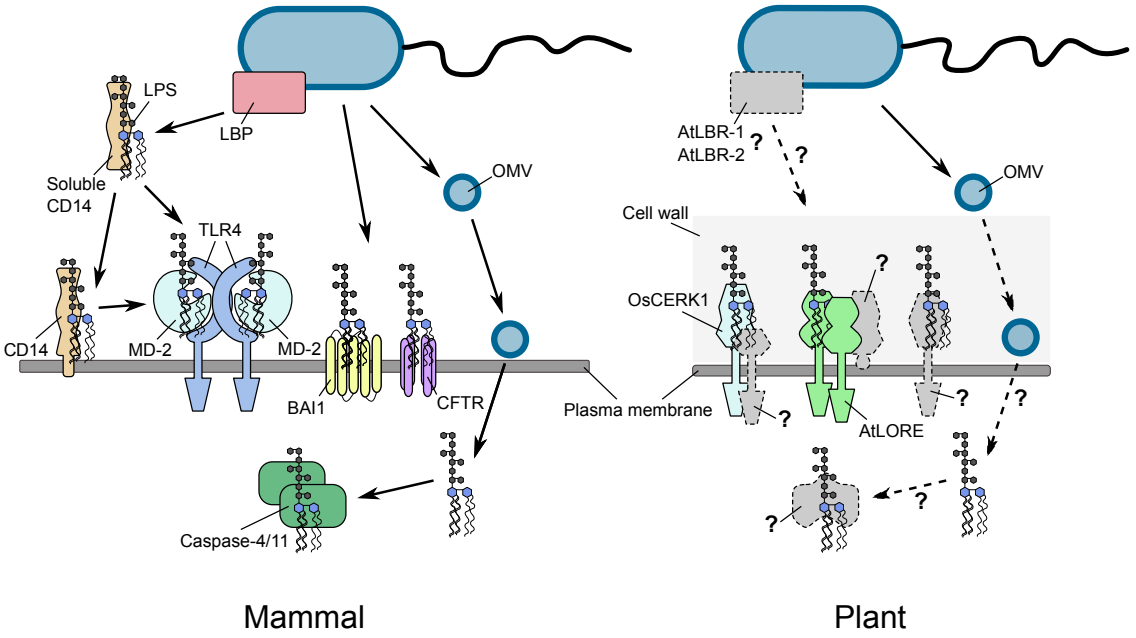

Figure 1.7: In mammals, LPS is sensed by different extracellular and intracellular immune receptors. In mammalian serum, LPS is extracted from the bacterial OM by LBP and transferred to soluble or membrane bound CD14. LPS can be further transferred to the TLR4-MD-2 complex. Additionally, the membrane proteins BAI1 and CFTR can perceive the core-OS of LPS. OMVs facilitate intracellular delivery of LPS for caspase-4/11 sensing. In plants, apoplastic *At*LBR proteins can bind LPS, but it is unknown if this facilitates LPS disaggregation or transfer to putative LPS receptors. In *A. thaliana*, the bulb-type lectin receptor-like kinase LORE is essential for the perception of Pseudomonas LA. In rice, the LysM-type receptor-like kinase CERK1 is required for LPS immune sensing. Further LPS receptors as well as additional signalling components have yet to be discovered (figure from publication I).

that LORE-dependent LPS sensing depends on the acyl chain length as well as on the acylation pattern. However, it remains to be resolved which structural features of lipid A are essential for the perception of LPS in *A. thaliana* and if/how it is influenced by the core-OS.

1.4.4 Dynamic lipopolysaccharide modifications as virulence strategy

The molecular structure of LPS influences the resilience to biotic and abiotic stresses of Gram-negative bacteria and is important for immune evasion and host colonization processes. Constitutive modifications of the LPS structure result from long-term adaption processes of bacteria to their natural habitats. Additionally, dynamic modifications also facilitate quick adaption in response to environmental changes and stresses (Li et al., 2012; Needham and Trent, 2013) (Fig. 1.8). Non-stoichiometric modifications of the core-OS and OPS such as phosphorylation, acetylation and addition of other substituents enhance resistance to CAMPs and to components of the mammalian adaptive and innate immune system (Knirel et al., 2006; Meredith et al., 2007). Dynamic remodelling and modification of the lipid A moiety leads to alterations of membrane integrity and fluidity, increased CAMP resistance and influences LPS immunogenicity. This enables the bacteria to rapidly switch

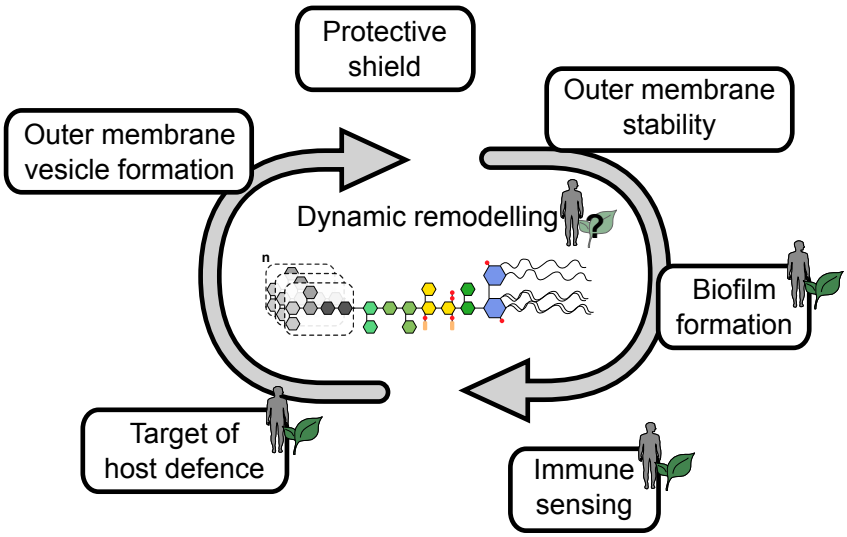

Figure 1.8: Functions of LPS substructures. LPS is fundamental to the physicochemical properties and biological functions of the OM. As part of an adaptation mechanism to changing environmental conditions, LPS structures can be dynamically remodelled. For mammalian pathogens, this is a well-known virulence strategy to promote host colonization.

between lifestyles to increase survival capabilities and promote pathogenesis (Li et al., 2012) (Fig. 1.8). The dynamic modifications are orchestrated in a highly responsive manner by a complex mechanism including transcriptional and post-translational control by two-component sensor systems. In *P. aeruginosa*, such two-component systems include PhoPQ, PmrAB, ParRS and CprRS. They regulate dynamic modifications such as the addition of aminoarabinose or phosphoethanolamine to the lipid A backbone by the transferases ArnT or EptA and alteration of the lipid A acylation by the 3-O-deacylase PagL and the palmitoyltransferase PagP in response to presence of specific CAMPs and changes in ion availability Needham and Trent (2013). PagL removes the 3-OH-C10 acyl chain from the lipid A and reduces LPS-triggered TLR4 activation in mammals. Expression of *pagL* is up-regulated by the PhoPQ regulation system in response to an increasing Mg^{2+} concentration or temperature, which might reflect conditions found in the host (Ernst et al., 2006, 2003; Needham and Trent, 2013). Accordingly, *P. aeruginosa* isolates from acute infections mainly produce penta-acylated lipid A due to an increased PagL activity. Upon transmission to the host, the bacteria might escape immune recognition to facilitate colonization and to establish an infection (Fig. 1.8). Interestingly, *P. aeruginosa* isolates from chronic infections show a loss of PagL function as part of adaption processes and thus produce hexa-acylated lipid A (Ernst et al., 2006).

Two-component systems described and characterized in *P. syringae* include RhpRS, CvsSR, CorSPR and GacSA. While RhpRS and CvsSR regulate the type III secretion system, CorSPR influences coronatine biosynthesis and GacSA regulates the synthesis of extracellular products (Deng et al., 2014; Fishman et al., 2018; Heeb and Haas, 2001; Ullrich et al., 1995). While putative homologs of the PhoPQ regulation system and PagL exist in *P. syringae*, experimental evidence for a functional conservation of the respective

genes and studies of potential dynamic remodelling of lipid A are lacking. First insights into specificity of LPS perception in plants hint towards an influence of lipid A acylation on immune recognition. Therefore, dynamic alterations in the lipid A acylation could not only contribute to adjustment of membrane properties but might facilitate an evasion of the immune system also in plants. However, before such effects of LPS remodelling *in planta* can be studied mechanistically, the mechanism and specificity of LPS perception in plants have to be elucidated.

1.5 Objectives

The aim of this thesis is to improve the understanding of the various roles of the different parts of LPS of plant-associated *Pseudomonas* bacteria in interaction with the plant host, with a focus on plant immune activation and virulence function during plant infection. To this end, the different LPS substructures were purified and their plant immune activation potential was analyzed. Mutant strains with modified LPS structures were generated, structurally analyzed and tested for their infection capability to assess their contribution to survival and virulence during plant infection. In the scope of this thesis, the following objectives were addressed in detail:

1. **Analysis of the biosynthesis and structure of LPS from plant-associated *Pseudomonas* strains**
   How is LPS synthesized in plant-associated *Pseudomonas* strains and are the genetic elements of LPS synthesis in *P. aeruginosa* conserved within the genus? What are structural features characteristic for *Pseudomonas* LPS?

2. **Specificity of LPS perception in *A. thaliana***
   What is the minimal epitope of LPS required for the recognition by LORE? Beyond the epitope, is perception specificity influenced by the overall LPS structure?

3. **Virulence function of LPS during plant colonization and infection**
   How does LPS affect virulence of plant-pathogenic bacteria beyond immune perception? Is LPS a general virulence factor in infections of plants by Gram-negative bacteria? How does the molecular structure of LPS contribute to its putative role in virulence?

2 Embedded Publications

2.1 The multifaceted functions of lipopolysaccharide in plant-bacteria interactions (Publication I)

Alexander Kutschera and Stefanie Ranf

Published 2018 in *Biochimie*, 159:93–98.

Summary

In Gram-negative bacteria, LPS constitutes the major component of the OM. Its structure includes three domains which are defined by their different chemical and biological properties: the lipophilic lipid A moiety, the negatively charged core-OS, and the hydrophilic OPS. LPS directly contributes to selective permeability and integrity stabilizing properties of the OM. Due to its outermost localisation on the bacterial surface, LPS takes part in interactions between bacteria and their environment. In this review we discuss recent findings in immune sensing of LPS in plants and draw comparisons to LPS perception in animals. Perception systems targeting different LPS domains and putative components which deliver LPS to immune receptors are elucidated. We summarize the multiple functions of LPS in plant colonization with focus on its role for the resistance of bacteria to biotic and abiotic stresses, attachment processes and release of OM vesicles. We briefly describe the complex coordination of LPS remodelling to adjust OM properties as response to environmental changes while simultaneously facilitating the bypass of plant immunity. Finally, we highlight open questions and point out possible obstacles for future studies of LPS function in plant-bacteria interactions.

Contributions

I drafted the manuscript and designed the figures. After critical revision of Stefanie Ranf, I finalized the manuscript and the figures for submission.

2.2 Loss of *wbpL* disrupts O-polysaccharide synthesis and impairs virulence of plant-associated *Pseudomonas* strains (Publication II)

Alexander Kutschera, Ursula Schombel, Michelle Wröbel, Nicolas Gisch and Stefanie Ranf

Published 2019 in *Molecular Plant Pathology*, 20:1535-1549.

Summary

OPS is the most variable substructure of LPS. It contributes to the stability of the cell envelope, provides protection against antimicrobial compounds, and is vital for immune evasion processes of mammalian pathogens. However, the function of OPS from plant-associated bacteria and its role during plant-microbe interactions remain largely unknown. In *P. aeruginosa*, independent gene clusters code for enzymes which are required for synthesis of its two OPS variants, CPA and OSA. To study OPS biosynthesis on a genomic level, we performed a comparative genome analysis on plant-associated *Pseudomonas* strains. The results indicated that the CPA cluster is only conserved in bacteria of the *P. fluorescens* group. While the putative locus of the OSA cluster could be identified in all analyzed *Pseudomonas* strains, no orthologs were found for most of the functionally relevant genes of OSA biosynthesis. Nevertheless, the *wbpL* and *wbpM* genes appeared to be conserved in the *Pseudomonas* spp. genomes. The glycosyltransferase WbpL initiates CPA as well as OSA synthesis in *P. aeruginosa* PAO1. Knockout of the *wbpL* orthologs in *Pst* and *Pseudomonas cichorii* ATCC10857/DSM50259 (*Pci*) resulted in OPS-deficient mutants and thus indicated a functional conservation of the enzyme. To understand the role of OPS in plant-bacteria interactions, we analyzed the virulence of the mutant strains in infection experiments. Less *Pst* Δ*wbpL* cells enter the leaf tissue of *A. thaliana* after surface inoculation and their apoplastic amplification is decreased. Similarly, disease symptoms caused by *Pci* Δ*wbpL* on lettuce (*Lactuca sativa*) were significantly reduced compared to the wild type. A reduced swarming motility of *Pst* and *Pci* Δ*wbpL* strains might be causal for the reduced virulence observed during infection experiments. In summary, our results indicate that an intact OPS is required for full virulence, possibly because of the protective properties of OPS against plant antimicrobial compounds. We hypothesize, due to its impact on cell-surface polarity, OPS influences bacterial motility and biofilm formation. This in turn affects epiphytic survival, tissue entry and dissemination in the plant apoplast.

Contributions

For this study, I developed a python script to perform multi-BLAST (blastp) experiments with a custom protein database to analyze and compare bacterial genomes. I then used the script to conduct genomic synteny analyses. Together with Stefanie Ranf, I developed a Golden Gate-compatible plasmid system for generating insertion deletions of genes in *Pseudomonas*. I performed genetic knockout and complementation of *wbpL* in *Pst*

and *Pci* as well as LPS isolation and analysis by SDS-PAGE. Finally, I conducted plant infection experiments and analyzed swarming motility of the bacteria strains. I analyzed the respective data sets and prepared figures for the manuscript. All results were interpreted and discussed by myself, Stefanie Ranf and Nicolas Gisch. I drafted the manuscript and critically revised with help of Stefanie Ranf and Nicolas Gisch. I performed all additional experiments and changes in the written manuscript suggested by the reviewers. I was responsible for the final preparation and submission of the manuscript to the journal as well as for correspondence with the journal editor and reviewers.

2.3 Community-led comparative genomic and phenotypic analysis of the aquaculture pathogen *Pseudomonas baetica* a390T sequenced by Ion semiconductor and Nanopore technologies (Publication III)

Ainsley Beaton, Cedric Lood, Edward Cunningham-Oakes, Alison MacFadyen, Alex J. Mullins, Walid El Bestawy, João Botelho, Sylvie Chevalier, Shannon Coleman, Chloe Dalzell, Stephen K. Dolan, Alberto Faccenda, Maarten G. K. Ghequire, Steven Higgins, Alexander Kutschera, Jordan Murray, Martha Redway, Talal Salih, Ana C. da Silva, Brian A. Smith, Nathan Smits, Ryan Thomson1, Stuart Woodcock, Martin Welch, Pierre Cornelis, Rob Lavigne, Vera van Noort and Nicolas P. Tucker

Published 2018 in *FEMS Microbiology Letters*, 365(9).

Summary

The collaboration for this publication was initiated as part of the genomics forum of the 16th international conference on *Pseudomonas* in September 2017. The genome of a recently described *Pseudomonas baetica* strain, isolated from infected wedge sole fish (*Dicologlossa cuneata*), was sequenced. The genome was analyzed mainly by PhD students and young postdocs in a collaborative project.

