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Fatty Acid Profiles in Plant Pathogenic Bacteria

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ABSTRACT: Plant pathogenic bacteria cause major crop losses globally, necessitating precise identification tools for effective management. Fatty acid profiling, particularly via fatty acid methyl ester (FAME) analysis, has long served as a valuable method for distinguishing closely related taxa based on membrane lipid signatures. Characteristic patterns, such as unsaturated fatty acids in *Pseudomonas syringae* and branched-chain fatty acids in *Clavibacter michiganensis*, offer stable biochemical markers. Recent advances in phospholipidomics using LC–MS/MS now enable structural resolution of intact phospholipids, including isomeric phosphatidylethanolamines, improving taxonomic precision and physiological insight. These developments mark a shift from bulk FAME profiling toward lipidomics-driven bacterial classification, revealing lipid adaptations to environmental and host-derived stresses. This review explores the diagnostic and ecological relevance of bacterial fatty acid profiles in light of emerging lipidomic technologies.

Keywords: Fatty acid profiling, Plant pathogenic bacteria, FAME, Lipidomics, LC–MS/MS, Bacterial taxonomy.

INTRODUCTION

Plant pathogenic bacteria are a diverse group of microorganisms that infect plants and cause a wide range of economically significant diseases, including leaf spots, blights, wilts, cankers, galls, and rots. These pathogens can colonize plant tissues by entering through natural openings such as stomata or hydathodes, or through wounds caused by mechanical damage or insect vectors. Once inside the host, they release a variety of virulence factors, including toxins, enzymes, and extracellular polysaccharides, which degrade plant cell walls, interfere with signaling pathways, and disrupt the normal transport of water and nutrients—ultimately resulting in visible disease symptoms and yield loss (Agrios, 2005). Plant pathogenic bacteria are responsible for a wide range of economically important diseases affecting crops worldwide. Effective identification and characterization of these pathogens are critical for disease surveillance, quarantine regulation, and the development of targeted control strategies. One promising phenotypic trait for bacterial identification and ecological insight is the composition of membrane fatty acids, particularly when analyzed through fatty acid methyl ester (FAME) profiling or more advanced lipidomic techniques.

Historically, FAME analysis via gas chromatography has been widely used for microbial systematics due to its ability to produce reproducible and taxonomically relevant fatty acid signatures (Laguerre *et al.*, 2020). This method provides valuable biochemical fingerprints distinguishing closely related taxa, particularly among genera such as *Pseudomonas*, *Xanthomonas*, and *Clavibacter*. For instance, Gram-negative bacteria like *Pseudomonas syringae* exhibit dominant unsaturated fatty acids such as 16:1 ω7c and 18:1 ω7c, while Gram-positive bacteria like *Clavibacter michiganensis* contain branched-chain fatty acids (BCFAs) such as iso-15:0 and anteiso-15:0 (Wang *et al.*, 2020; Liao *et al.*, 2021). Such compositional patterns can serve as stable taxonomic markers and are increasingly being revisited with the advent of high-resolution lipidomic technologies.

More recently, research has shifted toward comprehensive phospholipidomic profiling, utilizing liquid chromatography–mass spectrometry (LC–MS/MS). Rudt *et al*. (2024) introduced a tailored reversed-phase high-performance liquid chromatography (RP-HPLC)–MS/MS platform for analyzing intact phospholipids in plant-pathogenic bacteria, including species from *Xanthomonas*, *Pseudomonas*, and *Clavibacter* (Rudt *et al.*, 2024). This

method enabled the resolution of isomeric phosphatidylethanolamines (PEs) based on their BCFA content, a level of structural specificity not achievable through conventional FAME analysis. Furthermore, the researchers established a retention-based identification system transferable across lipid classes, including phosphatidylglycerol (PG) and cardiolipin (CL). Their integrative workflow, combining LC–MS/MS with GC–MS verification, allowed for the structural elucidation of phospholipidomes and subsequent classification of bacteria based on intact lipid profiles (Rudt *et al.*, 2024). These advancements signal a paradigm shift in the application of fatty acid profiling—from a bulk methyl ester approach to a lipidomics-centered strategy, which offers deeper insights into bacterial physiology, membrane adaptation, and taxonomic resolution. Notably, lipidomic responses to host-derived stress, environmental pressure, and antimicrobial treatment are increasingly viewed as key factors in understanding pathogenicity and resilience mechanisms (Nguyen *et al.*, 2023; Zhang *et al.*, 2023).

This review synthesizes current knowledge on fatty acid compositions in plant pathogenic bacteria, comparing FAME and phospholipid-based techniques, and highlighting their taxonomic, ecological, and diagnostic implications in light of recent technological advancements.

Structural Roles of Fatty Acids in Bacterial Membranes

Fatty acids are crucial components of the phospholipid bilayer in bacterial cell membranes. In plant pathogenic bacteria, as in other bacteria, they play a vital role in maintaining membrane fluidity, permeability, and functionality under varying environmental conditions (Denich *et al.*, 2003; Russell & Nichols 1999).

