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PHYTOPLASMA: UNDERSTANDING A STEALTHY PATHOGEN

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Abstract

Phytoplasmas are unique and enigmatic pathogens that cause devastating diseases in a wide range of plants, leading to significant economic losses in agriculture worldwide. Despite their small size and lack of cell wall, phytoplasmas exhibit remarkable complexity in their biology, transmission and interaction with host plants and insect vectors. This paper provides an overview of phytoplasma biology, including their morphology, genetic diversity, transmission mechanisms, and impact on plant health. Additionally, we discuss current methods for phytoplasma detection, diagnosis, and management strategies. Understanding phytoplasma pathogenesis and developing effective control measures are essential for sustainable agriculture and food security.

Keywords: *Phytoplasma; Morphology; Detection; Genetic diversity; Transmission; Colonization*

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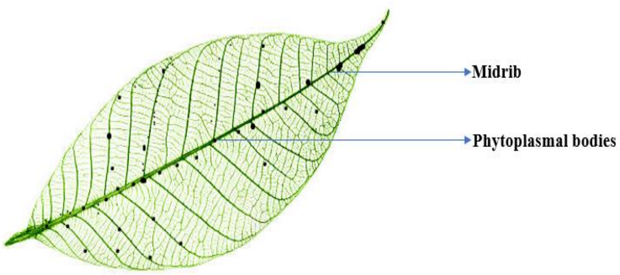
Introduction

In the recent years, plants are exposed to several threats posed both biotic and abiotic factors. Among biotic factors, fungi, bacteria, virus and phytoplasma are important agents. Though fungi, bacteria and virus are causing severe yield losses, phytoplasmal identification remains unknown. Since study on phytoplasma was narrower and knowledge was uncertain. So there is a need to know about it.



Phytoplasmas are bacterial pathogens belonging to the class Mollicutes, characterized by their small size, lack of cell wall, and dependence on host plants and insect vectors for survival and transmission (Hogenhout*et al.*, 2008). First discovered in the early 1960s, phytoplasmas have since been implicated in numerous plant diseases worldwide, affecting over 1,000 plant species across diverse agricultural and horticultural crops (Kumari *et al.*, 2019). Their ability to cause a wide range of symptoms, including leaf yellowing, stunting, witches' broom, and phyllody, poses significant challenges to crop production and food security. Despite their economic importance, many aspects of phytoplasma biology and pathogenesis remain poorly understood (Reddy *et al.*, 2023).

Morphology and Ultrastructure of Phytoplasma

The morphology and ultrastructure of phytoplasmas are distinct features that contribute to their unique biological characteristics. Phytoplasmas lack a cell wall and possess a relatively simple cellular organization compared to other bacteria.

Cell Shape and Size: Phytoplasmas exhibit pleomorphic shapes, ranging from spherical to elongated or filamentous structures. The size of phytoplasma cells typically falls within the range of 200 to 800 nm, although variations can occur depending on the specific strain and environmental conditions(Namba, 2019; Gamage*et al.*, 2023).

Cellular Components: Phytoplasma cells consist of a plasma membrane that encloses the cytoplasmic contents. Unlike typical bacteria, phytoplasmas lack a rigid cell wall, which distinguishes them as members of the class Mollicutes. This absence of a cell wall contributes to their flexibility and adaptability within host tissues (Razin, 2006).

Cytoplasmic Structures: Within the cytoplasm, phytoplasmas contain various organelle-like structures, including ribosomes, nucleoid regions containing the genetic material (DNA), and inclusion bodies (He *et al.*, 2023). These structures play essential roles in cellular metabolism, protein synthesis, and nucleic acid replication.

Ultrastructural Features: Transmission electron microscopy (TEM) has provided valuable insights into the ultrastructure of phytoplasmas. TEM images reveal the presence of electron-dense ribosomes dispersed throughout the cytoplasm, contributing to protein synthesis. Additionally, nucleoid regions can be observed, which contain the chromosomal DNA and associated proteins(Liu *et al.*, 2012; Malatesta, 2021).

Absence of Cell Wall: One of the most notable ultrastructural features of phytoplasmas is the absence of a cell wall. This characteristic distinguishes them from other bacteria, such as Gram-positive or Gram-negative species, which possess a peptidoglycan layer in their cell wall. The lack of a cell wall contributes to phytoplasmas' susceptibility to osmotic stress and sensitivity to certain antibiotics targeting cell wall synthesis(Christensen*et al.*, 2005)

Host-Pathogen Interactions: Understanding the ultrastructure of phytoplasmas is crucial for elucidating their interactions with host plants and insect vectors. Phytoplasmas colonize the phloem tissue of infected plants and can disrupt nutrient transport, leading to characteristic disease symptoms. Additionally, ultrastructural studies have revealed interactions between phytoplasmas and insect vectors, shedding light on transmission mechanisms and vector specificity(Inaba*et al.*, 2023).