P. baetica was classified as a member of the *P. fluorescens* group and was found to predominantly cause disease in the marine wedge sole fish. *P. baetica* was originally isolated from aquacultural fish and it is assumed to be an opportunistic pathogen. Our phylogenetic analyzes confirmed the previous classification. Genome comparison revealed the conservation of genus-specific genomic features. Analysis of orthologs of lipid A synthesis enzymes showed a high similarity to other *Pseudomonas* species and a conservation of the LpxA hydrocarbon ruler motif. Further analyses led to the identification of a putative core-OS biosynthesis cluster, which lacked a homolog of the core-OS heptose kinase WapP (PA5008) of *P. aeruginosa* PAO1. The overall conservation of lipid A and core-OS biosynthesis genes suggested a lipid A and core-OS similar to *P. aeruginosa* possibly with minor alteration e.g. reduced phosphorylation of the core-OS. In accordance with the general genus characteristics, the whole genome analysis indicated that *P. baetica* is a versatile and highly adaptive species, which is most likely an opportunistic pathogen not restricted to a marine environment.

Contribution

I identified lipid A and core-OS synthesis genes in the *P. baetica* a390T genome and compared them with the respective gene cluster of other *Pseudomonas* species. I interpreted the data and contributed the respective results section to the manuscript.

2.4 Bacterial medium chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants (Publication IV)

Alexander Kutschera*, Corinna Dawid*, Nicolas Gisch, Christian Schmid, Lars Raasch, Tim Gerster, Milena Schäffer, Elwira Smakowska-Luzan, Youssef Belkhadir, Corina Vlot, Courtney E. Chandler, Romain Schellenberger, Dominik Schwudke, Robert K. Ernst, Stephan Dorey, Ralph Hückelhoven, Thomas Hofmann and Stefanie Ranf (*authors contributed equally)

Published 2019 in *Science*, 364(6436):178–181.

Summary

In plants, LPS-sensing elements were unknown for a long time until the bulb-type lectin receptor-like kinase LORE was identified in *A. thaliana*. LORE mediates the induction of PTI responses upon treatment with LPS or lipid A preparations of *Pseudomonas* or *Xanthomonas*, while enterobacterial LPS or lipid A preparations did not trigger any immune responses in *A. thaliana*. Since lipid A was sufficient to induce LORE-dependent immune responses, we screened LPS preparations from various bacterial species with different lipid A structures to elucidate the sensing specificity of LORE. The presence of ester bound 3-hydroxy C10:0 acyl chains in the lipid A structure was found to be essential for activating LORE signalling. Furthermore, we showed that synthetic 3-hydroxy decanoic acid at nanomolar concentrations is sufficient to induce LORE-dependent immune responses in *A. thaliana*. Experiments with other synthetic 3-hydroxy fatty acids and synthetic derivatives indicated that immunogenicity depends on carbon chain length. Sensing required an unsubstituted 3-hydroxy group and only tolerated small substituents at the carboxyl group. Further analysis and re-purification of LPS preparations revealed that not LPS, but free 3-hydroxy decanoic acid, which presumably co-purifies with LPS, induces PTI in *A. thaliana*. Similarly, other bacterial compounds which contain 3-hydroxy fatty acid substructures such as rhamnolipids, lipopeptides, and acyl-homoserine-lactones did not trigger LORE-dependent immune responses. Free 3-hydroxy fatty acid could be released by various metabolic processes in bacteria. Therefore, we propose that the recognition of 3-hydroxy fatty acid by LORE constitutes a novel perception system for low-complexity metabolites in plants.

Contribution

For this study, I isolated LPS and analyzed it by urea SDS-PAGE. I developed and performed a heat-detergent-mediated re-purification method for LPS preparations to deplete free 3-hydroxy decanoic acid. I designed, performed and analyzed infection experiments of *A. thaliana* as well as calcium, reactive oxygen species (ROS) and peroxidase assays. For this data, I prepared the figure drafts and wrote the methods sections. I contributed ideas, interpreted and discussed the results and critically revised the manuscript.

2.5 Analysis of the core oligosaccharide structure and the genetic background of its biosynthesis in *Pseudomonas syringae* pv. *tomato* DC3000 (Publication V)

Alexander Kutschera, Ursula Schombel, Dominik Schwudke, Stefanie Ranf and Nicolas Gisch

Published 2021 in *International Journal of Molecular Sciences*, 22(6):3250.

LPS directly influences membrane stability and is essential for bacterial survival and virulence. The negatively charged residues of the core-OS enable cross-linking between LPS molecules via interactions with divalent cations. Genetics of core-OS synthesis and resulting structures have been thoroughly investigated in mammalian pathogens such as *E. coli* or *P. aeruginosa*. The genetic background of core-OS synthesis remains unresolved in plant-associated *Pseudomonas* strains and only a few core-OS structures have been analyzed to date. As part of this study, we identified the core-OS gene cluster in *Pst* by comparative synteny analysis utilizing the genetic information available for *P. aeruginosa*. We then used the corresponding gene and predicted protein sequences to conduct comparative genome analyses and to reveal differences and similarities within the *P. syringae* species complex and other plant-associated pseudomonads. The analysis showed a high conservation of genes involved in the synthesis of the inner core-OS but variations in the genes of glycosyltransferases which are responsible for outer core-OS synthesis. The findings suggest a structural conservation of the core-OS within the subgroups of the *P. syringae* complex. Structure elucidation of the *Pst* core-OS revealed an uncommonly high degree of phosphorylation, which is likely characteristic for the genus *Pseudomonas*. Further comparison of the structure to the core-OS of other *P. syringae* species confirmed the conservation indicated by the genomic analysis.

Contribution

I performed bioinformatic analyses and preparation of *Pst* Δ*wbpL* LPS samples for this study. I analyzed and interpreted NMR data with the help of Nicolas Gisch. All results were discussed and interpreted by myself, Nicolas Gisch and Stefanie Ranf. I wrote the first draft of the manuscript together with Nicolas Gisch, which was critically revised by Stefanie Ranf.

2.6 Variation of the O-polysaccharide length distribution in plant-associated *Pseudomonas* strains (Publication VI)

Alexander Kutschera and Stefanie Ranf

Published 2019 on *figshare*, doi:10.6084/m9.figshare.8208932.v2 (non peer-reviewed).

Summary

OPS is the distal substructure of LPS and usually accounts for the majority of the molecular weight of the glycolipid. The number of repeating units and thus the size of the polysaccharide can vary considerably on a single bacterial cell. Accordingly, LPS samples separated via urea SDS-PAGE display a distinct size distribution pattern. I extracted LPS from different plant-associated *Pseudomonas* species and analyzed it via urea SDS-PAGE and silver staining to characterize their size distribution. The result shows that the size distributions can differ significantly even between different isolates of *P. syringae* pathovars.

Contribution

I isolated the LPS samples and performed the urea SDS-PAGE as well as the silver staining. I wrote the description and submitted the figure to figshare, an open access repository for scientific images and figures, after critical revision by Stefanie Ranf.

2.7 Rhamnolipids and their 3-(3-hydroxyalkanoyloxy)alkanoic acid precursors activate *Arabidopsis* innate immunity through two independent mechanisms (Publication VII)

Romain Schellenberger, Jérôme Crouzet, Arvin Nickzad, Alexander Kutschera, Tim Gerster, Nicolas Borie, Corinna Dawid, Maude Cloutier, Sandra Villaume, Sandrine Dhondt-Cordelier, Jane Hubert, Sylvain Cordelier, Florence Mazeyrat-Gourbeyre, Christian Schmid, Marc Ongena, Jean-Hugues Renault, Arnaud Haudrechy, Thomas Hofmann, Fabienne Baillieul, Christophe Clément, Cyril Zipfel, Charles Gauthier, Eric Déziel, Stefanie Ranf and Stéphan Dorey

Published 2020 on *bioRxiv*, 2020.12.18.423392 (non peer-reviewed).

Summary

Rhamnolipids (RLs) are glycolipids which are considered to be biosurfactants. They are composed of a L-rhamnose mono- or disaccharide head group and a (*R*)-3-hydroxyalkanoate (HAA) moiety with varying chain length. RLs are secreted by different Gram-negative bacteria including *Pseudomonas* and *Burkholderia* species and are required for swarming motility and biofilm formation. As part of this study we showed that the lipid secretome of *P. aeruginosa* triggers immune responses in *A. thaliana*. It contains RLs, HAAs with medium chain (mc)-acyl chains and mc-3-hydroxy fatty acids. Perception of HAAs and mc-3-hydroxy fatty acids is LORE-dependent. RLs, however, trigger atypical immune responses in *A. thaliana*, probably through a non-canonical perception pathway independent of LORE. *A. thaliana* mutants with altered sphingolipid synthesis displayed a reduced long-term ROS response after treatment with RLs. These findings might indicate the sphingolipid content and/or order state of the plasma membranes possibly influences the perception of RLs.

Contribution

I performed and analyzed preliminary calcium, ROS and peroxidase assays with RL and HAA samples.

3 Discussion

3.1 Insights into immune recognition of lipopolysaccharide in plants

LPS is considered a MAMP, which is sensed by the plant immune system. First experiments showed that LPS preparations from different Gram-negative bacteria elicit immune responses in plants (Dow et al., 2000). In recent years, a lot of effort was put into identifying immune components of LPS perception in plants. Especially the search for an LPS receptor was pursued by many groups within the research field. With LORE, the first key component of LPS perception was allegedly identified in *A. thaliana* (Ranf et al., 2015). Later, Desaki et al. (2018) showed that CERK1 is required for LPS sensing in rice. The underlying mechanism as well as the respective epitope of LPS, however, remained largely unknown. In fact, the search for the minimal motif required for immune activation in *A. thaliana* revealed not LPS, but free 3-OH-C10 is sensed in an LORE-dependent manner (publication IV).

3.1.1 Lipopolysaccharide is not recognized by LORE

Ranf et al. (2015) found that certain acylation patterns of the lipid A correlated with immune responses in *A. thaliana*. For example, preparations of *E. coli* LPS with longer acyl chains (C12/C14) did not elicit a responses while preparations of *P. aeruginosa* LPS with shorter acyl chains (C10/C12) triggered immunity. Screening of LPS samples with different acylation patterns revealed the presence of a 3-OH-C10 acylation corresponded with strong PTI responses in *A. thaliana* seedlings. Experiments showed synthetic 3-OH-fatty acids are sufficient to induce immune responses in a chain length-dependent manner. Accordingly, 3-OH-C10 elicited responses at nanomolar concentrations, while for example 3-OH-C14 did not trigger PTI in *A. thaliana* (publication IV). This confirmed earlier findings that enterobacterial LPS with predominantly C14 acyl chains does not elicit LORE-dependent responses (Ranf et al., 2015). Screening of synthetic 3-OH-C10 derivatives showed an unsubstituted 3-hydroxy is necessary for elicitor activity and only small substituents are tolerated at the carboxyl group (publication IV and VII). In lipid A, however, the fatty acids are covalently bound to the di-glucosamine backbone or to the hydroxy group of primary acyl chains via an amide or ester bond. Thus, they cannot be directly perceived by *A. thaliana* (see section 1.3.2). Indeed, analysis of HPLC-purified lipid A and LPS samples which elicited LORE-dependent PTI responses with novel analytical methods, confirmed they contain free 3-OH-C10. Heat-detergent purified LPS of *P. aeruginosa* H4 and *Pst* void of free 3-OH-C10 neither elicited early immune responses nor induced resistance against *Pst* infection in *A. thaliana*. This shows not *Pseudomonas* LPS but free 3-OH-fatty acids are perceived by LORE (publication IV).

3.1.2 LORE-independent perception of lipopolysaccharide in plants

LPS-triggered immune activation is reported for monocotyledon and dicotyledon plants (see publication I and section 1.4.2). LPS from *E. coli*, *S. enterica* or *Burkholderia* spp. triggers immune responses in *A. thaliana*, which are likely independent of LORE. This suggests that other, possibly additional, LPS perception pathways might exist (Ranf, 2016; Zeidler et al., 2004). On the contrary, experiments of Ranf et al. (2015) showed neither an elevation of cytosolic Ca^{2+} concentration nor a ROS burst in *A. thaliana* after treatment with LPS samples from *B. cepacia*, *Burkholderia pseudomallei*, *E. coli* or *S. enterica*. These contradicting results could be caused by technical issues in the experimental setup which are discussed in detail in section 3.1.3.

Recent studies showed treatment of *A. thaliana* with LPS and lipid A samples resulted in a strong, late ROS burst, which was only partially dependent on LORE (Shang-Guan et al., 2018). However, experiments with *P. aeruginosa* LPS purified from free 3-OH-C10 showed no late ROS burst in *A. thaliana* (publication IV). Thus, not LPS but another substance in the LPS preparations could possibly be responsible for the induction of the late ROS burst. In mammals multiple pathways for the immune perception of LPS exist, which target different LPS substructures (see section 1.4.1). In plants, core-OS and/or OPS might be sensed as MAMP as well. It was reported that synthetic oligo-rhamnans which mimic the L-rhamnose-rich OPS of plant-associated *Pseudomonas* strains, the core-OS-OPS section of *B. cepacia* LPS, and the core-OS of *X. campestris* pv. *campestris* elicit immune responses in *A. thaliana* (Bedini et al., 2002; Madala et al., 2011; Silipo et al., 2005). The core-OS of *P. aeruginosa* H4, however, was not sufficient to induce PTI in *A. thaliana* (Ranf et al., 2015). Identification of components of a LPS perception pathway might answer how immune responses are triggered by LPS in plants. LPS-interactor candidates in *A. thaliana* were detected via affinity purification and mass spectrometry analysis but remain to be functionally confirmed in genetic and biochemical studies (Vilakazi et al., 2017). In rice plants, the perception of LPS appears to be mediated by the LysM receptor CERK1, which is a key component of chitin perception (see section 1.2.3). LPS and lipid A with diverse acylation patterns elicit ROS production in suspension-cultured rice cells (Desaki et al., 2018). Given the structural similarity between the N-acetylglucosamine polymer chitin and di-glucosamine, it seems possible that CERK1 mediates recognition of the lipid A backbone independent of its acylation. In *A. thaliana*, however, CERK1 and other LysM-proteins appear not to be involved in LPS perception (Desaki et al., 2018). It remains unclear whether further, yet unknown receptors or co-factors besides CERK1 are required for the LPS perception in rice.

Taken together, current data suggests multiple LPS perception systems with different sensing specificity might exist. It is still unclear whether they comply with a canonical perception pathway mediated by a PRR or follow a different mechanism. It is also possible that the observed effects of LPS-treatment are caused by non-specific stress reactions to LPS or potential contaminants (discussed in section 3.1.3). Future studies will reveal

potential receptors and components of the LPS-perception systems and thus might clarify how LPS perception evolved in plants.

3.1.3 Lipopolysaccharide is a complex and technically challenging elicitor

The results of different studies investigating LPS perception in plants appear to be contradicting (see section 3.1.2). This raised the general question whether technical issues with the experiments might have misled the research field in the past. The discovery that LORE does not mediate the perception of *Pseudomonas* LPS but senses free 3-OH-fatty acids brings previous findings into a new light and requires them to be revisited (publication IV). Beyond that, this demonstrates the technical problems of PTI experiments with LPS. MAMPs are usually highly immunogenic substances and trace amounts are sufficient to elicit immune responses in plants. This involves the risk that co-purified contaminants are the actual active compound in a preparation of the alleged elicitor, especially when they are used in high concentrations (Ranf et al., 2016). Besides the identification of 3-OH-fatty acids in LPS extractions, other prominent examples support this thesis. Flagellin and bacterial cold-shock protein were discovered to be the actual elicitors in a harpin protein extract from *P. syringae tabaci* or in a peptidoglycan preparation, respectively (Felix and Boller, 2003; Felix et al., 1999). Usually, synthetic compounds are used to exclude contaminants and confirm the MAMP-activity when a minimal motif is identified. LPS/lipid A, however, is a complex molecule, which is difficult to synthesize. Depending on the synthesis process, synthetic compounds may also contain contaminations, which trigger plant immunity. Additionally, state-of-the-art analytical methods can aid to confirm the purity of an elicitor preparation, or, as in the case of 3-OH-C10, facilitate the identification of the actual active compound (publication IV).