1. Modulation of Membrane Fluidity and Phase Behavior. The physical state of bacterial membranes is largely governed by the chain length, degree of saturation, and branching of constituent fatty acids. Shorter and unsaturated fatty acids (e.g., 16:1, 18:1) enhance fluidity and permeability, while longer or saturated fatty acids (e.g., 18:0, 20:0) increase rigidity (Fan *et al.*, 2024). Branched-chain fatty acids (BCFAs), especially anteiso-fatty acids, promote greater fluidity compared to iso-forms, particularly in Gram-positive bacteria such as *Bacillus subtilis* (Willdigg & Helmann 2021).

2. Adaptive Homeoviscous Regulation. Bacteria maintain optimal membrane viscosity through a process known as homeoviscous adaptation, which involves enzymatic modifications of fatty acid structures in response to external stressors such as temperature, pH, solvents, and antimicrobials (Willdigg & Helmann 2021). This includes:

- Desaturation to introduce double bonds,
- Branched-chain elongation, and
- cis–trans isomerization, which rapidly alters membrane packing to resist environmental damage (Fan *et al.*, 2024).

3. Cyclopropane Fatty Acids in Stress Response. Cyclopropane fatty acids (CFAs), produced by cyclopropane fatty acid synthase (CFA synthase), are important in stabilizing membranes during acid stress, osmotic shock, and host colonization. CFAs reduce proton permeability, enhancing bacterial resilience and pathogenicity. This is especially notable in pathogens like Mycobacterium tuberculosis and *Escherichia coli*.

4. Structural Lipid Diversity Across Bacterial Taxa. Major bacterial membrane lipids such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL), each incorporate two or more fatty acid chains with varied lengths, degrees of saturation, and branching (Strahl & Errington 2017). The resulting diversity impacts membrane domain formation, protein localization, and curvature stress. Gram-positive pathogens such as *Staphylococcus aureus* have also been shown to incorporate host-derived unsaturated fatty acids into their membranes, dynamically adjusting membrane structure and fluidity (Fan *et al.*, 2024).

5. Adaptation to Extreme Environments. In extreme environments such as cold or high-pressure ecosystems, bacteria modify their membrane fatty acid composition to maintain functional fluidity. For instance, Antarctic bacteria reduce fatty acid chain lengths and increase unsaturation at low temperatures to prevent membrane solidification (Akulava *et al.*, 2024). Similarly, high hydrostatic pressure induces adjustments in fatty acid saturation and branching to optimize membrane packing and permeability (Sharma *et al.*, 2022).

Overview of Bacterial Fatty Acids. Bacterial membranes are composed primarily of phospholipids, each containing fatty acids that determine the physical and functional characteristics of the cell envelope. These fatty acids vary significantly among bacterial taxa and are shaped by genetic regulation, environmental conditions, and adaptive pressures.

Types of Fatty Acids Commonly Found in Bacteria

1. Long-Chain Fatty Acids (LCFAs; C12–C20). LCFAs are key structural components and also serve as nutrients and signaling molecules. They are integral to membrane assembly and fluidity regulation, and can influence bacterial virulence, particularly in Gram-negative pathogens like *Salmonella*, *Pseudomonas*, and *Vibrio*.

2. Straight-Chain Fatty Acids (SCFAs). Straight-chain saturated (e.g., palmitic acid 16:0, stearic acid 18:0) and monounsaturated fatty acids (e.g., palmitoleic acid 16:1, oleic acid 18:1) are commonly found in Gram-negative bacteria such as *Pseudomonas*, *Escherichia coli*, and *Xanthomonas* species. These fatty acids form the structural core of the lipid bilayer and influence membrane fluidity and permeability (Zhang & Rock 2008; Parsons & Rock 2013).

3. Medium- and Very-Long-Chain Fatty Acids (MCFAs & VLCFAs). Adaptive remodeling under temperature stress is common. Antarctic bacteria increase VLCFA content and adjust MCFA/branched-SFA balance to maintain membrane fluidity at low temperatures (Akulava *et al.,* 2024).

4. Cyclopropane Fatty Acids (CFAs). CFAs confer membrane rigidity, enhance acid tolerance, and contribute to stress resilience and pathogenicity. Their synthesis via the *cfa* gene is a central adaptive mechanism, making CFA synthase a potential antimicrobial target (Cronan & Luk 2022). Cyclopropane fatty acids are formed by the addition of a methylene group to a double bond in unsaturated fatty acids via the enzyme cyclopropane fatty acid synthase. These fatty acids enhance membrane rigidity and are associated with acid tolerance and stress resistance in pathogens such as *Mycobacterium tuberculosis* and *Salmonella enterica* (Grogan & Cronan 1984).

5. Branched-Chain Fatty Acids (BCFAs). They are ordinarily found in Gram-positive bacteria, BCFAs modulate membrane fluidity and phase behavior. Biosynthesis is tightly regulated, with physiological implications under stress conditions. Branched-chain fatty acids are predominant in many Gram-positive bacteria, including *Bacillus*, *Listeria*, and *Clavibacter* species. These fatty acids occur mainly as iso and anteiso forms, derived from branched-chain amino acid precursors (valine, leucine, isoleucine). Anteiso-BCFAs typically enhance membrane fluidity more than iso-forms and are particularly important for cold adaptation (Kaneda, 1991; Willecke & Pardee 1971).