The variability in phytoplasma morphology underscores their diverse genetic makeup and adaptation to different host environments.

Genetic Diversity and Phylogenetics

Genetic diversity and phylogenetics are fundamental aspects of phytoplasma research, offering insights into their evolutionary history, taxonomy, and epidemiology. Phytoplasmas, as uncultivable bacteria, present unique challenges in studying their genetic diversity. However, advancements in molecular biology techniques have facilitated the characterization of phytoplasma genomes and the elucidation of their phylogenetic relationships.

Genetic Diversity**:** Phytoplasmas exhibit considerable genetic diversity, which manifests in variations in virulence, host range, and geographical distribution. This diversity arises from several mechanisms, including mutation, recombination, and horizontal gene transfer. The 16S rRNA gene, along with other protein-coding genes and non-coding regions, serves as a primary target for assessing genetic diversity due to its conserved nature interspersed with hypervariable regions. Additionally, whole-genome sequencing has provided comprehensive insights into the genetic makeup of phytoplasmas, revealing gene content variations and genomic rearrangements among different strains and species(Malembic-Maher*et al.*, 2011; Cho*et al.*, 2020; Weiand Zhao, 2022).

Phylogenetic Analysis: Phylogenetic analysis plays a crucial role in understanding the evolutionary relationships among phytoplasma strains and species. The construction of phylogenetic trees using molecular data enables researchers to infer the genetic relatedness and evolutionary history of phytoplasmas(Mardi *et al.*, 2016). Commonly employed molecular markers for phylogenetic analysis include the 16S rRNA gene, ribosomal protein genes (e.g., rp genes), and housekeeping genes (e.g., dnaA, tuf). Phylogenetic trees are reconstructed using various methods, such as maximum likelihood, Bayesian inference, and neighbor-joining algorithms, to infer evolutionary distances and phylogenetic relationships accurately(Shakya *et al.*, 2020; Tokuda*et al.*, 2023)

International Phytoplasma Classification Scheme: The International Phytoplasma Classification Group (IPCG) has developed a standardized classification scheme for phytoplasmas based on 16S rRNA gene sequence analysis. Phytoplasmas are categorized into distinct groups (e.g., 16SrI, 16SrII) and further subdivided into subgroups within each group based on sequence similarity (Zhao *et al.*, 2016; Kirdat*et al.*, 2023). This classification scheme provides a framework for organizing phytoplasma diversity and facilitates communication among researchers and plant health professionals worldwide. However, it is essential to note that advancements in genomic analysis may lead to periodic revisions and updates in phytoplasma taxonomy (Al-Subhi*et al.*, 2017; Dudukand Bertaccini, 2011).

Intraspecific Variation and Strain Differentiation: Within individual phytoplasma species, intraspecific variation and strain differentiation are commonly observed. This variation may arise due to geographical isolation, host specialization, or genetic drift(Pusz-Bochenska*et al.*, 2022). Molecular techniques, such as multilocus sequence typing (MLST) and multilocus variable number tandem repeat analysis (MLVA), are employed to characterize intraspecific diversity and differentiate between phytoplasma strains(Kong*et al.*, 2022; Pilet*et al.*, 2019). These methods allow for the identification of unique genetic markers and the comparison of genetic profiles among different isolates.

Implications for Disease Management: Understanding genetic diversity and phylogenetic relationships among phytoplasma strains has significant implications for disease management strategies. Knowledge of strain diversity and distribution can inform the development of molecular diagnostics, disease surveillance programs, and targeted control measures(Contaldo*et al.*, 2021;Janik*et al.*, 2023). Furthermore, insights into phylogenetic relationships can aid in tracing the origins and spread of phytoplasma-associated diseases, guiding efforts to mitigate their impact on agricultural crops and natural ecosystems (Rao, 2021).

How these Phytoplasma Bodies are moved Across the Insect and their Transmission through Vector?

Their transmission primarily occurs through insect vectors, which acquire phytoplasmas while feeding on infected plants and subsequently transmit them to healthy plants.

Vector-Mediated Transmission: Phytoplasmas rely on insect vectors for their transmission between plants. These vectors are typically sap-feeding insects belonging to various families, including leafhoppers (Cicadellidae), planthoppers (Delphacidae), psyllids

(Psyllidae), and others (Weintrauband Beanland, 2006; Trivelloneand Dietrich, 2021). When infected vectors feed on phytoplasma-infected plants, they ingest phytoplasmas along with phloem sap(Rojas-Martinez,2009). Phytoplasmas can then colonize the insect's digestive system, including the gut and salivary glands, where they multiply and persist. During subsequent feeding activities, infected vectors inoculate phytoplasmas into healthy plants through their saliva, thereby transmitting the pathogen.