The chemical properties of LPS itself cause difficulties when used in PTI experiments. Due to its amphiphilic nature, the glycolipid forms micelles when solved in polar substances such as water (Santos et al., 2003). This complicates the preparation of LPS solutions without the use of detergents or solvents harmful to plant cells. As a consequence, high LPS concentrations are often used in studies screening for possible LPS-receptors or perception components. This increases the risk of contamination, which can trigger unspecific PTI responses in plants (Ranf et al., 2016). Moreover, the studies of Nomura et al. (2008) indicate LPS can integrate into and disturb the integrity of lipid bilayers such as the plasma membrane of plant cells, which could be another cause of unspecific stress responses. LPS preparations contain a mixture of different LPS molecules, which makes it difficult to determine the molarity of the sample. While often different types of OPS can occur in parallel, also the size of the polysaccharide varies considerably on a single bacterial cell (see section 1.3.4 and publication II, VI). Besides non-stoichiometric modifications, LPS structure can be dynamically modulated according to the environmental conditions. Thus, cultivation conditions influence LPS structures and should be taken into consideration when experiments are planned and data is interpreted. Only a subfraction of the LPS

molecules with specific modifications could potentially be responsible for the elicitation of plant immunity (see section 1.4.4 and publication V) (Alexander and Rietschel, 2001; Needham and Trent, 2013). Again, this might result in the use of highly concentrated LPS samples, which potentially contain more contaminants as well (Ranf et al., 2016). Various pitfalls have to be avoided when LPS is used in plant immunity experiments and the risk of co-purification of immunogenic compounds has to be taken into account. Determination of the structure-activity relationship in combination with analytical methods can help to identify the active component in elicitor preparations (publication IV).

3.1.4 New perspectives on lipopolysaccharide perception in plants

With its conserved basic structure, high abundance and essential role for the viability of most Gram-negative bacteria, LPS possesses characteristic features of a MAMP. LPS recognition by the innate and adaptive immune system in mammals has been studied for several decades. Many insights into perception mechanisms from the animal field were gained in these years and research appeared to focus on finding similar features in plants. The studies conducted as part of this thesis aimed to identify the minimal MAMP-active motif of LPS, which is recognized by LORE. However, experiments revealed not LPS but mc-3-OH-fatty acids which are co-isolated during LPS extraction are sensed by *A. thaliana* through LORE. These findings confirm concerns in regard to technical challenges during the identification of MAMPs and question the interpretation of data from previous studies. LPS preparations used in these studies have to be re-analyzed with state-of-the-art analytical methods and experiments have to be repeated with re-purified samples. Beyond that, *A. thaliana lore* mutants can be used in PTI experiments in order to exclude an influence of 3-OH-fatty acids (publication IV). Current data shows neither re-purified LPS of *P. aeruginosa* H4 and *Pst* nor LPS of *E. coli* K12 elicit PTI responses or induce resistance against infection with *Pst* in *A. thaliana* (see 3.1.2 and publication IV) (Ranf et al., 2015). Likely, *Pseudomonas* lipid A is not recognized as a MAMP in *A. thaliana*. However, LPS might trigger non-canonical immune responses in plants (see section 3.1.3). This leaves CERK1 in rice as only known component of a putative LPS perception system in plants. Beyond *A. thaliana* other dicotyledons including *N. tabacum* and other solanaceous species respond to treatment with LPS preparations (see section 1.4.2). Genetic screens can reveal receptors and key components involved in immune sensing of LPS. Future structure-to-function studies could aid to identify the corresponding minimal-motifs, which are recognized by the plant and thereby prove that these plants perceive LPS as MAMP. The discovery of a novel key component of LPS sensing in dicotyledons could be a new starting point on the way to unravel the mechanism and evolution of LPS perception in plants.

3.2 Medium chain 3-hydroxy fatty acids as elicitors

Fatty acids are a basic building block in the composition of cell components and play an important role in metabolism. They take part in signalling pathways, are a source of energy and therefore also act as energy storage, modify proteins (acylation) and are a major component of lipid bilayers, which make up cell membranes (Rustan and Drevon, 2001). They are defined by a carboxyl group and an aliphatic chain of varying length which determines chemical properties of the molecule such as polarity and melting point. 3-OH-fatty acids, however, are not very common in animals but can occur for example as byproduct of an incomplete beta-oxidation in the lipid metabolism (Jin et al., 1992). They are closely associated with the LPS of Gram-negative bacteria and are therefore used as a biomarker for the presence of LPS in environmental samples (Saraf and Larsson, 1996; Szponar et al., 2002). However, also other bacterial molecules contain 3-OH-fatty acid building blocks (see 3.2.2). The discovery that LPS samples can contain free 3-OH-fatty acids also has an impact on the research field of human immunology.

3.2.1 3-Hydroxy fatty acids are sensed in mammalian cells

G-protein-coupled receptors (GPCRs) exist in a wide range of organisms and can perceive various types of ligands. In mammals, several GPCRs exist, which are known to perceive fatty acids and regulate corresponding metabolic fluxes (Blad et al., 2012). GPR84 is a pro-inflammatory receptor in human and mouse cells, which senses fatty acids with a chain length of C9 - C14 (Wang et al., 2006). Suzuki et al. (2013) could show 2-OH and 3-OH mc-fatty acids elicited stronger GPR84-dependent responses than non-hydroxylated ones. Consequently, mammalian cells could sense the free 3-OH-fatty acids contained in LPS preparations (publication IV). The medium effective concentration required for a response to 2-OH-C10, 3-OH-C10, 2-OH-C12, and 3-OH-C12 were 31 µM, 230 µM, 9.9 µM, and 13 µM respectively (Suzuki et al., 2013). The highest concentrations of free 3-OH-C10 in LPS samples measured in our study (publication IV) was around 2 µM in a solution of 100 µg/ml. However, LPS concentrations used in experiments with mammalian cells usually range between 10 ng - 1 µg/mL (Chentouh et al., 2018; Coats et al., 2005). This suggests corresponding levels of free 3-OH-C10 might be too low to be perceived by GPR84. Notably, when exposed to hydroxylated fatty acids, GPR84 promotes the cytokine production of human peripheral polymorphonuclear leukocytes, which were pretreated with LPS. Although the LPS concentration of 100 ng/ml presumably contains free 3-OH-C10 below the response-threshold, they could potentially lead to a preactivation of the corresponding signalling pathway (Suzuki et al., 2013). Analysis of the used LPS preparation to measure the actual content of free 3-OH-fatty acids and eventual re-purification of the samples is necessary to exclude an influence of co-purified free 3-OH-fatty acids.

GPCRs constitute one of the largest gene families but no close homologs of GPR84 with a similar function could be identified in humans (Blad et al., 2012; Suzuki et al., 2013). A GPR84 ortholog exists in mice and possibly in other mammals and no other perception component of hydroxylated mc-fatty acids has been described to date (Wang et al., 2006). In *A. thaliana*, only one putative GPCR, called GCR1, exists, which is likely a regulator of the cell-cycle (Colucci et al., 2002; Taddese et al., 2014). LORE, however, is a plant specific bulb-type lectin S-domain-1 receptor-like kinase and putative homologs only exist in some members of the plant family of Brassicaceae (Ranf et al., 2015). This indicates GPR84 and LORE evolved independently to sense mc-3-OH-fatty acids.

3.2.2 3-Hydroxy fatty acids could originate from multiple bacterial compounds

The functional classification of mc-3-OH-fatty acid perception in plants depends on the origin of this elicitor. Although 3-OH-fatty acids co-purify with LPS and are an essential building block of lipid A, they do not necessarily originate from LPS molecules. Microbial compounds comprising 3-OH-C10 building blocks are highly abundant in bacteria of the genus *Pseudomonas* but not exclusive to them. Such compounds are: N-acyl homoserine lactones (AHLs), Lipopeptides (LPs), Polyhydroxyalkanoates (PHAs), RLs and LPS (publication IV and VII). While LPS, for example, does not exist in Gram-positive bacteria, some strains produce PHAs, which might contain mc-3-OH-fatty building blocks as well (Valappil et al., 2007). Beyond that, 3-OH-acyl moieties can occur bound to ACP or coenzyme A (CoA) as metabolic intermediates of metabolic pathways such as lipid synthesis or degradation. Notably, (*S*)-3-OH-C10 elicits weaker responses than (*R*)-3-OH-C10, which is presumably the preferred configuration in bacteria. Accordingly, mc-3-OH-fatty acid building blocks in bacterial compounds such as lipid A and PHAs are usually in (*R*)-configuration (publication IV)(Brandl et al., 1990). However, current data indicates, the mc-3-OH-fatty acids are sensed only in their free form and display no elicitor activity when the carboxyl or hydroxy groups are bound to a sterically complex substituent. Nevertheless, also precursors of bacterial compounds comprising 3-OH-C10 building blocks can be sensed if the 3-hydroxy group is not blocked and the substituent at the carboxyl group does not hinder the perception (publication IV). Indeed, for example HAAs, the precursors of RLs which consist of two 3-OH-fatty acids linked via an ester bond, trigger LORE-dependent PTI responses in *A. thaliana*. Yet, up to 50 times higher concentrations of HAAs are required to elicit similar responses as 3-OH-C10 (publication VII). A similar effect was also observed with other 3-OH-fatty acid esters (publication IV). LPS, AHLs and RLs comprising 3-OH-C10 moieties did not elicit LORE-dependent responses. The LP Massetolide A induced a minor elevation of cytosolic Ca^{2+} concentration, which could be attributed to small amounts of free 3-OH-C10 identified in this sample (see section 3.1.1 and publication IV). Thus, mc-3-OH-fatty acids have to be released to be immunogenic in *A. thaliana*.

AHLs are diffusible quorum sensing molecules released by various bacteria. They enter neighbouring cells and influence gene expression depending on their concentration (Shiner et al., 2005). Also host organisms including plants can perceive AHLs secreted by bacteria into their environment (Shiner et al., 2005; Teplitski et al., 2011). Bacteria modulate their quorum sensing through AHL degradation by endogenous acylases which releases free fatty acids. Plant-associated *Pseudomonas* spp., for example, produce 3-OH-C10-AHL or 3-oxo-C12-AHL in concentrations as high as 10 µM under laboratory conditions (Ortori et al., 2011; Shiner et al., 2005). Due to keto-enol tautomerism, 3-oxo-fatty acids can also be present as unsaturated 3-OH-fatty acids and trigger LORE-dependent immune responses in *A. thaliana* (publication IV). Degradation of such AHLs might therefore be a possible source for free mc-3-OH-fatty acids sensed by LORE. Furthermore, bacteria produce various types of LPs. Some of them function as surfactants while others are toxins or potent antimicrobial substances released to fend off competing microbes (Tran et al., 2007). Massetolide A, an LP comprising a 3-OH-C10-acyl moiety, is released by *P. fluorescens* during the colonization of host-plants (de Bruijn et al., 2008). Free 3-OH-C10 might be cleaved off Massetolide A by hydrolysis of the peptide bond via proteolytic enzymes. In plants, a broad range of such enzymes exist, which, among others, play a role in defense responses (Sebastián et al., 2018; Xia, 2004). To date, however, hydrolysis of LPs and release of the acyl group *in planta* has not been reported yet. Similarly, PHAs and RLs, which function as carbon storage or surfactant respectively, comprise ester or ether bound mc-3-OH-acyl moieties, which can be released via enzymatic hydrolysis (Abdel-Mawgoud et al., 2010; Jendrossek and Handrick, 2002). PHAs are commonly composed of short chain 3-OH-fatty acids (<C8) but PHAs with longer acyl-moieties are also produced in bacteria (Steinbüchel and Valentin, 1995; Verlinden et al., 2007). Notably, 3-OH-C10 fatty acid is the major component of PHAs in *P. aeruginosa* (Soberón-Chávez et al., 2005). An active degradation of these molecules *in planta* has not been reported to date. Although treatment with RLs, Massetolide A and AHLs elicited neither an elevation of cytosolic Ca^{2+} concentration nor a ROS burst, free mc-3-OH-fatty acids might be released by plant enzymes over time (publication IV). Further experiments analyzing later immune responses and systemic resistance induced by theses compounds could reveal whether possible immune responses are LORE-dependent and are thus caused by released mc-3-OH-fatty acids. However, current data suggests that re-purified LPS samples do not elicit LORE-dependent early or late immune responses in *A. thaliana* (publication IV).

3.2.3 Medium chain 3-hydroxy fatty acids are metabolic byproducts of bacteria

Besides being released from bacterial compounds *in planta*, free mc-3-OH-fatty acids might occur as byproduct of bacterial metabolism. LPS, for example, is synthesized with a hexa-acylated lipid A, but the acylation pattern is often modified post-synthesis (see section 1.3.2 and 1.4.4) (Needham and Trent, 2013). The PagL deacylase removes the 3-OH-C10 acyl chain at position 3 of *P. aeruginosa* LPS to hamper recognition by TLR4

in humans (Rutten et al., 2006). The resulting high percentage of penta-acylated LPS is characteristic for *Pseudomonas* species including *Pst* (see section 1.8). The fate of the released 3-OH-C10 is unclear, but it seems likely that, due to a constant turnover of LPS molecules, a fraction of free 3-OH-C10 remains in the periplasm or is integrated into the cell membrane. However, while the LPS samples of *Pst* Δ*pagL* mutants comprise only hexa-acylated LPS molecules, they still contained free 3-OH-C10. This finding indicates that other sources of free 3-OH-C10 exist (publication IV). Similarly, PHAs are degraded by PHA-depolymerases, which fragment the polymer into oligomers, dimers and monomers including 3-OH-fatty acids (Jendrossek and Handrick, 2002). Furthermore, ACP- or CoA-bound mc-3-OH-acyl moieties occur in several primary metabolic pathways such as fatty acid synthesis or beta-oxidation, which could be released by thioesterase activity or non-enzymatically when released to the environment. In general, free mc-3-OH-fatty acids in the cytoplasma or periplasma originating from one of these sources could be released into the environment upon cell lysis. Additionally, bacteria constantly shed-off OMVs, which play an important role in colonization processes, including the directed delivery of virulence factors into the host cell (Bomberger et al., 2009; O'Donoghue et al., 2017). Outer inner membrane vesicles released by bacteria also contain fractions of the cytosol and could deliver free mc-3-OH-fatty acids to the plant plasma membrane (publication I). Beyond that, surfactants such as RLs influence the cell-surface properties of bacteria by extraction of LPS from the outer membrane (Al-Tahhan et al., 2000). Secretion of RLs might therefore contribute to the release of mc-3-OH-fatty acids associated to the LPS. Finally, free fatty acids can diffuse through membranes and could be passively released into the periphery of the bacteria (Hamilton et al., 2001). Taken together, it is conceivable that mc-3-OH-fatty acids are not produced by one specific pathway but through different pathways which might vary depending on the bacterial species. The fatty acids are likely released into the environment via different routes. In the case of plant-associated bacteria, released mc-3-OH-fatty acids can be sensed by the cell-surface receptor LORE and activate PTI in *A. thaliana*.