- **iso-fatty acids**: Branch at the penultimate carbon (e.g., iso-15:0, iso-17:0)
- **anteiso-fatty acids**: Branch at the antepenultimate carbon (e.g., anteiso-15:0, anteiso-17:0).

6. Phospholipid-Derived Fatty Acids (PLFAs). PLFA biomarkers vary by bacterial group: Gram-negatives show monounsaturated and cyclopropane FAs under stress; Gram-positives are enriched in iso/anteiso BCFAs and 10-methyl PLFAs (Actinomycetes), reflecting taxonomic and ecological diversity.

7. Unsaturated Fatty Acids (UFAs). Common unsaturated fatty acids include 16:1Δ9 and 18:1Δ11, which increase membrane fluidity. These are especially prevalent in Gram-negative bacteria and are regulated through desaturase enzymes, particularly under cold or nutrient-limited conditions (Zhang & Rock 2008).

8. Polyunsaturated Fatty Acids (PUFAs). Though rare in most mesophilic bacteria, PUFAs such as eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) are found in some marine psychrophilic bacteria like *Shewanella* and *Moritella*. These fatty acids maintain membrane flexibility under extreme cold and high pressure (Yano *et al.,* 2015).

Functions of Fatty Acids in Cell Membrane Structure and Physiology

1. Membrane Fluidity Regulation

- Fatty acids modulate membrane fluidity depending on their saturation and chain length. (Zhang & Rock 2008).
- Unsaturated fatty acids introduce kinks, increasing fluidity and flexibility.
- Saturated fatty acids pack tightly, reducing fluidity and enhancing rigidity (Denich *et al*., 2003).
- This balance is essential for membrane protein function and cellular response to temperature (Russell & Nichols, 1999)
- Cyclopropane fatty acids (CFAs) introduce rigid rings into acyl chains, increasing membrane order and stability under stress, while still allowing fluidity. Molecular dynamics simulations show that CFAs both stabilize the lipid bilayer and increase lateral diffusion, especially under acid or cold shock conditions (Poger & Mark 2015; Maiti *et al.,* 2023; Fan *et al*., 2024).
- During bacterial stationary phase and acid stress, up to 40% of *E. coli* membrane lipids are CFAs, which enhance thickness, reduce proton permeability, and increase resistance to oxidation (Fan *et al*., 2024).

2. Adaptation to Environmental Stress

- Bacteria adjust their fatty acid composition in response to temperature, pH, osmotic pressure, and host-derived signals (Sato *et al*., 2000; Sohlenkamp & Geiger 2016).
- Increased unsaturation helps maintain membrane function under cold or osmotic stress (Russell & Nichol 1999).
- Branched-chain fatty acids improve membrane performance under low-nutrient or low-temperature conditions (Kaneda, 1991).
- Bacteria actively regulate desaturation, branching, and cis–trans isomerization of their fatty acids to maintain optimal membrane viscosity across conditions (Willdigg & Helmann 2021; Fan *et al.,* 2024).
- Specifically, Gram-negative species like *Pseudomonas* and *Vibrio* increase trans-unsaturated fatty acids up to ~40% under solvent or osmotic stress, enhancing membrane packing and reducing permeability (Fan *et al*., 2024).
- In *Bacillus subtilis*, temperature or detergent stress activates sigma factor σW, decreasing branched-chain FA synthesis and increasing straight-chain FA and chain length, thus stiffening membranes for enhanced stress resistance (Willdigg & Helmann 2021).

3. Permeability Barrier and Selective Transport

- Fatty acids form the hydrophobic core of the membrane, controlling the diffusion of solutes (Zhang & Rock 2008)
- Altering fatty acid types affects permeability to ions, antibiotics, and plant defense compounds (Sohlenkamp & Geiger 2016; Denich *et al*., 2003).
- CFAs produced by cyclopropane fatty acid synthase (CfaS) are essential for survival in acidic environments. Overexpression of *cfaS* in *E. coli* increased cyclopropane FA content 3.5-fold and substantially enhanced acid resistance.

4. Support for Membrane Protein Function

- Proper membrane fluidity and structure allow integral membrane proteins (e.g., transporters, receptors) to function correctly (Russell & Nichols 1999; Sato *et al*., 2000).
- Disruption in fatty acid composition can impair signal transduction and transport activity (Zhang & Rock, 2008).

5. Role in Cell Division and Growth

- Fatty acid composition affects membrane curvature and flexibility, influencing processes like septum formation and cytokinesis (Zhang & Rock 2008; Sohlenkamp & Geiger 2016).
- Some bacteria use specific fatty acids for membrane expansion during growth and division (Kaneda, 1991).

6. Integration of Environmental Fatty Acids

- Pathogens such as *S. aureus* and *S. pneumoniae* incorporate exogenous fatty acids from their environment, dynamically altering membrane composition. In *S. aureus*, uptake of environmental UFAs reduced branched FA content but increased membrane order due to co-associated pigments (Fan *et al.*, 2024).