Persistent Propagative Transmission: Phytoplasmas undergo persistent propagative transmission within insect vectors, meaning they persistently infect and multiply in the vector's body without causing apparent harm. Infected vectors can retain phytoplasmas for the duration of their lifespan, allowing for multiple opportunities for transmission to new host plants(Chen*et al.,* 2017). The ability of phytoplasmas to persistently infect insect vectors is facilitated by their adaptation to the vector's physiology and the presence of specific molecular interactions between the phytoplasma and insect host (Ghosh*et al.,* 2019;Blanc*et al.,* 2019)

Circulative Transmission Pathway: The transmission of phytoplasmas within insect vectors follows a circulative pathway, wherein the pathogen circulates through the insect's hemolymph (circulatory fluid) and other tissues(Heck, 2018). After ingestion, phytoplasmas traverse the insect's digestive system and cross the gut barrier to enter the hemolymph. Once in the hemolymph, phytoplasmas can spread to various organs, including the salivary glands, where they accumulate and become available for transmission to new host plants during feeding (King and Hillyer, 2012).

Salivary Inoculation: Transmission of phytoplasmas to healthy plants occurs when infected insect vectors feed on phloem sap and inoculate phytoplasmas into the plant's vascular system through their saliva (Hemmati *et al.,* 2019). Phytoplasmas injected into the plant tissue can colonize the phloem, where they establish infection and induce disease symptoms. The presence of phytoplasmas in the plant's phloem disrupts nutrient transport and cellular processes, leading to characteristic disease symptoms such as yellowing, stunting, or abnormal growth (Sugio*et al.,* 2011).

Vector Specificity and Efficiency: The transmission efficiency and specificity of phytoplasmas depend on various factors, including the vector species, vector competence, and phytoplasma strain. Different insect vectors exhibit varying degrees of vector competence, with some species capable of transmitting specific phytoplasma strains more efficiently than others. Vector specificity also influences the epidemiology and geographic distribution of phytoplasma diseases, as certain vector species may be restricted to specific regions or host plants (Boscoand D'Amelio, 2009).

The transmission of phytoplasmas primarily occurs through insect vectors, which acquire the pathogen from infected plants and transmit it to healthy plants during feeding activities. The persistent propagative transmission of phytoplasmas within insect vectors, combined with their ability to circulate through the insect's body and be inoculated into new host plants via saliva, facilitates the spread of phytoplasma diseases in agricultural and natural ecosystems. Understanding the transmission mechanisms of phytoplasmas is essential for developing effective strategies for disease management and vector control.

How Phytoplasma Colonizes the Living Tissue?

Phytoplasmas, as obligate intracellular bacteria, cause disease in plants through complex interactions with their host's physiology and immune responses.

Colonization of Plant Tissues: Phytoplasmas typically enter plants through the feeding activities of insect vectors, such as leafhoppers, planthoppers, or psyllids. Once inside the plant, phytoplasmas colonize the phloem tissue, where they establish themselves and multiply

(Alma*et al.*,2019). The presence of phytoplasmas disrupts normal plant physiological processes, leading to disease symptoms.

Phloem Disruption and Nutrient Imbalance: Phytoplasmas disrupt the function of the phloem, which is responsible for transporting nutrients and signalling molecules throughout the plant. This disruption interferes with the distribution of essential nutrients, such as sugars, amino acids, and hormones, leading to nutrient imbalances within the plant. As a result, affected plant tissues may exhibit chlorosis (yellowing), necrosis (tissue death), or abnormal growth patterns (Buoso*et al.*, 2022).

Production of Phytopathogenic Molecules: Phytoplasmas produce and secrete various phytopathogenic molecules, including effectors, toxins, and cell wall-degrading enzymes, which contribute to disease development. These molecules can manipulate host cell processes, suppress plant defences, and facilitate the colonization and spread of phytoplasmas within the plant(Nishad*et al.*, 2020). For example, phytoplasma effectors may interfere with plant hormone signalling pathways or modulate the plant's immune responses to promote their own survival and proliferation.

Induction of Abnormal Developmental Responses: Phytoplasma infections often induce abnormal developmental responses in plants, leading to characteristic disease symptoms. For example, phytoplasma-infected plants may exhibit witches' broom (excessive branching), phyllody (conversion of floral organs into leaf-like structures), virescence (greening of floral parts), or proliferation of shoots. These developmental abnormalities result from disruptions in hormone signalling pathways and cellular differentiation processes caused by phytoplasma infection(Bertaccini, 2022).