3.2.4 Release of medium chain 3-hydroxy fatty acids by the host

Beyond bacteria, plants themselves could be a possible source of free mc-3-OH-fatty acids. Each plant cell synthesizes fatty acids as part of the triacylglycerol metabolism (lipid metabolism). Since they also produce hydroxylated fatty acids, intermediates such as CoA-bound mc-3-OH-fatty acids could possibly occur in the plastids (Harwood, 1988; Xu and Shanklin, 2016). Upon tissue damage they could enter the apoplastic space where free mc-3-OH-fatty acids could be released enzymatically or non-enzymatically, which are subsequently perceived by neighbouring cells as DAMPs. Although pathways with 3-OH fatty acid intermediates have been described in Brassicaceae, plants usually synthesize non-hydroxylated long-chain (> C14) fatty acids (Bach et al., 2008; Li et al., 2018). In the past mc-3-OH-fatty acids isolated from plant material have been associated with bacteria (Smith, 1971). Therefore, it remains elusive whether mc-3-OH-acyl intermediates

originating from the plant actually occur in sufficient concentrations that the corresponding free mc-3-OH-fatty acids can elicit responses in neighbouring cells.

Another way of mc-3-OH-fatty acids release through the plant host is the degradation of microbial compounds containing 3-OH-acyl moieties (see section 3.2.2). These compounds are either actively secreted or released by bacteria as part of colonization processes (LPS, RLs, LPs and AHLs) or could be released upon cell lysis (PHAs and ACP- or CoA-bound mc-3-OH-acyl intermediates). Specific enzymes of the host might hydrolyse these compounds and mc-3-OH-fatty acids could become available for perception. For example, quorum sensing is disturbed by host-organisms through enzymatic degradation of AHLs. In *A. thaliana*, the fatty acid amide hydrolase FAAH cleaves AHLs and thereby releases free fatty acids (Ortíz-Castro et al., 2008). Indeed, FAAH appears to play a role in plant immunity and FAAH overexpressing lines are more susceptible to infection by adapted and nonhost pathogens (Kang et al., 2008). However, succeeding studies indicate that this effect is probably independent from FAAH activity and knockout mutants showed the same susceptibility as wild type plants (Kang et al., 2008; Kim et al., 2009). It remains to be elucidated whether FAAH activity is connected to LORE-dependent perception of mc-3-OH-fatty acids.

3.2.5 3-Hydroxy fatty acids act as antimicrobial agents

Multiple putative sources of free mc-3-OH-fatty acids such as microbial compounds or metabolic intermediates exist. Nevertheless, bacteria might actively secrete mc-3-OH-fatty acids into their environment. 3-OH-C10 can integrate into fungal membranes and damage them by altering the permeability of the cell wall. They are therefore considered to be a potent anti-fugal agent. The fatty acid 3-OH-C10 was described first in this context when it was detected in the royal jelly of honey bees (Weaver et al., 1968). Beyond that, leaf-cutting ants appear to protect their fungal gardens from contamination and harvesting ants prevent collected seed from germination by secreting 3-OH-C10 (Schildknecht and Koob, 1971). Similarly, Sjögren et al. (2003) reported that 3-OH-C10 produced and released by *Lactobacillus plantarum* (Gram-positive) has anti-fungal properties. Screens for RLs in the lipid secretome of *Pseudomonas* revealed 3-OH-C10 and other mc-3-OH-fatty acids are released (publication VII). Thus, bacteria might as well overcome possible microbial competitors during plant colonization by secreting mc-3-OH-fatty acids as antimicrobial agents. Analyses of the secretome from other plant-associated bacteria will reveal if 3-OH-C10 release is a common trait or only occurs in specific strains. Additionally, further elucidation of the putative antimicrobial activity and its relevance during plant-colonization is necessary.

3.2.6 Brassicaceae might monitor their microbiota by sensing medium chain 3-hydroxy fatty acids

LPS is not the only source of mc-3-OH-fatty acids. Other bacterial compounds and metabolic intermediates exist, which contain mc-3-OH-acyl moieties. Free mc-3-OH-fatty acids could be released from these compounds or metabolic mediates by endogenous enzymes, host enzymes or non-enzymatically upon cell lysis. 3-OH-C10 might as well be secreted by some bacteria as antimicrobial agent (see section 3.2.2, 3.2.3 and 3.2.5). Oyaizu and Komagata (1983) classified various *Pseudomonas* strains on the basis of their 3-OH-fatty acid profile. They found most of the analyzed strains produce mc-3-OH-fatty acids. Interestingly, those strains which appeared to lack 3-OH-C10 such as *Pseudomonas cepacia* (today: *Burkholderia cepacia*) were later classified as non-*Pseudomonas* strains. Other plant-associated bacteria such as *Xanthomonas* spp. produce microbial compounds, which comprise mc-3-OH-fatty acid building blocks and feature fatty acid profiles containing mc-3-OH-fatty acids as well (Stead, 1989, 1992). Thus the presence of many plant-associated bacteria could be sensed by *A. thaliana* in a LORE-dependent manner. Since not only pathogenic but also commensal and beneficial bacteria could be the origin of this elicitor, the level of free mc-3-OH-fatty acids might exceed a certain threshold when bacteria invade plant tissue and propagate aggressively. Therefore, it possible that perception of mc-3-OH-fatty acids could serve the purpose to monitor the plant microbiota and mount defense responses when infection attempts occur.

Phylogenetic analysis indicates putative orthologs of LORE only exist in the plant family of Brassicaceae (Ranf et al., 2015). Accordingly, *Eutrema salsugineum* and *Capsella rubella* respond to treatment with LPS preparations similar as *A. thaliana*. Thus, these species likely sense mc-3-OH-fatty acids as well. Screens of other Brassicaceae will reveal whether the function and sensing specificity of LORE orthologs is conserved or whether other chain length preferences exist.

3.3 Heterogeneity of lipopolysaccharide in plant-associated bacteria

LPS contributes to essential colonization processes and immune evasion strategies of bacteria including biofilm formation (Murphy et al., 2014) and complement resistance (Murray et al., 2006) in mammalian hosts. It confers resistance against environmental stresses and antimicrobial compounds such as CAMPs (Alexander and Rietschel, 2001). Molecular LPS structures of *Pseudomonas* spp. suggest a genus wide conservation of characteristic structural features. These include lipid A acylation with relatively short acyl chains (C10/C12) and the presence of heptose residues in the inner core-OS region (publication I)(King et al., 2009). *Pseudomonas* spp. are considered to be versatile and can inhabit hostile environments. The putative conserved LPS structures might increase resistance against biotic and abiotic stresses and, thus, could partially be responsible for the distinctive traits of the genus (Silby et al., 2011). Structural information about LPS

from plant-associated *Pseudomonas* strains is scarce and little is known about possible differences in LPS biosynthesis within the genus. Genome analyses for components of LPS biosynthesis in *Pseudomonas* of the *P. syringae* species complex indicate lipid A biosynthesis is conserved and probably only minor differences in core-OS synthesis exist (publication III and V). The OPS gene clusters of *P. aeruginosa*, however, are not conserved in most plant-associated *Pseudomonas* strains. Hence, synthesis might be regulated by a yet unknown set of genes (publication II).

3.3.1 Lipid A biosynthesis is conserved in *Pseudomonas* spp.

The search for orthologs of genes involved in the lipid A biosynthesis in *P. aeruginosa* suggests that the content of the respective gene cluster is generally conserved in *Pseudomonas* (publication III). Homologs of the *E. coli* acyltransferase LpxM could not be identified in the analyzed *Pseudomonas* genomes. This is in accordance with their classification as group II Gammaproteobacteria, which all lack a LpxM homolog (Opiyo et al., 2010). Putative orthologs of the *P. aeruginosa* acyltransferases HtrB1 and HtrB2, which are responsible for secondary lipid A acylation, are present in the genomes (publication III, figure 1.4). In-depth analysis revealed high sequence similarities and conservation of the hydrocarbon ruler motif in the LpxA orthologs and possibly in the LpxD orthologs as well. Consequently, these findings suggest a conservation of a hexa-acylated lipid A structure with C10 and C12 primary acyl chains at position 3/3' and 2/2', respectively. Similarly, the conserved hydroxylases LpxO1 and LpxO2 indicate a common acylchain 3-hydroxylation. Lipid A structures of *Pseudomonads* resolved to date follow this pattern and confirm the bioinformatic studies (Lam et al., 2011; Molinaro et al., 2009). Post-synthetic modifications in response to environmental conditions influence the final lipid A structure and usually lead to diversification *in vivo* (publication I and IV).

3.3.2 Composition of the core oligosaccharide within the genus *Pseudomonas* is highly similar

Further analysis of *Pseudomonas* spp. genomes with a focus on the *P. syringae* species complex revealed that also the core-OS gene cluster is generally conserved in these species, but differs compared to other bacteria (publication V). Comparison of the results of the genomic analysis with the analyzed core-OS structure of *Pst* (publication V) and *P. syringae* pv. *phaseolicola* (Zdorovenko et al., 2004a) suggest that it might be possible to estimate the core-OS composition from the respective gene cluster content. While the cluster content with regard to inner core-OS synthesis is highly similar in most *P. syringae* pathovars, variations in the genes responsible for outer core-OS synthesis even occur within the species complex. However, published core-OS structures of *P. syringae* strains suggest that this part of the core-OS is defined by the presence of a proximal D-GalN and a terminal L-Rha residue (publication V)(Zdorovenko et al., 2004a,b, 2007). The conservation of the GalN-transferase *WaaG* in the core-OS synthesis cluster of analyzed genomes indicates

that D-GalN might also be a common structural feature. On the other hand, a putative Rha transferase gene homologous to *P. aeruginosa migA* was found to be only conserved in *P. syringae* pathovars but not in other species of the *P. syringae* complex (publication V). In *P. aeruginosa*, the position of the terminal L-Rha residue determines whether an OPS is linked to the core-OS during LPS synthesis. Transfer of L-Rha 1→6 by MigA leads to the synthesis of OPS-deficient LPS while transfer of L-Rha 1→3 by WapR results in LPS with OPS (Knirel et al., 2006; Kocincova and Lam, 2011). The ligation site of OPS to the core-OS has not been determined for *P. syringae* strains to date, because the high OPS:core-OS ratio impedes the analysis of the combined structure via NMR. Therefore, usually OPS-deficient mutant strains are used or the OPS is chemically hydrolyzed from the core-OS and analyzed separately (publication V). The presence of a terminal L-Rha residue in core-OS structures of plant-associated *Pseudomonas* strains suggests that it might function as an OPS ligation site. Interestingly, the *P. syringae* pathovars *phaseolicola* and *tomato* DC3000 posses two different core-OS glycoforms, with one lacking a terminal L-Rha completely (Molinaro et al. (2009); Zdorovenko et al. (2004a) and publication V). Analogously to *P. aeruginosa*, this could result in a capped and uncapped core-OS (i. e. with or without OPS), which would influence the amount of OPS-decorated LPS produced in this strain. However, it is also possible that the terminal β-GlcNAc residue acts as OPS ligation site: Contrary to L-Rha it is stoichiometrically present in the analyzed *Pst* core-OS. Genetic knockout of the respective Rha transferase (possibly PSPTO_1330 in *Pst* DC3000) could prove this. Additionally, the ratio of LPS molecules with or without OPS molecules is possibly influenced by the specificity of the required OPS-ligase and could be altered in response to environmental changes such as pH or temperature (publication I and II).

3.3.3 A high degree of core oligosaccharide phosphorylation is common for *Pseudomonas* spp.

The phosphate groups of the lipid A backbone and inner core OS mediate LPS cross-links via divalent cations, which are essential for cell wall integrity. Notably, compared to core-OS from other bacteria analyzed to date, the *P. aeruginosa* core-OS is the most phosphorylated one with up to five phosphate groups at the heptose residues (Kocincova and Lam, 2011). The high conservation of the core-OS gene cluster responsible for the inner core-OS synthesis revealed in the genome analyses suggests that the analyzed *Pseudomonas* strains could display a similar phosphorylation pattern. Especially the conservation of the *P. aeruginosa* heptose kinases WaaQ, WapP and WaaP corroborates that a high degree of core-OS phosphorylation might be common to *Pseudomonads* (publication V). Since this feature is associated with general resistance against environmental influences and intrinsic drug resistance, it probably contributes to the versatility and adaptation potential of these bacteria (Raetz and Whitfield, 2002; Silby et al., 2011) (see section 1.3.6). Disruption of core-OS phosphorylation in *P. aeruginosa* leads to the synthesis of truncated LPS molecules and disturbs LPS transport to the OM (Delucia et al., 2011). While *waaP* mutants of *S. enterica* and *E. coli* are viable, this is not the case for *P. aeruginosa* (Delucia et al., 2011;

Yethon et al., 2000, 1998). This further underlines the putative importance of the inner core-OS structure for the viability of *P. aeruginosa*, which is likely a common feature of the genus *Pseudomonas* (publication V).

OPS forms a steric barrier to shield off antimicrobial agents targeting the cross-links which are considered to be a weak point of the bacterial cell wall (Raetz and Whitfield, 2002; Silby et al., 2011). Several studies reported that interference with OPS synthesis has a negative impact on the virulence of plant pathogens and attenuates symbiosis capability of plant root symbionts (publication II). However, OPS-deficient Δ*wbpL* strains of *Pst* and *Pci* can multiply *in planta* and cause disease symptoms (publication II). The finding shows that although OPS protects the ionic cross-links between LPS molecules, loss of OPS does not necessarily result in avirulence. Polar effects of gene knockout might be responsible for the often strongly reduced virulence observed in other studies. Indeed, disruption of OPS synthesis by knockout of genes involved in monosaccharide precursor synthesis can affect other pathways such as exopolysaccharide synthesis and protein glycosylation (publication II) (Delucia et al., 2011; Liao et al., 2014). The OPS-deficient *P. syringae* pv. *syringae* 61 Δ*galU* strain for example was used to study effects on plant-bacteria interactions (Deng et al., 2010). Knockout of *galU* disrupts the synthesis of the monosaccharide precursor UDP-glucose, which results in truncation of the core-OS and hence loss of OPS. However, due to the possible polar effects on other pathways it is not possible to clearly attribute the observed effects such as a strongly reduced survivability *in planta* to either the truncated core-OS or the loss of OPS. Knockout of genes specific to OPS synthesis such as *wbpL* avoid any effects on core-OS synthesis (publication II and V). Further analyses of such OPS-deficient strains are necessary to elucidate whether the high degree of core-OS phosphorylation in *Pseudomonas* spp. itself mediates resistance against antimicrobials and if OPS is of less importance in this case.