Methods for Analyzing Fatty Acid Composition. Fatty acid analysis is typically performed using gas chromatography (GC) following their derivatization into fatty acid methyl esters (FAMEs), which facilitates more efficient separation and quantification compared to intact triglycerides or free fatty acids (Sasser, 2001; Zhang & Rock 2008). Most analytical protocols involve saponification, a process that hydrolyzes complex lipids such as triglycerides and phospholipids to release free fatty acids for subsequent methylation (Kozlova *et al*., 2011). The analysis of fatty acid composition in bacteria is essential for understanding their taxonomy, physiology, and adaptive strategies (Kaneda, 1991; O'Leary & Wilkinson 1981). Various analytical techniques are employed, each with distinct advantages in terms of sensitivity, specificity, and resolution. Among these, FAME profiling using gas chromatography remains the most widely accepted and standardized method, particularly for bacterial identification and classification (Sasser, 2001).

1. Gas Chromatography (GC). Gas chromatography is the most widely used method for analyzing bacterial fatty acids. It involves converting fatty acids to fatty acid methyl esters (FAMEs), which are then separated based on chain length, degree of saturation, and branching. GC with flame ionization detection (GC-FID) offers high resolution and sensitivity, making it suitable for quantitative and qualitative analyses (Sasser, 1990; Kampfer & Kroppenstedt 1996).

2. Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS combines the separation power of GC with the structural identification capability of mass spectrometry. It is especially useful for identifying uncommon or novel fatty acids and determining their molecular structures. GC-MS provides detailed profiles and is commonly used in fatty acid research involving plant pathogenic bacteria (Christie, 1989; Bligh & Dyer 1959). FAME profiling via GC–FID remains the gold standard for routine FA analysis. Highly resolved analysis of cis/trans isomers depends on specialized polar columns (e.g., CP-Sil 88, BPX-70) that enhance separation performance (Fan *et al*., 2024). GC–MS further improves identification accuracy through mass spectral matching against libraries like NIST; however, distinguishing isomeric double bonds still requires specialized derivatization techniques (Fan *et al*., 2024). Two-dimensional GC (GC×GC–TOFMS) systems achieve exceptional separation of complex mixtures, allowing rapid profiling of multiple FAs within ~1 hour.

3. High Performane Liquid Chromatography: HPLC is an alternative to GC, particularly when analyzing polar or thermally labile lipids. It is often used in complex lipid studies where intact phospholipids or glycolipids need to be separated and quantified. Though less common for fatty acid methyl esters, HPLC can be coupled with UV, ELSD, or MS detectors for enhanced analysis (Kates, 1986).

4. Fatty Acid Methyl Ester (FAME) Profiling using the MIDI System: The MIDI Sherlock Microbial Identification System is a standardized GC-based method used for bacterial identification through fatty acid profiling. The system uses a reference library to match FAME profiles and is widely adopted in microbial ecology and taxonomy (Sasser, 1990).

5. Thin Layer Chromatography(TLC): TLC is a simple and cost-effective method used for the initial separation of lipid classes. It is especially useful in preliminary screening before more advanced techniques such as GC or MS. While limited in resolution, TLC provides useful qualitative insights into the presence of major lipid types (Kates, 1986).

6. Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR spectroscopy is used to determine the structural configuration of fatty acids, including double bond positions and isomeric forms. Though less commonly employed for routine analysis due to its cost and complexity, it is valuable in detailed lipidomics studies (Gunstone, 1991).

7. Sample Preparation & Extraction Methods. High-quality FA analysis depends on optimized sample prep. Reviews recommend Bligh–Dyer or Folch extraction for comprehensive lipid recovery, often followed by solid-phase extraction (SPE) to concentrate analytes or remove matrix interferences (Furse *et al*., 2015; Fan *et al*., 2024). For clinical and bacterial samples, automated workflows improve throughput and reproducibility (Fan *et al*., 2024).

8. Method Validation and Quality Control. Stringent validation—covering linearity, precision, detection limits (LoD/LoQ), and recovery studies—is essential, especially for high-throughput or clinical applications (Trivedi *et al*., 2022; Fan *et al*., 2024). Certified

standards (e.g., NIST SRMs) and batch-level QC samples are recommended to ensure consistent data quality across experiments (Fan *et al.*, 2024).

9. Emerging Techniques. GC×GC–TOFMS offers unparalleled separation for complex mixtures, significantly reducing co-elution issues. LC–MS/MS platforms optimized for intact lipid profiling and fast targeted FA panels are gaining popularity due to improved specificity and sensitivity (Trivedi *et al*., 2022; Fan *et al*., 2024).

Fatty Acid Profiles of Key Plant Pathogenic Genera. Fatty acid (FA) profiling of various plant pathogenic bacteria reveals distinct patterns that can aid in identification, taxonomy, and understanding of physiological adaptation. Branched-chain fatty acids (BCFAs), particularly iso- and anteiso- forms, are prevalent in genera like *Xanthomonas*, *Clavibacter*, and *Streptomyces*, while they are notably absent in *Acidovorax* and *Pseudomonas* species (Kaneda, 1991; Kozlova *et al.*, 2011; O'Leary & Wilkinson 1981).