Secondary Infections and Stress Susceptibility: Phytoplasma infections weaken plant defences and increase susceptibility to secondary infections by other pathogens, such as fungi, bacteria, or viruses (Ahmed et al., 2022). Additionally, phytoplasma-infected plants may experience increased susceptibility to environmental stresses, such as drought, heat, or nutrient deficiencies, further exacerbating disease symptoms. These synergistic effects contribute to the overall decline in plant health and productivity associated with phytoplasma diseases.

Vector-Mediated Transmission: Phytoplasmas rely on insect vectors for their transmission between plants. Infected insects acquire phytoplasmas during feeding on infected plants and subsequently transmit them to healthy plants during subsequent feeding activities(Galetto et al., 2023) This vector-mediated transmission facilitates the spread of phytoplasma diseases over large distances and across diverse plant species, contributing to their widespread impact on agriculture and natural ecosystems.

Phytoplasmas cause disease in plants by disrupting phloem function, inducing nutrient imbalances, producing phytopathogenic molecules and triggering abnormal developmental responses. The complex interactions between phytoplasmas, their plant hosts, and insect vectors contribute to disease development and spread, posing significant challenges for disease management and crop protection efforts. Understanding the mechanisms by which phytoplasmas cause disease is essential for developing effective strategies to mitigate their impact on agricultural crops and natural ecosystems.

Impact on Plant Health

Phytoplasma infections can result in a wide range of symptoms, including chlorosis, necrosis, proliferation of shoots, and abnormal floral development, ultimately leading to reduced yield and quality of agricultural crops. The severity of phytoplasma diseases depends on factors such as host susceptibility, environmental conditions, and vector abundance.

Integrated pest management (IPM) strategies combining cultural practices, chemical control, and resistant cultivars are essential for mitigating phytoplasma-associated losses

Whether it Possible to Detect and Diagnose the Phytoplasma?

Detecting and diagnosing phytoplasma infections in plants can be challenging but is crucial for implementing appropriate management strategies.

Accurate and timely detection of phytoplasma infections is crucial for disease management. Molecular techniques, including polymerase chain reaction (PCR), nested PCR, quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP), are commonly used for phytoplasma detection and identification in plant tissues and insect vectors. Serological methods, such as enzyme-linked immunosorbent assay (ELISA), are also employed for large-scale screening of phytoplasma-infected plants.

Management Strategies

Effective management of phytoplasma diseases requires an integrated approach incorporating cultural, chemical, and biological control measures. Cultural practices, such as rouging infected plants, controlling insect vectors, and promoting plant health through proper nutrition and irrigation, are essential for reducing disease incidence. Chemical control using insecticides can suppress vector populations, while biological control agents, such as natural enemies and entomopathogenic fungi, offer sustainable alternatives for vector management.

Conclusion

In conclusion, phytoplasmas present a formidable challenge to global agriculture, underscoring the necessity for concerted research endeavors to comprehend and mitigate these elusive pathogens. Through unraveling the complexities of phytoplasma biology, transmission mechanisms, and pathogenesis, researchers can pave the way for innovative strategies in disease prevention and control, ultimately safeguarding agricultural productivity and livelihoods worldwide. Advancements in genomic technologies, including high-throughput sequencing, genome editing, and metagenomics, offer promise in deciphering the molecular mechanisms underpinning phytoplasma virulence and host interactions. Understanding the genetic basis of phytoplasma pathogenicity and host susceptibility will facilitate the development of resistant cultivars and targeted control strategies. Furthermore, research focused on elucidating the ecology of phytoplasma transmission and the impact of climate change on disease dynamics is paramount for predicting and mitigating future disease outbreaks. Integrated pest management strategies, combining cultural, chemical, and biological control measures, are essential for effective disease management. In summary, by comprehensively understanding phytoplasma biology and ecology, along with deploying innovative control measures, we can combat the threat posed by these stealthy pathogens, ensuring sustainable agriculture and food security for generations to come.

Future Directions

Advancements in genomic technologies, including high-throughput sequencing, genome editing and metagenomics, hold promise for deciphering the molecular mechanisms underlying phytoplasma pathogenesis and host interactions. Understanding the genetic basis of phytoplasma virulence and host susceptibility will facilitate the development of resistant cultivars and targeted control strategies. Furthermore, research efforts focused on elucidating the ecology of phytoplasma transmission and the impact of climate change on disease dynamics are essential for predicting and mitigating future disease outbreaks. Conclusion: Phytoplasmas represent a significant threat to global food security, highlighting the need for concerted research efforts to understand and manage these elusive pathogens. By unravelling the complexities of phytoplasma biology, transmission, and pathogenesis, researchers can develop innovative strategies for disease prevention and control, ultimately safeguarding agricultural productivity and livelihoods worldwide.

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