3.3.4 O-polysaccharide gene cluster are not conserved in plant-associated *Pseudomonas*

The OPS composition of mammalian pathogens is highly variable, because its diversification is mainly driven by the need to overcome the adaptive immune system of their hosts (Whitfield and Trent, 2014) (see section 1.4.1). In contrast, OPS of plant-associated bacteria displays a low variability in saccharide composition and usually consists of a L-Rha backbone with lateral saccharide residues (Molinaro et al., 2009; Zdorovenko et al., 2001; Zdorovenko and Veremeichenko, 2001). The OPS of *Pst*, for example, comprises a linear L-Rha backbone with lateral N-acetyl-D-fucosamine residues (Knirel et al., 1998, 1993). Little is known about the genetic background of OPS synthesis in these bacteria. In *P. aeruginosa*, two distinct gene clusters responsible for the synthesis of CPA and OSA exist (see section 1.3.4). The CPA is a D-Rha homopolymer synthesized via the ABC-transporter-dependent pathway (Greenfield and Whitfield, 2012). Due to the resemblance in the rhamnose-rich composition of plant-associated *Pseudomonas* strains, a putative

genetic analogy was discussed in previous studies (Lam et al., 2011). However, most OPS structures of these strains analyzed to date contain L-Rha and not D-Rha, which might be assembled in a different pathway (Molinaro et al., 2009). Indeed, genetic analysis revealed homologs of a CPA cluster exist only in few plant-associated *Pseudomonas* species (publication II). The heteropolymeric OPS structure of these bacteria opposes a synthesis via the ABC transporter-dependent pathway or *S. enterica* serovar Borreze-specific synthase-dependent pathway and suggest a Wzx/Wzy-dependent synthesis instead (Islam and Lam, 2014; Kalynych et al., 2011; Raetz and Whitfield, 2002). In *P. aeruginosa* the OSA is synthesized through the Wzx/Wzy-dependent pathway. The variable content of additional glycosyltransferases in the corresponding gene cluster is responsible for the structural differentiation of the polysaccharide (DebRoy et al., 2016; Lam et al., 2011). In most plant-associated *Pseudomonas* strains, a putative locus for the OSA-cluster could be identified, but only gene homologs of the epimerase WbpM and glycosyltransferase WbpL were conserved in all of them (publication II). In *P. aeruginosa*, WbpL initiates synthesis of CPA as well as OSA. Previous studies with *P. putida* (Junker et al., 2001) and knockout and complementation of *wbpL* orthologs in *Pst* and *Pci* (publication II) suggest a functional conservation in *Pseudomonas* strains. However, the lack of key genes such as *wzx*, *wzy* or *wzz* in fact suggests that OPS is not synthesized via the Wzx/Wzy-dependent pathway in these strains (publication II). The putative conservation of the cluster locus might point to a loss of most of the genes during adaption processes to plant hosts (discussed in section 3.4.2). The conservation of WbpL indicates initiation of OPS synthesis by the enzyme might be independent of the subsequent synthesis pathway (publication II). Nevertheless, it is still unclear which genes determine the OPS synthesis in plant-associated bacteria. Analyses of additional genomes for glycosyltransferases correlated with co-expression data of e.g. *wbpL* might reveal genes, which have not been associated with OPS synthesis and the synthesis mechanism yet.

3.4 Influence of the O-polysaccharide structure on plant-bacteria interactions

LPS covers the surface of Gram-negative bacteria and is in direct contact with the environment. It contributes to various interaction processes such as cell to cell or cell to surface adhesion (Raetz and Whitfield, 2002). The hydrophilic OPS usually constitutes the majority of the molecular weight of LPS. Due to its distal location, OPS directly contributes to the polarity of the cell-surface. It influences the attachment to surfaces, biofilm formation and surface motility (publication II) (Bogino et al., 2013; Clifford et al., 2013). Specialised *Pseudomonas* strains can be found on a wide range of host such as mammals or fish and many *Pseudomonas* species appear to be able to colonize the plant rhizosphere and phyllosphere (Beaton et al., 2018; Levy et al., 2018; Molinaro et al., 2009; Silby et al., 2011). While for most plant-associated *Pseudomonas* spp. only an epiphytic and commensal lifestyle is reported, some species also invade the tissue of their plant host,

proliferate and cause disease (Silby et al., 2011). It is conceivable that LPS-related features of the bacterial cell such as cell wall composition and surface properties might promote the colonization of plants (Quiñones et al., 2005). Dynamic adaption to environmental changes in hostile environments or during colonization processes such as biofilm formation or tissue infiltration are crucial for the viability of bacteria (publication I). Variations in the OPS chain length and possibly also subtle differences in the molecular OPS structure between pathovars influences interactions with plants, and might contribute to host-pathogen compatibility (publication II). Furthermore, OPS potentially delays immune recognition of bacteria by the plant host.

3.4.1 Immune recognition of bacteria is influenced by the O-polysaccharide

While my studies show OPS might not be essential for the viability of plant-associated bacteria, many reports on the negative effects of OPS-loss on colonization, infection or symbiotic interactions with hosts exist (publication I and II). The described effects are usually linked to a higher sensitivity to external factors such as antimicrobials or other defense responses of the plant immune system. However, another factor could contribute to the reduction of virulence. Rapicavoli et al. (2018) describe an earlier immune recognition of an OPS-defective *X. fastidiosa* strain by the host plant. Initial amplification of bacteria within the plant might be delayed until the pathogen can overcome early immune responses with type III-secreted effectors like AvrPtoB and AvrPto and eventually cause disease (Macho and Zipfel, 2015; Wei and Collmer, 2018). This effect might also contribute to the reduced amplification *in planta* of OPS-deficient Δ*wbpL Pst* strains which was observed in infection experiments of *A. thaliana* (publication II). The mechanism of how OPS might protect the bacteria from immune recognition is not yet elucidated. The steric shield formed by the OPS could not only provide protection against external factors, but could also prevent the release of immune elicitors to the environment (Ranf et al., 2015). This trait is possibly linked to OPS composition and might be an additional reason for the low variation and often rhamnose-rich OPS composition of *P. syringae* pathovars (publication II). Further analyses of immune responses to viable bacteria and their respective OPS-deficient mutant strains are therefore necessary. *In vitro* experiments might help to decipher the effect of a generally higher sensitivity of the bacteria to e.g. antimicrobial metabolites of the plant from the effect of an earlier immune recognition and resulting immediate defense responses by the host.

3.4.2 O-polysaccharide composition affects host-interactions

Following the example of *P. aeruginosa* classification, Ovod et al. (1997) made a systematic investigation and proposed a novel serological classification of *P. syringae* pathovars based on the reaction of core-OS and OPS to monoclonal antibodies. Although the saccharide diversity of the OPS composition is low compared to *P. aeruginosa*, many pathovars of *P. syringae* could be differentiated on the basis of their OPS structure. Beyond that, Rudolph

(2001) proposed that the structural differences of the OPS might determine the narrow host specificity of *P. syringae* pathovars. However, *P. syringae* pathovars with different host ranges but identical OPS structures exist. When Rudolph (2001) performed infection experiments with *P. syringae* pv. *tomato* and *apii*, which possess a common OPS-structure, both caused disease on tomato and celery. Rudolph (2001) suggests compatibility of the OPS structure with host pectin possibly enables colonization and infection. While the genetic background of OPS synthesis is still unclear (publication II), optimization for OPS compatibility with plant hosts might be an opposing driver compared to the structural diversification due to immune evasion processes in vertebrate hosts. Therefore, OPS synthesis in plant-associated bacteria might differ from pathways known from bacteria with primarily mammalian hosts such as *E. coli* and *P. aeruginosa*. Indeed, most of the plant-associated *Pseudomonas* strains lack genes which are characteristic for any of the known OPS synthesis pathways (publication II). This data suggests OPS synthesis in these strains could be controlled by a yet unknown set of genes, possibly organized in a cluster (publication II). Further studies of the genetic background of OPS synthesis in *Pst* might not only shed light on how OPS is produced in this strain but might uncover a novel OPS synthesis pathway, which could be specific to plant-associated bacteria.

3.4.3 Modulation of O-polysaccharide chain length as fine-tuning of cell-surface polarity

The size of OPS varies on a single cell and results in a specific chain length distribution. Between bacterial species, strains and pathovars extensive variation of the chain length distribution can occur, which can also be observed within the *P. syringae* species complex (publication I, II and VI). Studies show a loss of OPS is accompanied by an increased attachment to nonpolar surfaces, which seems to result in reduced motility and increased biofilm formation (Huang et al., 2006; Kim and Surette, 2005; Lee et al., 2010; Petrocelli et al., 2012; Toguchi et al., 2000). Bacterial motility is essential for entering the host tissue and epiphytic fitness. Alterations often impair virulence (Haefele and Lindow, 1987; Tans-Kersten et al., 2001; Zeng et al., 2010). Accordingly, motility of OPS-deficient Δ*wbpL Pst* and *Pci* strains is reduced and virulence on their respective host plants is compromised (publication II). Not only the complete loss, but also alterations in the OPS chain-length appear to have an impact on colonization processes. Clifford et al. (2013) described a *X. fastidiosa* Δ*wzy* mutant, which is defective in OPS-synthesis and displays a reduced abundance of LPS with long-chain OPS. In their experiments, they detected an altered cell-cell and cell-surface attachment as well as a reduced virulence for the mutant strain (Clifford et al., 2013). This suggests that the observed variations in the OPS chain length distribution in strains of the *P. syringae* species complex probably lead to subtle differences in cell-surface polarity (publications II and VI). While the saccharide composition of OPS possibly affects compatibility with host pectins (discussed in section 3.4.2), the chain length distribution might be another factor influencing the adaption to host-specific surface conditions. Beyond colonization, the transmission to a plant host

is potentially influenced by the OPS. Experiments with the *X. fastidiosa* Δ*wzy* mutant revealed a reduced attachment to its insect vector *Graphocephala atropunctata* (Rapicavoli et al., 2015). Plant-associated bacteria experience very different surface conditions during transmission processes (attachment to vectors) or environment transitions during tissue infiltration. Also the need for cell-cell attachment (biofilm formation) varies in different stages of plant colonization (Bogino et al., 2013). Therefore, it seems likely that the OPS chain length can be dynamically modulated to adjust the cell-surface polarity according to environmental conditions and the stage of colonization. Indeed, Kwan and Isaacson (1998) report that *S. typhimurium* can switch between an adhesive phenotype, which displays shorter OPS, and a non-adhesive phenotype with at least eightfold longer OPS. The phenotypes are considered to correspond to the virulent and environmental form, respectively. Similar observations have been made for Rhizobia which are plant root symbionts. They alter their OPS chain length distribution in response to osmotic, ionic or heat stress as well as exposure to host root exudates (Lerouge and Vanderleyden, 2002; Lloret et al., 1995; Reuhs et al., 1994; Tao et al., 1992; Zahran et al., 1994). Consequently, OPS chain length distribution of the *Pseudomonas* strains shown in publication VI might just be a snapshot of a specific condition. Unfortunately, modulation of OPS size has not yet been studied for bacteria of the *P. syringae* species complex. Therefore an analysis of variations of OPS sizes in different environments and colonization stages is necessary.

A dynamic modulation of the average OPS chain length in response to environmental stimuli requires regulation. During OPS synthesis via the Wzx/Wzy-dependent pathway, the polysaccharide size is regulated by the copolymerase Wzz (Islam and Lam, 2014; Kalynych et al., 2011). However, the respective genes of *P. aeruginosa wzz* or *wzz2* are not conserved in *Pst* and in closely related strains (publication II). Indeed, some strains e.g. *P. syringae* pv. *maculicola* 438 and *P. corrugata* display OPS chain length distributions, which are similar to *wzz*/*wzz2* knockout mutants and correspond to an unregulated OPS synthesis as suggested by the model of Goldman and Hunt (1990) (publication II and VI) (Kintz et al., 2008; Whitfield et al., 1997). Therefore, this phenotype might have originated from an evolutionary loss-of-gene event which caused specific beneficial effects for these particular strains. Nevertheless, OPS chain length distribution of other analyzed strains indicates OPS size might be regulated (publication II and VI). Variation of the OPS chain length does not necessarily require a specific enzyme but is also influenced by the relative abundance of components of OPS synthesis. In *E. coli* O9a, OPS size is described as being regulated by the expression level of the glycosyltransferase WbdA during synthesis via the ABC transporter-dependent pathway (King et al., 2014). Increased abundance of ABC-transporter components relative to polysaccharide synthesis enzymes due to transient expression leads to a reduction of the average OPS length (Bronner et al., 1994). Similarly, phase transition and the corresponding OPS phenotype of *S. typhimurium* appear to be influenced by the expression level of the OPS-ligase *rfaL*/*waaL* (Kwan and Isaacson, 1998). Analysis of complemented Δ*wbpL Pci* and *Pst* mutants suggests the expression level of *wbpL* might determine the frequency of OPS initiation and thus impacts the ratio of LPS

species with and without OPS (publication II). These findings show a precise coordination of the ratio of OPS synthesis components is required for OPS synthesis and possibly also influences average OPS chain length. Further studies of the genetic background of OPS synthesis in plant-associated *Pseudomonas* strains might not only shed light on how OPS is produced, but uncover the regulation of OPS chain length. Identification and biochemical investigation of genetic key components of OPS synthesis regulation systems could reveal the mechanism behind the varying OPS chain length distributions of these strains. Finally, genetic modification and directed regulation of OPS synthesis can provide proof for the importance of host-specific OPS chainlength and compositions during plant colonization processes.

4 Concluding remarks

This thesis revealed that not LPS, but free mc-3-OH-fatty acids are perceived by the PRR LORE. The discovery of this novel elicitor further advances the understanding of plant immunity. Free mc-3-OH could be released from various bacterial compounds or occur as metabolic byproduct. Future studies may resolve whether mc-3-OH-fatty acids sensed by LORE predominantly originate from a single source. Beyond that, the perception of mc-3-OH-fatty acids further proves that a metabolite with low-complexity can be sufficient to induce immune responses. Future studies with plants of the Brassicaceae family and beyond will provide a better understanding of the evolutionary background of mc-3-OH-fatty acid perception in plants.

Studies performed as part of this thesis contribute novel insights into LPS synthesis processes in plant-associated *Pseudomonas* species. The elucidation of the *Pst* core-OS and genome analyses provided further evidence that certain features of *Pseudomonas* LPS, such as a highly phosphorylated core, might be characteristic for this genus. In combination, these studies could be a basis for predictions of lipid A and core-OS structures in other Pseudomonads. Furthermore, this thesis advanced the understanding of LPS functions during plant-bacteria interactions beyond a possible immune perception by the plant. This study provides novel insights into the function of OPS. The OPS composition could be part of a fine-tuning mechanism for long term host adaption while modulation of the OPS chain length might facilitate rapid adaption to environmental changes. Finally, this new perspective highlights the importance of LPS for interactions between Gram-negative bacteria and their plant hosts.

References

Abdel-Mawgoud, A. M., Lépine, F., and Déziel, E. (2010). Rhamnolipids: diversity of structures, microbial origins and roles. *Appl. Microbiol. Biotechnol.*, 86(5):1323–1336.

Abeyrathne, P. D., Daniels, C., Poon, K. K. H., Matewish, M. J., and Lam, J. S. (2005). Functional characterization of WaaL, a ligase associated with linking O-antigen polysaccharide to the core of *Pseudomonas aeruginosa* lipopolysaccharide. *J. Bacteriol.*, 187(9):3002–3012.

Al-Tahhan, R. A., Sandrin, T. R., Bodour, A. A., and Maier, R. M. (2000). Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. *Appl. Environ. Microbiol.*, 66(8):3262–3268.

Alexander, C. and Rietschel, E. T. (2001). Bacterial lipopolysaccharides and innate immunity. *J. Endotoxin Res.*, 7(3):167–202.

Aslam, S. N., Erbs, G., Morrissey, K. L., Newman, M.-A., Chinchilla, D., Boller, T., Molinaro, A., Jackson, R. W., and Cooper, R. M. (2009). Microbe-associated molecular pattern (MAMP) signatures, synergy, size and charge: influences on perception or mobility and host defence responses. *Mol. Plant Pathol.*, 10(3):375–387.

Bach, L., Michaelson, L. V., Haslam, R., Bellec, Y., Gissot, L., Marion, J., Da Costa, M., Boutin, J.-P., Miquel, M., Tellier, F., Domergue, F., Markham, J. E., Beaudoin, F., Napier, J. A., and Faure, J.-D. (2008). The very-long-chain hydroxy fatty acyl-CoA dehydratase PASTICCINO2 is essential and limiting for plant development. *Proc. Natl. Acad. Sci. U. S. A.*, 105(38):14727–14731.

Baltrus, D. A., McCann, H. C., and Guttman, D. S. (2017). Evolution, genomics and epidemiology of *Pseudomonas syringae*: Challenges in bacterial molecular plant pathology. *Mol. Plant Pathol.*, 18(1):152–168.

Bartling, C. M. and Raetz, C. R. H. (2009). Crystal structure and acyl chain selectivity of *Escherichia coli* LpxD, the *N*-acyltransferase of lipid A biosynthesis. *Biochemistry*, 48(36):8672–8683.