1. *Xanthomonas* spp. & *Acidovorax citrulli* (Gram-negative). A detailed GC-MS survey encompassing six plant pathogens including four *Xanthomonas* strains (X. campestris pv. campestris, X. perforans), Acidovorax citrulli, Pseudomonas syringae pv. tomato, *Clavibacter michiganensis*, and *Streptomyces scabies* reported 12–31 fatty acids per species, totaling 44 distinct FAs. Among these:

Xanthomonas campestris and *X. perforans* exhibited fatty acid profiles dominated by branched-chain iso- (i15:0, i16:0) and anteiso-fatty acids (a15:0).

Acidovorax citrulli, in contrast,contained only saturated and monounsaturated FAs, devoid of branched forms.

2. Pseudomonas syringae (Gram-negative). In the same study, *Pseudomonas syringae* exhibited a fatty acid profile dominated by saturated and monounsaturated chains, such as 16:0 and 18:1 ω7c (a monounsaturated fatty acid with a cis double bond at the seventh carbon from the methyl end of the chain), with no detectable branched-chain fatty acids—similar to the profile observed in *Acidovorax*.

3. Clavibacter michiganensis & Streptomyces scabies (Gram-positive). *Clavibacter michiganensis* and *S. scabies* displayed diverse profiles with a strong prevalence of iso- and anteiso-fatty acids (i15:0, a15:0, i16:0), consistent with general trends in Gram-positive bacteria.

Notably, the study identified more fatty acid species in *C. michiganensis* than previously reported, indicating broader lipid diversity

4. Intact Lipid Phospholipidome Profiling: LC–MS/MS Approach. Rudt *et al*. (2024) advanced understanding by analyzing intact phospholipids (PE, PG, CL) via RP–HPLC-MS/MS in the same six plant pathogens, enabling classification based on the number of bound branched-chain fatty acids (BCFAs). They achieved structural resolution of isomeric PEs and demonstrated retention behaviors transferable across lipid classes.

By integrating both hydrolyzed FA profiles (via GC–MS) and intact lipid phospholipidome patterns (via LC–MS/MS), these studies provide a comprehensive understanding of membrane composition across major plant-pathogenic genera.

The total number of fatty acids detected across these strains ranges from 12 to 31 distinct molecules, with individual FA contributions varying from 0.01% up to 43.8% of the total composition (Kozlova *et al.*, 2011). The presence or absence of branched-chain fatty acids provides a taxonomic and ecological signature that distinguishes genera and may correlate with their adaptation strategies and pathogenic potential.

Factors Influencing Fatty Acid Composition. The fatty acid composition of plant pathogenic bacteria is not fixed; it is dynamically influenced by environmental and physiological conditions. These factors alter the membrane lipid profile to help bacteria maintain membrane fluidity, permeability, and survivability under different stresses (Denich *et al*., 2003; Russell & Nichols 1999; Zhang & Rock 2008). The most significant influencing factors include:

1. Growth Temperature. Temperature strongly affects the saturation level and branching of fatty acids:

- Low temperatures increase unsaturated and shorter-chain fatty acids to maintain membrane fluidity.
- High temperatures promote saturated and longer-chain fatty acids for membrane stability (Kaneda, 1991; Russell & Nichols 1999; Sato *et al*., 2000).
- Chain length & branching: Low temperature environments (e.g., *Micrococcus cryophilus*, *Shewanella oneidensis*, *Escherichia coli*) trigger production of shorter or branched-chain fatty acids like iso- and anteiso-BCFAs to preserve fluidity. Conversely, deep-sea barophiles increase polyunsaturated fatty acids (PUFAs) in very cold, high-pressure habitats.
- Barophilic species from trenches (e.g., *Shewanella violacea*, *Oleispira antarctica*) adjust FA chain length, increase unsaturation, incorporate PUFAs and hydroxy-FAs to maintain membrane integrity under extreme pressure and low temperature (Frontiers in Microbiology, 2020; Wikipedia for *S. violacea*).

2. Growth Phase. Fatty acid composition varies between logarithmic (exponential) and stationary phases:

- Exponential phase: more unsaturated fatty acids to support rapid membrane activity.
- Stationary phase: increase in cyclopropane fatty acids (e.g., C19:0 cyclo ω8c) for stress resistance (Zhang & Rock 2008; Grogan & Cronan 1984).

3. Culture Medium Composition. The type and availability of carbon and nitrogen sources, along with ions and pH of the medium, influence fatty acid synthesis:

• Rich media may promote branched-chain FA synthesis, depending on precursor availability.
• Minimal media often reduce lipid diversity (Kaneda, 1991; Denich *et al*., 2003).

4. Oxygen Availability. Aerobic vs anaerobic growth conditions affect the desaturation of fatty acids:
• Aerobic bacteria can introduce double bonds (unsaturation).
• Anaerobic conditions limit desaturase activity, often leading to more saturated FAs. (Zhang & Rock 2008; Sato *et al*., 2000)

5. Host Environment (In planta vs In vitro). The plant host environment can induce unique changes in bacterial fatty acid profiles:
• Presence of plant signals or defense molecules can trigger remodeling of bacterial membranes (Sohlenkamp & Geiger 2016).
• Certain lipids may only appear during plant infection stages. Certain lipids may be uniquely expressed or upregulated during plant colonization or infection stages (Kozlova *et al*., 2011).

6. Genetic Regulation. Mutations or natural variations in genes encoding fatty acid synthase (FAS), acyltransferases, or desaturases result in different profiles among strains and species (Zhang & Rock 2008; Sohlenkamp & Geiger 2016).

Below are specific examples highlighting genus-specific FA profiles among plant pathogenic bacteria:

1. *Xanthomonas* spp.

Characteristic fatty acids:
— High levels of branched-chain fatty acids (BCFAs): iso-C15:0, iso-C16:0, anteiso-C15:0
— Presence of unsaturated fatty acids like C16:1 ω7c
• These BCFAs contribute to membrane fluidity and stress tolerance.
• Used to distinguish *Xanthomonas* from other Gram-negative phytopathogens (Kozlova *et al*., 2011; Kaneda, 1991).

2. *Clavibacter michiganensis*
• They belong to the Gram-positive Actinobacteria.
• Dominated by branched-chain saturated fatty acids, especially iso-C14:0, iso-C16:0, and anteiso-C15:0.
• No unsaturated FAs typically present (Kaneda, 1991; O'Leary & Wilkinson 1981).

3. *Pseudomonas syringae*
• Lacks branched-chain FAs altogether.
• Dominated by straight-chain saturated and monounsaturated FAs, e.g.
— C16:0, C18:1 ω7c, C17:0 cyclo ω7c
• Cyclopropane fatty acids increase under stress (Grogan & Cronan 1984; Zhang & Rock 2008).

4. *Streptomyces scabies*
• Produces abundant branched-chain fatty acids, typical of Gram-positive filamentous actinomycetes.
• Contains iso-C16:0, anteiso-C15:0, and minor amounts of hydroxylated FAs (Kaneda, 1991; Sohlenkamp & Geiger 2016).

5. *Acidovorax citrulli*
• Distinct from *Xanthomonas* and lacks branched FAs.
• Fatty acid profile includes C16:0, C17:0 cyclo, and C18:1 ω7c, but no iso- or anteiso-FAs (Kozlova *et al.*, 2011).

These findings support the use of FA profiling as a reliable chemotaxonomic marker, reflecting:
• Genetic differences in fatty acid biosynthesis and desaturation pathways (Zhang & Rock 2008).
• Adaptive responses to environmental niches such as host plant and temperature (Sato *et al*., 2000; Denich *et al*., 2003).
• Evolutionary divergence between Gram-positive and Gram-negative bacterial lineages (Kaneda, 1991; Sohlenkamp & Geiger 2016).

Composition of fatty acids in plant pathogenic bacteria is influenced by species and environmental conditions

1. Species-Dependent Variation. Different bacterial species exhibit distinct fatty acid biosynthesis pathways, determined by specific enzymes such as desaturases, cyclopropane synthases, and fatty acid synthases (Kaneda, 1991; Zhang & Rock 2008). These pathways govern their baseline fatty acid profile, creating species-specific signatures.

Examples:
• *Xanthomonas campestris*: Dominated by branched-chain fatty acids (BCFAs) such as iso-C15:0, iso-C16:0, and anteiso-C15:0, which are typical for many members of this genus (Kozlova *et al*., 2011; Kaneda, 1991).
• *Pseudomonas syringae*: Contains primarily straight-chain saturated and monounsaturated FAs, e.g., C16:0, C18:1 ω7c, and cyclopropane FA (C17:0 cyclo), but lacks BCFAs (Zhang & Rock 2008; Grogan & Cronan 1984).
• *Clavibacter michiganensis*: As a Gram-positive Actinobacterium, it shows a fatty acid profile rich in iso- and anteiso-BCFAs, with no unsaturated FAs, distinguishing it from Gram-negative plant pathogens (Kaneda, 1991).

2. Environmental Influences on Fatty Acid Composition. Bacteria adjust their fatty acid profiles in response to environmental factors to maintain membrane fluidity, integrity, and function (Denich *et al*., 2003; Sohlenkamp & Geiger 2016).

a. Temperature
• Lower temperatures induce higher levels of unsaturated fatty acids to maintain membrane fluidity.
• Example: *Pseudomonas* spp. increase C18:1 ω7c under cold stress (Russell & Nichols, 1999; Sato *et al.*, 2000).

b. Nutrient Limitation and Stationary Phase
• Many bacteria, including *Pseudomonas*, increase cyclopropane fatty acids (CFAs) (e.g., C17:0 cyclo) in the stationary phase as a stress adaptation strategy (Grogan & Cronan 1984; Zhang & Rock 2008).

c. Osmotic or pH Stress

- Stress conditions often lead to elevated saturated or cyclic fatty acids.
- These changes make membranes less permeable and more stable under osmotic pressure (Denich *et al*., 2003; Sohlenkamp & Geiger 2016).

d. Host Interaction / Rhizosphere Environment

- FA composition can shift in response to plant root exudates or defense molecules.
- Example: *Ralstonia solanacearum* shows altered membrane lipid profiles when infecting tomato roots, possibly to evade host immunity or oxidative stress (Figueiredo *et al.*, 2021).