Beaton, A., Lood, C., Cunningham-Oakes, E., MacFadyen, A., Mullins, A. J., Bestawy, W. E., Botelho, J., Chevalier, S., Coleman, S., Dalzell, C., Dolan, S. K., Faccenda, A., Ghequire, M. G. K., Higgins, S., Kutschera, A., Murray, J., Redway, M., Salih, T., da Silva, A. C., Smith, B. A., Smits, N., Thomson, R., Woodcock, S., Welch, M., Cornelis, P., Lavigne, R., van Noort, V., and Tucker, N. P. (2018). Community-led comparative genomic and phenotypic analysis of the aquaculture pathogen *Pseudomonas baetica* a390T sequenced by ion semiconductor and nanopore technologies. *FEMS Microbiol. Lett.*, 365(9).

Bedini, E., Parrilli, M., and Unverzagt, C. (2002). Oligomerization of a rhamnanic trisaccharide repeating unit of O-chain polysaccharides from phytopathogenic bacteria. *Tetrahedron Lett.*, 43(49):8879–8882.

Bertani, B. and Ruiz, N. (2018). Function and biogenesis of lipopolysaccharides. *EcoSal Plus*, 8(1).

Blad, C. C., Tang, C., and Offermanns, S. (2012). G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nat. Rev. Drug Discov.*, 11(8):603–619.

Bogino, P. C., Oliva, M. d. l. M., Sorroche, F. G., and Giordano, W. (2013). The role of bacterial biofilms and surface components in plant-bacterial associations. *Int. J. Mol. Sci.*, 14(8):15838–15859.

Boller, T. and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.*, 60:379–406.

Boller, T. and He, S. Y. (2009). Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, 324(5928):742–744.

Bomberger, J. M., Maceachran, D. P., Coutermarsh, B. A., Ye, S., O'Toole, G. A., and Stanton, B. A. (2009). Long-distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vesicles. *PLoS Pathog.*, 5(4):e1000382.

Boutrot, F. and Zipfel, C. (2017). Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annu. Rev. Phytopathol.*, 55:257–286.

Brandl, H., Gross, R. A., Lenz, R. W., and Fuller, R. C. (1990). Plastics from bacteria and for bacteria: Poly(β-hydroxyalkanoates) as natural, biocompatible, and biodegradable polyesters. In *Microbial Bioproducts*, pages 77–93. Springer Berlin Heidelberg.

Braun, S. G., Meyer, A., Holst, O., Pühler, A., and Niehaus, K. (2005). Characterization of the *Xanthomonas campestris* pv. *campestris* lipopolysaccharide substructures essential for elicitation of an oxidative burst in tobacco cells. *Mol. Plant. Microbe. Interact.*, 18(7):674–681.

Bronner, D., Clarke, B. R., and Whitfield, C. (1994). Identification of an ATP-binding cassette transport system required for translocation of lipopolysaccharide O-antigen side-chains across the cytoplasmic membrane of *Klebsiella pneumoniae* serotype O1. *Mol. Microbiol.*, 14(3):505–519.

Buist, G., Steen, A., Kok, J., and Kuipers, O. P. (2008). LysM, a widely distributed protein motif for binding to (peptido)glycans. *Mol. Microbiol.*, 68(4):838–847.

Cao, Y., Liang, Y., Tanaka, K., Nguyen, C. T., Jedrzejczak, R. P., Joachimiak, A., and Stacey, G. (2014). The kinase LYK5 is a major chitin receptor in arabidopsis and forms a chitin-induced complex with related kinase CERK1. *Elife*, 3:e03766.

Chentouh, R., Fitting, C., and Cavaillon, J.-M. (2018). Specific features of human monocytes activation by monophosphoryl lipid a. *Sci. Rep.*, 8(1):7096.

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., and Felix, G. (2006). The *Arabidopsis* receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. *Plant Cell*, 18(2):465–476.

Clifford, J. C., Rapicavoli, J. N., and Roper, M. C. (2013). A rhamnose-rich O-antigen mediates adhesion, virulence, and host colonization for the xylem-limited phytopathogen *Xylella fastidiosa*. *Mol. Plant. Microbe. Interact.*, 26(6):676–685.

Coats, S. R., Pham, T.-T. T., Bainbridge, B. W., Reife, R. A., and Darveau, R. P. (2005). MD-2 mediates the ability of tetra-acylated and penta-acylated lipopolysaccharides to antagonize *Escherichia coli* lipopolysaccharide at the TLR4 signaling complex. *J. Immunol.*, 175(7):4490–4498.

Colucci, G., Apone, F., Alyeshmerni, N., Chalmers, D., and Chrispeels, M. J. (2002). GCR1, the putative *Arabidopsis* G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolishes seed dormancy and shortens time to flowering. *Proc. Natl. Acad. Sci. U. S. A.*, 99(7):4736–4741.

Das, S., Owen, K. A., Ly, K. T., Park, D., Black, S. G., Wilson, J. M., Sifri, C. D., Ravichandran, K. S., Ernst, P. B., and Casanova, J. E. (2011). Brain angiogenesis inhibitor 1 (BAI1) is a pattern recognition receptor that mediates macrophage binding and engulfment of Gram-negative bacteria. *Proc. Natl. Acad. Sci. U. S. A.*, 108(5):2136–2141.

de Bruijn, I., de Kock, M. J. D., de Waard, P., van Beek, T. A., and Raaijmakers, J. M. (2008). Massetolide A biosynthesis in *Pseudomonas fluorescens*. *J. Bacteriol.*, 190(8):2777–2789.

DebRoy, C., Fratamico, P. M., Yan, X., Baranzoni, G., Liu, Y., Needleman, D. S., Tebbs, R., O'Connell, C. D., Allred, A., Swimley, M., Mwangi, M., Kapur, V., Raygoza Garay, J. A., Roberts, E. L., and Katani, R. (2016). Comparison of O-antigen gene clusters of all O-serogroups of *Escherichia coli* and proposal for adopting a new nomenclature for O-typing. *PLoS One*, 11(1):e0147434.

Delucia, A. M., Six, D. A., Caughlan, R. E., Gee, P., Hunt, I., Lam, J. S., and Dean, C. R. (2011). Lipopolysaccharide (LPS) inner-core phosphates are required for complete LPS synthesis and transport to the outer membrane in *Pseudomonas aeruginosa* PAO1. *MBio*, 2(4).

Deng, W.-L., Lin, Y.-C., Lin, R.-H., Wei, C.-F., Huang, Y.-C., Peng, H.-L., and Huang, H.-C. (2010). Effects of *galU* mutation on *Pseudomonas syringae*-plant interactions. *Mol. Plant. Microbe. Interact.*, 23(9):1184–1196.

Deng, X., Liang, H., Chen, K., He, C., Lan, L., and Tang, X. (2014). Molecular mechanisms of two-component system RhpRS regulating type III secretion system in *Pseudomonas syringae*. *Nucleic Acids Res.*, 42(18):11472–11486.

Desaki, Y., Kouzai, Y., Ninomiya, Y., Iwase, R., Shimizu, Y., Seko, K., Molinaro, A., Minami, E., Shibuya, N., Kaku, H., and Nishizawa, Y. (2018). OsCERK1 plays a crucial role in the lipopolysaccharide-induced immune response of rice. *New Phytol.*, 217(3):1042–1049.

Dongari-Bagtzoglou, A. (2008). Pathogenesis of mucosal biofilm infections: challenges and progress. *Expert Rev. Anti. Infect. Ther.*, 6(2):201–208.

Dow, M., Newman, M.-A., and von Roepenack, E. (2000). The induction and modulation of plant defense responses by bacterial lipopolysaccharides. *Annu. Rev. Phytopathol.*, 38:241–261.

Dubiella, U., Seybold, H., Durian, G., Komander, E., Lassig, R., Witte, C.-P., Schulze, W. X., and Romeis, T. (2013). Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proc. Natl. Acad. Sci. U. S. A.*, 110(21):8744–8749.

Erbs, G. and Newman, M. A. (2012). The role of lipopolysaccharide and peptidoglycan, two glycosylated bacterial microbe-associated molecular patterns (MAMPs), in plant innate immunity. *Mol. Plant Pathol.*, 13(1):95–104.

Ernst, R. K., Adams, K. N., Moskowitz, S. M., Kraig, G. M., Kawasaki, K., Stead, C. M., Trent, M. S., and Miller, S. I. (2006). The *Pseudomonas aeruginosa* lipid A deacylase: selection for expression and loss within the cystic fibrosis airway. *J. Bacteriol.*, 188(1):191–201.

Ernst, R. K., Hajjar, A. M., Tsai, J. H., Moskowitz, S. M., Wilson, C. B., and Miller, S. I. (2003). *Pseudomonas aeruginosa* lipid A diversity and its recognition by Toll-like receptor 4. *J. Endotoxin Res.*, 9(6):395–400.

Erridge, C., Bennett-Guerrero, E., and Poxton, I. R. (2002). Structure and function of lipopolysaccharides. *Microbes Infect.*, 4(8):837–851.

Felix, G. and Boller, T. (2003). Molecular sensing of bacteria in plants. the highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. *J. Biol. Chem.*, 278(8):6201–6208.

Felix, G., Duran, J. D., Volko, S., and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.*, 18(3):265–276.

Fishman, M. R., Zhang, J., Bronstein, P. A., Stodghill, P., and Filiatrault, M. J. (2018). Ca^{2+}-induced two-component system CvsSR regulates the type III secretion system and the extracytoplasmic function sigma factor AlgU in *Pseudomonas syringae* pv. *tomato* DC3000. *J. Bacteriol.*, 200(5).

Flemming, H.-C. and Wingender, J. (2010). The biofilm matrix. *Nat. Rev. Microbiol.*, 8(9):623–633.

Frirdich, E. and Whitfield, C. (2005). Lipopolysaccharide inner core oligosaccharide structure and outer membrane stability in human pathogens belonging to the Enterobacteriaceae. *J. Endotoxin Res.*, 11(3):133–144.

Gibbons, H. S., Reynolds, C. M., Guan, Z., and Raetz, C. R. H. (2008). An inner membrane dioxygenase that generates the 2-hydroxymyristate moiety of *Salmonella* lipid A. *Biochemistry*, 47(9):2814–2825.

Goldman, R. C. and Hunt, F. (1990). Mechanism of O-antigen distribution in lipopolysaccharide. *J. Bacteriol.*, 172(9):5352–5359.

Gómez-Gómez, L. and Boller, T. (2000). FLS2: An LRR receptor–like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell*, 5(6):1003–1011.

Gozdziewicz, T. K., Lugowski, C., and Lukasiewicz, J. (2014). First evidence for a covalent linkage between enterobacterial common antigen and lipopolysaccharide in *Shigella sonnei* phase II ECALPS. *J. Biol. Chem.*, 289(5):2745–2754.

Greenfield, L. K. and Whitfield, C. (2012). Synthesis of lipopolysaccharide O-antigens by ABC transporter-dependent pathways. *Carbohydr. Res.*, 356:12–24.

Gust, A. A., Biswas, R., Lenz, H. D., Rauhut, T., Ranf, S., Kemmerling, B., Götz, F., Glawischnig, E., Lee, J., Felix, G., and Nürnberger, T. (2007). Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in *Arabidopsis*. *J. Biol. Chem.*, 282(44):32338–32348.

Haefele, D. M. and Lindow, S. E. (1987). Flagellar motility confers epiphytic fitness advantages upon *Pseudomonas syringae*. *Appl. Environ. Microbiol.*, 53(10):2528–2533.

Hamilton, J. A., Johnson, R. A., Corkey, B., and Kamp, F. (2001). Fatty acid transport. *J. Mol. Neurosci.*, 16(2):99–108.

Harwood, J. L. (1988). Fatty acid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 39(1):101–138.

Heeb, S. and Haas, D. (2001). Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. *Mol. Plant. Microbe. Interact.*, 14(12):1351–1363.

Hittle, L. E., Powell, D. A., Jones, J. W., Tofigh, M., Goodlett, D. R., Moskowitz, S. M., and Ernst, R. K. (2015). Site-specific activity of the acyltransferases HtrB1 and HtrB2 in *Pseudomonas aeruginosa* lipid A biosynthesis. *Pathog. Dis.*, 73(8):ftv053.

Hong, Y. and Reeves, P. R. (2014). Diversity of O-antigen repeat unit structures can account for the substantial sequence variation of Wzx translocases. *J. Bacteriol.*, 196(9):1713–1722.

Huang, T.-P., Somers, E. B., and Wong, A. C. L. (2006). Differential biofilm formation and motility associated with lipopolysaccharide/exopolysaccharide-coupled biosynthetic genes in *Stenotrophomonas maltophilia*. *J. Bacteriol.*, 188(8):3116–3120.

Iizasa, S., Iizasa, E., Matsuzaki, S., Tanaka, H., Kodama, Y., Watanabe, K., and Nagano, Y. (2016). *Arabidopsis* LBP/BPI related-1 and -2 bind to LPS directly and regulate *PR1* expression. *Sci. Rep.*, 6:27527.

Iizasa, S., Iizasa, E., Watanabe, K., and Nagano, Y. (2017). Transcriptome analysis reveals key roles of AtLBR-2 in LPS-induced defense responses in plants. *BMC Genomics*, 18(1):995.

Islam, S. T. and Lam, J. S. (2014). Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Can. J. Microbiol.*, 60(11):697–716.

Jendrossek, D. and Handrick, R. (2002). Microbial degradation of polyhydroxyalkanoates. *Annu. Rev. Microbiol.*, 56:403–432.

Jin, S. J., Hoppel, C. L., and Tserng, K. Y. (1992). Incomplete fatty acid oxidation. the production and epimerization of 3-hydroxy fatty acids. *J. Biol. Chem.*, 267(1):119–125.

Jones, J. D. G. and Dangl, J. L. (2006). The plant immune system. *Nature*, 444(7117):323–329.

Junker, F., Rodríguez-Herva, J. J., Duque, E., Ramos-González, M. I., Llamas, M., and Ramos, J. L. (2001). A WbpL mutant of *Pseudomonas putida* DOT-T1E strain, which lacks the O-antigenic side chain of lipopolysaccharides, is tolerant to organic solvent shocks. *Extremophiles*, 5(2):93–99.

Kagan, J. C. (2017). Lipopolysaccharide detection across the kingdoms of life. *Trends Immunol.*, 38(10):696–704.

Kalynych, S., Ruan, X., Valvano, M. A., and Cygler, M. (2011). Structure-guided investigation of lipopolysaccharide O-antigen chain length regulators reveals regions critical for modal length control. *J. Bacteriol.*, 193(15):3710–3721.

Kang, L., Wang, Y.-S., Uppalapati, S. R., Wang, K., Tang, Y., Vadapalli, V., Venables, B. J., Chapman, K. D., Blancaflor, E. B., and Mysore, K. S. (2008). Overexpression of a fatty acid amide hydrolase compromises innate immunity in *Arabidopsis*. *Plant J.*, 56(2):336–349.

Keck, M., Gisch, N., Moll, H., Vorhölter, F.-J., Gerth, K., Kahmann, U., Lissel, M., Lindner, B., Niehaus, K., and Holst, O. (2011). Unusual outer membrane lipid composition of the Gram-negative, lipopolysaccharide-lacking myxobacterium *Sorangium cellulosum* So ce56. *J. Biol. Chem.*, 286(15):12850–12859.

Keenleyside, W. J. and Whitfield, C. (1996). A novel pathway for O-polysaccharide biosynthesis in *Salmonella enterica* serovar *Borreze*. *J. Biol. Chem.*, 271(45):28581–28592.

Khan, M., Subramaniam, R., and Desveaux, D. (2016). Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. *Curr. Opin. Microbiol.*, 29:49–55.

Kieser, K. J. and Kagan, J. C. (2017). Multi-receptor detection of individual bacterial products by the innate immune system. *Nat. Rev. Immunol.*, 17(6):376–390.

Kim, S., Patel, D. S., Park, S., Slusky, J., Klauda, J. B., Widmalm, G., and Im, W. (2016). Bilayer properties of lipid a from various Gram-negative bacteria. *Biophys. J.*, 111(8):1750–1760.

Kim, S.-C., Kang, L., Nagaraj, S., Blancaflor, E. B., Mysore, K. S., and Chapman, K. D. (2009). Mutations in *Arabidopsis* fatty acid amide hydrolase reveal that catalytic activity influences growth but not sensitivity to abscisic acid or pathogens. *J. Biol. Chem.*, 284(49):34065–34074.

Kim, W. and Surette, M. G. (2005). Prevalence of surface swarming behavior in *Salmonella*. *J. Bacteriol.*, 187(18):6580–6583.

King, J. D., Berry, S., Clarke, B. R., Morris, R. J., and Whitfield, C. (2014). Lipopolysaccharide O antigen size distribution is determined by a chain extension complex of variable stoichiometry in *Escherichia coli* O9a. *Proc. Natl. Acad. Sci. U. S. A.*, 111(17):6407–6412.

King, J. D., Kocíncová, D., Westman, E. L., and Lam, J. S. (2009). Review: Lipopolysaccharide biosynthesis in *Pseudomonas aeruginosa*. *Innate Immun.*, 15(5):261–312.

Kintz, E., Scarff, J. M., DiGiandomenico, A., and Goldberg, J. B. (2008). Lipopolysaccharide O-antigen chain length regulation in *Pseudomonas aeruginosa* serogroup O11 strain PA103. *J. Bacteriol.*, 190(8):2709–2716.

Knirel, Y. A., Bystrova, O. V., Kocharova, N. A., Zähringer, U., and Pier, G. B. (2006). Conserved and variable structural features in the lipopolysaccharide of *Pseudomonas aeruginosa*. *J. Endotoxin Res.*, 12(6):324–336.

Knirel, Y. A., Grosskurth, H., Helbig, J. H., and Zähringer, U. (1995). Structures of decasaccharide and tridecasaccharide tetraphosphates isolated by strong alkaline degradation of O-deacylated lipopolysaccharide of *Pseudomonas fluorescens* strain ATCC 49271. *Carbohydr. Res.*, 279:215–226.

Knirel, Y. A., Ovod, V. V., Zdorovenko, G. M., Gvozdyak, R. I., and Krohn, K. J. (1998). Structure of the O polysaccharide and immunochemical relationships between the lipopolysaccharides of *Pseudomonas syringae* pathovar *tomato* and pathovar *maculicola*. *Eur. J. Biochem.*, 258(2):657–661.

Knirel, Y. A., Shashkov, A. S., Paramonov, N. A., Zdorovenko, G. M., Solyanic, L. P., and Yakovleva, L. M. (1993). Somatic antigens of pseudomonads: structure of the O-specific polysaccharide of *Pseudomonas syringa*e pv. *tomato* 140(R). *Carbohydr. Res.*, 243(1):199–204.

Kobe, B. and Deisenhofer, J. (1994). The leucine-rich repeat: a versatile binding motif. *Trends Biochem. Sci.*, 19(10):415–421.

Kocincova, D. and Lam, J. S. (2011). Structural diversity of the core oligosaccharide domain of *Pseudomonas aeruginosa* lipopolysaccharide. *Biochemistry*, 76(7):755–760.

Kuhn, H. M., Meier-Dieter, U., and Mayer, H. (1988). ECA, the enterobacterial common antigen. *FEMS Microbiol. Rev.*, 4(3):195–222.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., and Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in arabidopsis plants. *Plant Cell*, 16(12):3496–3507.

Kutschera, A. and Ranf, S. (2019). The multifaceted functions of lipopolysaccharide in plant-bacteria interactions. *Biochimie*, 159:93–98.

Kwan, L. Y. and Isaacson, R. E. (1998). Identification and characterization of a phase-variable nonfimbrial *Salmonella typhimurium* gene that alters O-antigen production. *Infect. Immun.*, 66(12):5725–5730.

Lam, J. S., Taylor, V. L., Islam, S. T., Hao, Y., and Kocíncová, D. (2011). Genetic and functional diversity of *Pseudomonas aeruginosa* lipopolysaccharide. *Front. Microbiol.*, 2:118.

Lee, C. H. and Tsai, C. M. (1999). Quantification of bacterial lipopolysaccharides by the purpald assay: measuring formaldehyde generated from 2-keto-3-deoxyoctonate and heptose at the inner core by periodate oxidation. *Anal. Biochem.*, 267(1):161–168.

Lee, Y.-W., Jeong, S.-Y., In, Y.-H., Kim, K.-Y., So, J.-S., and Chang, W.-S. (2010). Lack of O-polysaccharide enhances biofilm formation by *Bradyrhizobium japonicum*. *Lett. Appl. Microbiol.*, 50(5):452–456.

Lerouge, I. and Vanderleyden, J. (2002). O-antigen structural variation: mechanisms and possible roles in animal/plant-microbe interactions. *FEMS Microbiol. Rev.*, 26(1):17–47.

Levy, A., Salas Gonzalez, I., Mittelviefhaus, M., Clingenpeel, S., Herrera Paredes, S., Miao, J., Wang, K., Devescovi, G., Stillman, K., Monteiro, F., Rangel Alvarez, B., Lundberg, D. S., Lu, T.-Y., Lebeis, S., Jin, Z., McDonald, M., Klein, A. P., Feltcher, M. E., Rio, T. G., Grant, S. R., Doty, S. L., Ley, R. E., Zhao, B., Venturi, V., Pelletier, D. A., Vorholt, J. A., Tringe, S. G., Woyke, T., and Dangl, J. L. (2018). Genomic features of bacterial adaptation to plants. *Nat. Genet.*, 50(1):138–150.

Li, B., Meng, X., Shan, L., and He, P. (2016). Transcriptional regulation of pattern-triggered immunity in plants. *Cell Host Microbe*, 19(5):641–650.

Li, X., Teitgen, A. M., Shirani, A., Ling, J., Busta, L., Cahoon, R. E., Zhang, W., Li, Z., Chapman, K. D., Berman, D., Zhang, C., Minto, R. E., and Cahoon, E. B. (2018). Discontinuous fatty acid elongation yields hydroxylated seed oil with improved function. *Nat Plants*, 4(9):711–720.

Li, Y., Powell, D. A., Shaffer, S. A., Rasko, D. A., Pelletier, M. R., Leszyk, J. D., Scott, A. J., Masoudi, A., Goodlett, D. R., Wang, X., Raetz, C. R. H., and Ernst, R. K. (2012). LPS remodeling is an evolved survival strategy for bacteria. *Proc. Natl. Acad. Sci. U. S. A.*, 109(22):8716–8721.

Liao, C.-T., Du, S.-C., Lo, H.-H., and Hsiao, Y.-M. (2014). The galU gene of *Xanthomonas campestris* pv. *campestris* is involved in bacterial attachment, cell motility, polysaccharide synthesis, virulence, and tolerance to various stresses. *Arch. Microbiol.*, 196(10):729–738.

Liu, T., Liu, Z., Song, C., Hu, Y., Han, Z., She, J., Fan, F., Wang, J., Jin, C., Chang, J., Zhou, J.-M., and Chai, J. (2012). Chitin-induced dimerization activates a plant immune receptor. *Science*, 336(6085):1160–1164.

Lloret, J., Bolanos, L., Lucas, M. M., Peart, J. M., Brewin, N. J., Bonilla, I., and Rivilla, R. (1995). Ionic stress and osmotic pressure induce different alterations in the lipopolysaccharide of a *Rhizobium meliloti* strain. *Appl. Environ. Microbiol.*, 61(10):3701–3704.

Macho, A. P. and Zipfel, C. (2014). Plant PRRs and the activation of innate immune signaling. *Mol. Cell*, 54(2):263–272.

Macho, A. P. and Zipfel, C. (2015). Targeting of plant pattern recognition receptor-triggered immunity by bacterial type-III secretion system effectors. *Curr. Opin. Microbiol.*, 23:14–22.

Madala, N. E., Molinaro, A., and Dubery, I. A. (2011). Distinct carbohydrate and lipid-based molecular patterns within lipopolysaccharides from *Burkholderia cepacia* contribute to defense-associated differential gene expression in *Arabidopsis thaliana*. *Innate Immun.*, 18(1):140–154.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., and Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.*, 13(6):614–629.

Meredith, T. C., Mamat, U., Kaczynski, Z., Lindner, B., Holst, O., and Woodard, R. W. (2007). Modification of lipopolysaccharide with colanic acid (M-antigen) repeats in *Escherichia coli*. *J. Biol. Chem.*, 282(11):7790–7798.

Molinaro, A., Newman, M.-A., Lanzetta, R., and Parrilli, M. (2009). The structures of lipopolysaccharides from plant-associated Gram-negative bacteria. *Eur. J. Org. Chem.*, 2009(34):5887–5896.

Murphy, K., Park, A. J., Hao, Y., Brewer, D., Lam, J. S., and Khursigara, C. M. (2014). Influence of O polysaccharides on biofilm development and outer membrane vesicle biogenesis in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.*, 196(7):1306–1317.

Murray, G. L., Attridge, S. R., and Morona, R. (2006). Altering the length of the lipopolysaccharide O antigen has an impact on the interaction of *Salmonella enterica* serovar typhimurium with macrophages and complement. *J. Bacteriol.*, 188(7):2735–2739.

Needham, B. D. and Trent, M. S. (2013). Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. *Nat. Rev. Microbiol.*, 11(7):467–481.

Netea, M. G., van Deuren, M., Kullberg, B. J., Cavaillon, J.-M., and Van der Meer, J. W. M. (2002). Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? *Trends Immunol.*, 23(3):135–139.

Nomura, K., Inaba, T., Morigaki, K., Brandenburg, K., Seydel, U., and Kusumoto, S. (2008). Interaction of lipopolysaccharide and phospholipid in mixed membranes: solid-state ^{31}P-NMR spectroscopic and microscopic investigations. *Biophys. J.*, 95(3):1226–1238.

Nürnberger, T., Brunner, F., Kemmerling, B., and Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol. Rev.*, 198(1):249–266.

O'Donoghue, E. J., Sirisaengtaksin, N., Browning, D. F., Bielska, E., Hadis, M., Fernandez-Trillo, F., Alderwick, L., Jabbari, S., and Krachler, A. M. (2017). Lipopolysaccharide structure impacts the entry kinetics of bacterial outer membrane vesicles into host cells. *PLoS Pathog.*, 13(11):e1006760.

Opiyo, S. O., Pardy, R. L., Moriyama, H., and Moriyama, E. N. (2010). Evolution of the Kdo_2-lipid A biosynthesis in bacteria. *BMC Evol. Biol.*, 10(1):362.

Ortíz-Castro, R., Martínez-Trujillo, M., and López-Bucio, J. (2008). N-acyl-L-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. *Plant Cell Environ.*, 31(10):1497–1509.

Ortori, C. A., Dubern, J.-F., Chhabra, S. R., Cámara, M., Hardie, K., Williams, P., and Barrett, D. A. (2011). Simultaneous quantitative profiling of N-acyl-L-homoserine lactone and 2-alkyl-4(1H)-quinolone families of quorum-sensing signaling molecules using LC-MS/MS. *Anal. Bioanal. Chem.*, 399(2):839–850.

Ovod, V., Rudolph, K., and Krohn, K. (1997). Serological classification of *Pseudomonas syringae* pathovars based on mononoclonal antibodies towards the lipopolysaccharide O-chains. In Rudolph, K., Burr, T. J., Mansfield, J. W., Stead, D., Vivian, A., and von Kietzell, J., editors, *Pseudomonas syringae Pathovars and Related Pathogens*, pages 526–531. Springer Netherlands, Dordrecht.

Owens, T. W., Taylor, R. J., Pahil, K. S., Bertani, B. R., Ruiz, N., Kruse, A. C., and Kahne, D. (2019). Structural basis of unidirectional export of lipopolysaccharide to the cell surface. *Nature*.

Oyaizu, H. and Komagata, K. (1983). Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J. Gen. Appl. Microbiol.*, 29(1):17–40.

Park, B. S., Song, D. H., Kim, H. M., Choi, B.-S., Lee, H., and Lee, J.-O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature*, 458(7242):1191–1195.

Petrocelli, S., Tondo, M. L., Daurelio, L. D., and Orellano, E. G. (2012). Modifications of *Xanthomonas axonopodis* pv. *citri* lipopolysaccharide affect the basal response and the virulence process during citrus canker. *PLoS One*, 7(7):e40051.

Quiñones, B., Dulla, G., and Lindow, S. E. (2005). Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. *Mol. Plant. Microbe. Interact.*, 18(7):682–693.

Raetz, C. R. H., Reynolds, C. M., Trent, M. S., and Bishop, R. E. (2007). Lipid A modification systems in Gram-negative bacteria. *Annu. Rev. Biochem.*, 76:295–329.

Raetz, C. R. H. and Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.*, 71:635–700.

Ranf, S. (2016). Immune sensing of lipopolysaccharide in plants and animals: Same but different. *PLoS Pathog.*, 12(6):e1005596.

Ranf, S., Gisch, N., Schäffer, M., Illig, T., Westphal, L., Knirel, Y. A., Sánchez-Carballo, P. M., Zähringer, U., Hückelhoven, R., Lee, J., and Scheel, D. (2015). A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in *Arabidopsis thaliana*. *Nat. Immunol.*, 16(4):426–433.

Ranf, S., Scheel, D., and Lee, J. (2016). Challenges in the identification of microbe-associated molecular patterns in plant and animal innate immunity: a case study with bacterial lipopolysaccharide. *Mol. Plant Pathol.*, 17(8):1165–1169.

Rapicavoli, J. N., Blanco-Ulate, B., Muszyński, A., Figueroa-Balderas, R., Morales-Cruz, A., Azadi, P., Dobruchowska, J. M., Castro, C., Cantu, D., and Roper, M. C. (2018). Lipopolysaccharide O-antigen delays plant innate immune recognition of *Xylella fastidiosa*. *Nat. Commun.*, 9(1):390.

Rapicavoli, J. N., Kinsinger, N., Perring, T. M., Backus, E. A., Shugart, H. J., Walker, S., and Roper, M. C. (2015). O antigen modulates insect vector acquisition of the bacterial plant pathogen *Xylella fastidiosa*. *Appl. Environ. Microbiol.*, 81(23):8145–8154.

Reuhs, B. L., Kim, J. S., Badgett, A., and Carlson, R. W. (1994). Production of cell-associated polysaccharides of *Rhizobium fredii* USDA205 is modulated by apigenin and host root extract. *Mol. Plant. Microbe. Interact.*, 7(2):240–247.

Rudolph, K. (2001). Thirty-five years of phytobacteriology research with special emphasis on pathogenicity of *Pseudomonas syringae*. In De Boer, S. H., editor, *Plant Pathogenic Bacteria: Proceedings of the 10th International Conference on Plant Pathogenic Bacteria, Charlottetown, Prince Edward Island, Canada, July 23–27, 2000*, pages 109–117. Springer Netherlands, Dordrecht.

Rustan, A. C. and Drevon, C. A. (2001). Fatty acids: Structures and properties. In John Wiley & Sons, Ltd, editor, *Encyclopedia of Life Sciences*, volume 36, page 783. John Wiley & Sons, Ltd, Chichester.

Rutten, L., Geurtsen, J., Lambert, W., Smolenaers, J. J. M., Bonvin, A. M., de Haan, A., van der Ley, P., Egmond, M. R., Gros, P., and Tommassen, J. (2006). Crystal structure and catalytic mechanism of the LPS 3-O-deacylase PagL from *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.*, 103(18):7071–7076.