Fatty acid composition in plant pathogenic bacteria is a dynamic trait shaped by both genetic determinants (species-specific traits) and environmental stimuli such as temperature, pH, growth phase, and host interaction. These changes allow pathogens to optimize membrane performance and survivability in diverse ecological niches (Zhang & Rock 2008).

Compiled data on fatty acid (FA) composition serve as a powerful tool for both the identification and the functional understanding of membrane biology in plant pathogenic bacteria. Each bacterial species or genus tends to exhibit a characteristic fatty acid profile determined by its genetic and enzymatic capabilities. For example, *Xanthomonas* spp. are typically rich in iso- and anteiso-branched chain fatty acids such as iso-C15:0 and anteiso-C15:0, while *Pseudomonas syringae* and *Ralstonia solanacearum* contain mainly straight-chain saturated and monounsaturated fatty acids like C16:0, C18:1 ω7c, and cyclopropane derivatives such as C17:0 cyclo. These genus-specific profiles are widely used for chemotaxonomic classification, aiding in the rapid identification of phytopathogens through techniques such as fatty acid methyl ester (FAME) analysis (Sasser, 2001; Kozlova *et al.*, 2011). In addition, fatty acid data reveal critical insights into membrane adaptation strategies during environmental stress. For instance, *Pseudomonas putida* and *Escherichia coli* are known to increase their cyclopropane fatty acid content during the stationary phase or under low-pH and osmotic stress, which enhances membrane rigidity and survival under adverse conditions (Grogan & Cronan 1984; Zhang & Rock 2008).

Similarly, *Ralstonia solanacearum* shows altered membrane lipid composition in response to host plant interactions, which may facilitate evasion of plant defenses. Such data, when compiled across multiple species and conditions, enable researchers to not only distinguish pathogens but also to understand how membrane fluidity, permeability, and structure are regulated in the context of pathogenesis. Thus, fatty acid profiling bridges microbial taxonomy and functional membrane biology in phytopathogens (Figueiredo *et al.*, 2021**).** A broader review on *Pseudomonas* spp. reported that environmental variables—such as temperature, pH, culture medium, and growth stage—significantly alter fatty acid (FA) profiles, affecting membrane fluidity and permeability. For instance, higher temperatures and simpler media favor saturated straight-chain FAs, which contribute to more rigid membranes. In contrast, cooler temperatures, lower pH, or growth in complex media increase the proportions of unsaturated or branched-chain FAs, thereby enhancing membrane fluidity (Segura *et al.*, 2022).

In *Bacillus subtilis*, a plant-associated gram-positive, lower growth temperatures (25 °C vs. 37 °C) resulted in a 30-fold increase in the lipopeptide mycosubtilin, with a higher proportion of odd-numbered anteiso-C17 FAs, suggesting cold-induced shifts in FA biosynthesis.

4. Functional implications

- Alteration in fatty acid (FA) saturation and branching adjusts membrane fluidity and permeability, thereby influencing pathogen fitness parameters such as host adhesion, stress resistance, and antibiotic tolerance (Segura *et al*., 2022).
- Oxylipin signaling—derived from oleic (18:1), linoleic (18:2), and linolenic (18:3) acids—functions as a cross-kingdom communication mechanism, modulating both pathogen virulence and plant host defense responses (Beccaccioli *et al.*, 2022).

CONCLUSIONS

Fatty acid profiles are diverse and often genus-specific. Fatty acid (FA) composition is increasingly used as a chemotaxonomic tool to distinguish bacterial genera and species (Kaneda, 1991; Sasser, 2001; Kozlova *et al.*, 2011). The types and proportions of saturated, unsaturated, branched, hydroxylated, and cyclopropane fatty acids vary significantly across genera. These differences reflect evolutionary adaptations to niche environments, membrane requirements, and biosynthetic capabilities unique to each genus.

The types and proportions of saturated, unsaturated, branched-chain, hydroxylated, and cyclopropane fatty acids vary significantly across bacterial genera.

These variations reflect:

- Evolutionary adaptations to ecological niches (Sohlenkamp & Geiger 2016).
- Differences in membrane functionality and structural demands (Zhang & Rock 2008).
- Genetically determined biosynthetic capabilities, such as the presence or absence of specific desaturases, methyltransferases, or hydroxylases (Kaneda, 1991; Grogan & Cronan 1984).

For instance:

- *Xanthomonas* species exhibit a high proportion of branched-chain and hydroxy FAs,
- *Pseudomonas* are rich in unsaturated and cyclopropane FAs,
- *Clavibacter* and *Streptomyces* are dominated by iso-/anteiso-BCFAs, characteristic of Gram-positive bacteria (Kozlova *et al*., 2011).

These genus-specific fatty acid fingerprints are widely employed in FAME (Fatty Acid Methyl Ester) analysis, enabling rapid identification and differentiation of phytopathogenic bacteria based on their membrane lipid signatures (Sasser, 2001; Zhang & Rock 2008).