Santos, N. C., Silva, A. C., Castanho, M. A. R. B., Martins-Silva, J., and Saldanha, C. (2003). Evaluation of lipopolysaccharide aggregation by light scattering spectroscopy. *Chembiochem*, 4(1):96–100.

Saraf, A. and Larsson, L. (1996). Use of gas chromatography/ion-trap tandem mass spectrometry for the determination of chemical markers of microorganisms in organic dust. *J. Mass Spectrom.*

Savary, S., Bregaglio, S., Willocquet, L., Gustafson, D., Mason D'Croz, D., Sparks, A., Castilla, N., Djurle, A., Allinne, C., Sharma, M., Rossi, V., Amorim, L., Bergamin, A., Yuen, J., Esker, P., McRoberts, N., Avelino, J., Duveiller, E., Koo, J., and Garrett, K. (2017). Crop health and its global impacts on the components of food security. *Food Security*, 9(2):311–327.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nat Ecol Evol*.

Schildknecht, H. and Koob, K. (1971). Myrmicacin, the first insect herbicide. *Angew. Chem. Int. Ed Engl.*, 10(2):124–125.

Schroeder, T. H., Lee, M. M., Yacono, P. W., Cannon, C. L., Gerçeker, A. A., Golan, D. E., and Pier, G. B. (2002). CFTR is a pattern recognition molecule that extracts *Pseudomonas aeruginosa* lps from the outer membrane into epithelial cells and activates NF-κB translocation. *Proc. Natl. Acad. Sci. U. S. A.*, 99(10):6907–6912.

Sebastián, D., Guevara, M. G., Rocío, T. F., and Virginia, T. C. (2018). An overview of plant proteolytic enzymes. In Guevara, M. G. and Daleo, G. R., editors, *Biotechnological Applications of Plant Proteolytic Enzymes*, pages 1–19. Springer International Publishing, Cham.

Sels, J., Mathys, J., De Coninck, B. M. A., Cammue, B. P. A., and De Bolle, M. F. C. (2008). Plant pathogenesis-related (PR) proteins: a focus on PR peptides. *Plant Physiol. Biochem.*, 46(11):941–950.

Seltmann, G. and Holst, O. (2010). *The Bacterial Cell Wall*. Springer Berlin Heidelberg.

Shang-Guan, K., Wang, M., Myint Phyu Sin Htwe, N., Li, P., Li, Y., Qi, F., Zhang, D., Cao, M., Kim, C., Weng, H., Cen, H., Black, I. M., Azadi, P., Carlson, R. W., Stacey, G., and Liang, Y. (2018). Lipopolysaccharides trigger two successive bursts of reactive oxygen species at distinct cellular locations. *Plant Physiol.*

Sherman, D. J., Xie, R., Taylor, R. J., George, A. H., Okuda, S., Foster, P. J., Needleman, D. J., and Kahne, D. (2018). Lipopolysaccharide is transported to the cell surface by a membrane-to-membrane protein bridge. *Science*, 359(6377):798–801.

Shi, J., Zhao, Y., Wang, Y., Gao, W., Ding, J., Li, P., Hu, L., and Shao, F. (2014). Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature*, 514(7521):187–192.

Shiner, E. K., Rumbaugh, K. P., and Williams, S. C. (2005). Interkingdom signaling: Deciphering the language of acyl homoserine lactones. *FEMS Microbiol. Rev.*, 29(5):935–947.

Silby, M. W., Winstanley, C., Godfrey, S. A. C., Levy, S. B., and Jackson, R. W. (2011). Pseudomonas genomes: diverse and adaptable. *FEMS Microbiol. Rev.*, 35(4):652–680.

Silipo, A., Molinaro, A., Sturiale, L., Dow, J. M., Erbs, G., Lanzetta, R., Newman, M.-A., and Parrilli, M. (2005). The elicitation of plant innate immunity by lipooligosaccharide of *Xanthomonas campestris*. *J. Biol. Chem.*, 280(39):33660–33668.

Silipo, A., Sturiale, L., Garozzo, D., Erbs, G., Jensen, T. T., Lanzetta, R., Dow, J. M., Parrilli, M., Newman, M.-A., and Molinaro, A. (2008). The acylation and phosphorylation pattern of lipid A from *Xanthomonas campestris* strongly influence its ability to trigger the innate immune response in *Arabidopsis*. *Chembiochem*, 9(6):896–904.

Sjögren, J., Magnusson, J., Broberg, A., Schnürer, J., and Kenne, L. (2003). Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. *Appl. Environ. Microbiol.*, 69(12):7554–7557.

Smith, C. R. (1971). Occurrence of unusual fatty acids in plants. *Prog. Chem. Fats Other Lipids*, 11:137–177.

Soberón-Chávez, G., Lépine, F., and Déziel, E. (2005). Production of rhamnolipids by *Pseudomonas aeruginosa*. *Appl. Microbiol. Biotechnol.*, 68(6):718–725.

SOPI (2014). Situation and outlook for primary industries 2014. Technical report, Ministry for Primary Industries, Government of New Zealand.

SOPI (2018). Situation and outlook for primary industries november 2018. Technical report, Ministry for Primary Industries, Government of New Zealand.

Stead, D. E. (1989). Grouping of *Xanthomonas campestris* pathovars of cereals and grasses by fatty acid profiling. *EPPO Bulletin*, 19(1):57–68.

Stead, D. E. (1992). Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *Int. J. Syst. Evol. Microbiol.*

Steeghs, L., den Hartog, R., den Boer, A., Zomer, B., Roholl, P., and van der Ley, P. (1998). Meningitis bacterium is viable without endotoxin. *Nature*, 392(6675):449–450.

Steinbüchel, A. and Valentin, H. E. (1995). Diversity of bacterial polyhydroxyalkanoic acids. *FEMS Microbiol. Lett.*, 128(3):219–228.

Suzuki, M., Takaishi, S., Nagasaki, M., Onozawa, Y., Iino, I., Maeda, H., Komai, T., and Oda, T. (2013). Medium-chain fatty acid-sensing receptor, GPR84, is a proinflammatory receptor. *J. Biol. Chem.*, 288(15):10684–10691.

Szponar, B., Norin, E., Midtvedt, T., and Larsson, L. (2002). Limitations in the use of 3-hydroxy fatty acid analysis to determine endotoxin in mammalian samples. *J. Microbiol. Methods*, 50(3):283–289.

Taddese, B., Upton, G. J. G., Bailey, G. R., Jordan, S. R. D., Abdulla, N. Y., Reeves, P. J., and Reynolds, C. A. (2014). Do plants contain G protein-coupled receptors? *Plant Physiol.*, 164(1):287–307.

Tans-Kersten, J., Huang, H., and Allen, C. (2001). *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.*, 183(12):3597–3605.

Tao, H., Brewin, N. J., and Noel, K. D. (1992). *Rhizobium leguminosarum* CFN42 lipopolysaccharide antigenic changes induced by environmental conditions. *J. Bacteriol.*, 174(7):2222–2229.

Teplitski, M., Mathesius, U., and Rumbaugh, K. P. (2011). Perception and degradation of N-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. *Chem. Rev.*, 111(1):100–116.

Thordal-Christensen, H. (2003). Fresh insights into processes of nonhost resistance. *Curr. Opin. Plant Biol.*, 6(4):351–357.

Toguchi, A., Siano, M., Burkart, M., and Harshey, R. M. (2000). Genetics of swarming motility in *Salmonella enterica* serovar typhimurium: critical role for lipopolysaccharide. *J. Bacteriol.*, 182(22):6308–6321.

Tran, H., Ficke, A., Asiimwe, T., Höfte, M., and Raaijmakers, J. M. (2007). Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytol.*, 175(4):731–742.

Trent, M. S., Stead, C. M., Tran, A. X., and Hankins, J. V. (2006). Diversity of endotoxin and its impact on pathogenesis. *J. Endotoxin Res.*, 12(4):205–223.

Ullrich, M., Peñaloza-Vázquez, A., Bailey, A. M., and Bender, C. L. (1995). A modified two-component regulatory system is involved in temperature-dependent biosynthesis of the *Pseudomonas syringae* phytotoxin coronatine. *J. Bacteriol.*, 177(21):6160–6169.

Valappil, S. P., Boccaccini, A. R., Bucke, C., and Roy, I. (2007). Polyhydroxyalkanoates in Gram-positive bacteria: insights from the genera *Bacillus* and *Streptomyces*. *Antonie Van Leeuwenhoek*, 91(1):1–17.

van Loon, L. C., Rep, M., and Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.*, 44(1):135–162.

Vanneste, J. L. (2017). The scientific, economic, and social impacts of the New Zealand outbreak of bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*). *Annu. Rev. Phytopathol.*, 55:377–399.

Verlinden, R. A. J., Hill, D. J., Kenward, M. A., Williams, C. D., and Radecka, I. (2007). Bacterial synthesis of biodegradable polyhydroxyalkanoates. *J. Appl. Microbiol.*, 102(6):1437–1449.

Vilakazi, C. S., Dubery, I. A., and Piater, L. A. (2017). Identification of lipopolysaccharide-interacting plasma membrane-type proteins in *Arabidopsis thaliana*. *Plant Physiol. Biochem.*, 111:155–165.

Vinogradov, E., Egbosimba, E. E., Perry, M. B., Lam, J. S., and Forsberg, C. W. (2001). Structural analysis of the carbohydrate components of the outer membrane of the lipopolysaccharide-lacking cellulolytic ruminal bacterium *Fibrobacter succinogenes* S85: Polysaccharides of *Fibrobacter succinogenes* S85. *Eur. J. Biochem.*, 268(12):3566–3576.

Voss, B. J. and Stephen Trent, M. (2018). LPS transport: Flipping out over MsbA. *Curr. Biol.*, 28(1):R30–R33.

Walsh, A. G., Matewish, M. J., Burrows, L. L., Monteiro, M. A., Perry, M. B., and Lam, J. S. (2000). Lipopolysaccharide core phosphates are required for viability and intrinsic drug resistance in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, 35(4):718–727.

Wang, J., Wu, X., Simonavicius, N., Tian, H., and Ling, L. (2006). Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. *J. Biol. Chem.*, 281(45):34457–34464.

Weaver, N., Johnston, N. C., Benjamin, R., and Law, J. H. (1968). Novel fatty acids from the royal jelly of honeybees (*Apis mellifera*, l.). *Lipids*, 3(6):535–538.

Wei, H.-L. and Collmer, A. (2018). Defining essential processes in plant pathogenesis with *Pseudomonas syringae* pv. *tomato* DC3000 disarmed polymutants and a subset of key type III effectors. *Mol. Plant Pathol.*, 19(7):1779–1794.

White, D. C., Sutton, S. D., and Ringelberg, D. B. (1996). The genus *Sphingomonas*: physiology and ecology. *Curr. Opin. Biotechnol.*, 7(3):301–306.

Whitfield, C., Amor, P. A., and Köplin, R. (1997). Modulation of the surface architecture of Gram-negative bacteria by the action of surface polymer:lipid A–core ligase and by determinants of polymer chain length. *Mol. Microbiol.*, 23(4):629–638.

Whitfield, C. and Trent, M. S. (2014). Biosynthesis and export of bacterial lipopolysaccharides. *Annu. Rev. Biochem.*, 83:99–128.

Willmann, R., Lajunen, H. M., Erbs, G., Newman, M.-A., Kolb, D., Tsuda, K., Katagiri, F., Fliegmann, J., Bono, J.-J., Cullimore, J. V., Jehle, A. K., Götz, F., Kulik, A., Molinaro, A., Lipka, V., Gust, A. A., and Nürnberger, T. (2011). Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl. Acad. Sci. U. S. A.*, 108(49):19824–19829.

Wyckoff, T. J. O., Lin, S., Cotter, R. J., Dotson, G. D., and Raetz, C. R. H. (1998). Hydrocarbon rulers in UDP-N-acetylglucosamine acyltransferases. *J. Biol. Chem.*, 273(49):32369–32372.

Xia, Y. (2004). Proteases in pathogenesis and plant defence. *Cell. Microbiol.*, 6(10):905–913.

Xin, X.-F., Kvitko, B., and He, S. Y. (2018). *Pseudomonas syringae*: what it takes to be a pathogen. *Nat. Rev. Microbiol.*, 16(5):316–328.

Xu, C. and Shanklin, J. (2016). Triacylglycerol metabolism, function, and accumulation in plant vegetative tissues. *Annu. Rev. Plant Biol.*, 67:179–206.

Yethon, J. A., Gunn, J. S., Ernst, R. K., Miller, S. I., Laroche, L., Malo, D., and Whitfield, C. (2000). *Salmonella enterica* serovar typhimurium *waaP* mutants show increased susceptibility to polymyxin and loss of virulence in vivo. *Infect. Immun.*, 68(8):4485–4491.

Yethon, J. A., Heinrichs, D. E., Monteiro, M. A., and others (1998). Involvement of *waaY*, *waaQ*, and *waaP* in the modification of *Escherichia coli* lipopolysaccharide and their role in the formation of a stable outer membrane. *Journal of Biological*.

Yu, X., Feng, B., He, P., and Shan, L. (2017). From chaos to harmony: Responses and signaling upon microbial pattern recognition. *Annu. Rev. Phytopathol.*, 55:109–137.

Zahran, H. H., Räsänen, L. A., Karsisto, M., and Lindström, K. (1994). Alteration of lipopolysaccharide and protein profiles in SDS-PAGE of rhizobia by osmotic and heat stress. *World J. Microbiol. Biotechnol.*, 10(1):100–105.

Zdorovenko, E. L., Vinogradov, E., Zdorovenko, G. M., Lindner, B., Bystrova, O. V., Shashkov, A. S., Rudolph, K., Zähringer, U., and Knirel, Y. A. (2004a). Structure of the core oligosaccharide of a rough-type lipopolysaccharide of *Pseudomonas syringae* pv. *phaseolicola*. *Eur. J. Biochem.*, 271(23-24):4968–4977.

Zdorovenko, E. L., Zatonsky, G. V., Zdorovenko, G. M., Pasichnyk, L. A., Shashkov, A. S., and Knirel, Y. A. (2001). Structural heterogeneity in the lipopolysaccharides of *Pseudomonas syringae* with O-polysaccharide chains having different repeating units. *Carbohydr. Res.*, 336(4):329–336.

Zdorovenko, G. M., Varbanets, L. D., Zdorovenko, E. L., Vinarskaya, N. V., and Yakovleva, L. M. (2004b). Chemical and biological characterization of lipopolysaccharides from the *Pseudomonas syringae* pv. *maculicola* IMV 381 collection culture and its dissociants. *Microbiology*, 73(6):678–688.

Zdorovenko, G. M. and Veremeichenko, S. N. (2001). Comparative characterization of the lipopolysaccharides of different *Pseudomonas fluorescens* biovar I strains. *Microbiology*, 70(4):441–450.

Zdorovenko, G. M., Zdorovenko, E. L., and Varbanets, L. D. (2007). Composition, structure, and biological properties of lipopolysaccharides from different strains of *Pseudomonas syringae* pv. *atrofaciens*. *Microbiology*, 76(6):683–697.

Zeidler, D., Zähringer, U., Gerber, I., Dubery, I., Hartung, T., Bors, W., Hutzler, P., and Durner, J. (2004). Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. *Proc. Natl. Acad. Sci. U. S. A.*, 101(44):15811–15816.

Zeng, W., Melotto, M., and He, S. Y. (2010). Plant stomata: a checkpoint of host immunity and pathogen virulence. *Curr. Opin. Biotechnol.*, 21(5):599–603.

Zhang, G., Meredith, T. C., and Kahne, D. (2013). On the essentiality of lipopolysaccharide to Gram-negative bacteria. *Curr. Opin. Microbiol.*, 16(6):779–785.

Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends Immunol.*, 35(7):345–351.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G., and Boller, T. (2004). Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature*, 428(6984):764–767.

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