Fatty acid (FA) profiling in plant pathogenic bacteria offers valuable insights into their taxonomy, physiology, adaptability, and pathogenicity. Species-specific differences in FA composition—such as the dominance of iso- and anteiso-branched fatty acids in Gram-positive pathogens and the prevalence of straight-chain saturated and monounsaturated fatty acids in Gram-negatives—highlight the taxonomic and structural diversity among these organisms (Wiedmaier-Czerny *et al.*, 2021). The advent of advanced lipidomic techniques like LC–MS/MS has further refined our understanding by resolving complex isomeric structures of membrane phospholipids, allowing more precise characterization of microbial membrane architecture (Rudt *et al.*, 2024).

Environmental conditions, including temperature, pH, nutrient composition, and growth stage, markedly influence FA composition. These adaptations affect membrane fluidity, stress resilience, and antibiotic resistance—traits critical for bacterial survival and virulence (Segura *et al*., 2022). Additionally, certain fatty acid derivatives, such as oxylipins, act as signaling molecules that regulate host–pathogen interactions and cross-kingdom communication, playing an emerging role in the modulation of plant immunity and microbial pathogenicity (Beccaccioli *et al.*, 2022).

In summary, fatty acid profiles serve not only as biochemical fingerprints for identification and classification but also as dynamic biomarkers reflecting the ecological adaptability and virulence potential of plant pathogenic bacteria. Future research integrating lipidomics, genomics, and metabolomics will enhance our understanding of bacterial pathogenesis and may open new avenues for developing targeted disease management strategies.

FUTURE SCOPE

Despite significant advancements in the characterization of fatty acid (FA) profiles in plant pathogenic bacteria, several key areas remain underexplored. Future research should focus on the following directions**:**

1. Enhancing Bacterial Identification through Fatty Acid Profiling

Fatty acid methyl ester (FAME) analysis has proven effective in bacterial identification and can be further enhanced by integration with molecular tools such as PCR, 16S rRNA sequencing, or whole-genome analysis. Combining lipidomics and genomics may yield a more accurate and high-throughput diagnostic approach for identifying plant pathogens, particularly in agricultural diagnostics and microbial ecology (Sasser, 2001; Kozlova *et al*., 2011; Zhang & Rock 2008).

Integration of Multi-Omics Approaches: Combining lipidomics with genomics, transcriptomics, and metabolomics can provide a comprehensive understanding of FA biosynthetic pathways, regulation mechanisms, and their roles in host-pathogen interactions. Such integrative studies could uncover novel biomarkers for early disease detection or targets for antibacterial strategies (Rudt *et al*., 2024).

2. Understanding Environmental Adaptation Mechanisms. Changes in fatty acid composition serve as markers of bacterial adaptation to temperature, pH, oxidative stress, and nutrient availability. Investigating these dynamics in plant pathogenic bacteria can help forecast pathogen behavior and outbreaks in response to climate change and other environmental pressures (Denich *et al*., 2003; Russell & Nichols 1999; Sohlenkamp & Geiger 2016).

More dynamic studies under simulated environmental stress—such as temperature fluctuations, drought, and agrochemical exposure—could clarify how FA modulation enables pathogen survival under agricultural field conditions. This would aid in predicting pathogen outbreaks under climate change scenarios (Beccaccioli *et al*., 2022).

3. Development of Fatty Acid-Based Diagnostic Tools. Species-specific FA profiles act as chemotaxonomic markers and hold promise for development of rapid, cost-effective, and field-deployable pathogen detection kits using biosensors or portable GC-MS systems (Kozlova *et al.*, 2011; Sasser, 2001).

Species-specific FA fingerprints especially when combined with advanced machine learning and portable mass spectrometry tools could be harnessed for rapid, on-site identification of bacterial plant pathogens in agricultural settings (Wiedmaier-Czerny *et al*., 2021).

4. Linking Lipid Composition to Virulence and Host Specificity. Future research may explore how FA profiles influence virulence traits such as motility, biofilm formation, host specificity, and immune evasion. Understanding these connections can offer insights into how lipid composition supports bacterial pathogenicity and interaction with host plants (Zhang & Rock 2008; Figueiredo *et al*., 2021).

Expanding the study of oxylipins and other FA-derived signals may reveal new aspects of cross-kingdom communication. Deciphering how bacterial FAs or lipids modulate plant defense responses can lead to development of lipid-based plant immunity modulators or resistance inducers (Beccaccioli *et al.*, 2022).

5. Designing Lipid-Targeted Disease Control Strategies. Elucidating the membrane lipid architecture of plant pathogens could enable the development of lipid-targeting antimicrobials or compounds that disrupt fatty acid synthesis, offering novel disease control options with reduced resistance risk (Parsons & Rock 2013; Sohlenkamp & Geiger 2016).

6. Antimicrobial target discovery. Since membrane integrity and FA composition are essential for bacterial viability and virulence, targeting FA biosynthetic enzymes offers a promising antimicrobial strategy. Selective inhibition of FA pathways unique to phytopathogens may yield environmentally safe biocontrol agents.

Conflict of Interest. None.

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