PHYTOPLASMA

Abstract

Phytoplasmas is a class of bacteria that is pathogenic to a wide variety of plant species, and was first identified in 1967. It is characterized by its small genome size, ranging from 0.3 to 1.2 micrometers, and polymorphic polymorphism. It is important for agriculture to identify the factors that contribute to its pathogenicity, as well as to identify effective measures for the control of its diseases. Despite its economic importance, Phytoplasm remains the least characterized plant pathogen, largely due to the lack of in vitro cultivation, gene transfer, or mutagenesis. It is capable of infecting the eggs of insect vectors, and has been known to cause disease in more than 1,000 plant species. As it is not possible to culture the bacterium, most of the information regarding its morphology can be derived from the analysis of serologically thin sections of infected plant sieve tubes under a dark field light microscope. Phytoplasmas with well-studied host plant ranges show a wide range of host species, with some infecting multiple crops or non-crop species.

Keywords: Phytoplasma, Mollicute, Electron microscope

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I. INTRODUCTION

Phylogenetically related to low G+C Gram-positive bacteria, phytoplasmas are prokaryotic plants pathogens that belongs to the Mollicutes class of bacteria. Due to their morphological and ultrastructural resemblance to mycoplasmas, which were already recognized as the causative agents of various animal and human diseases, phytoplasmas were first identified in 1967 and were given the moniker mycoplasma-like organisms (MLOs). MLOs were created as a coherent, genus-level taxon after the use of molecular technologies, and this taxon was given the name "Candidatus Phytoplasma." Groups and subgroups within this new clade have been defined, and many of them are now regarded as species. Small single-celled polymorphic mollicutes with a size range of 0.3 to 1.2 mm, phytoplasmas are among the smallest known self-replicating organisms. They are also known for their trans-kingdom parasitic lifestyle and their small genomes, which range from 530 to 1350 kb. They could only thrive and proliferate in hysotonic environments, like the phloem of plants or the haemolymph of insects. So, they are strictly host- dependent. They could multiply in insect vectors and also infect their eggs. Phytoplasmas were known to be pathogenic to more than a thousand plant species. The phytoplasmas continuously cycle between plants and insects and require both organisms forsurvival and dispersal in nature. This requirement necessitates the adaptation to a broad range of environments, including the phloem of their plant hosts and the gut lumen, haemolymph, saliva and endocellular niches in various organs of their insect hosts. The majority of the information regarding the morphology of Phytoplasma can be derived from the examination of the serial thin sections of Phloem Sieve Tubes of Infected Plants under an electron microscope, as it has not yet been possible to culture the plant. The cell wall degrading enzymes, cellulase, and macerase, can be employed to differentiate the phytoplatmas from the intact phloem Tubes. The presence of Phytoplatmas in the sieve Tubes of infected Plants can be determined through the use of Dark Field Light Microscopy (Figure 1).

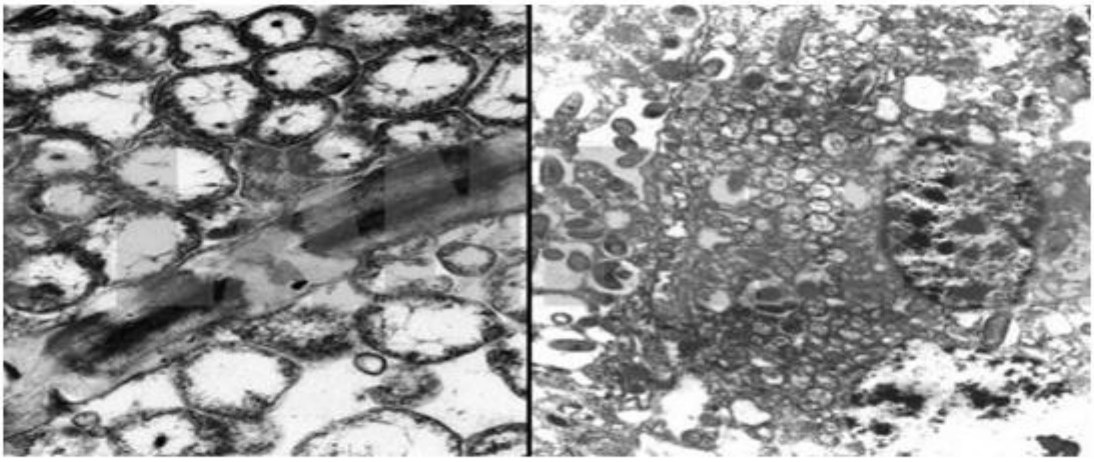

Figure 1: Transmission electron micrograph (TEM) showing Phytoplasma bodies that are found in Plant phloem sieve plates. (Courtesy - www.costphytoplasma.ipwgnet.org)

As a result, Phytoplasmas morphology can be studied at different stages of the crop's development. In the initial stages of yellow diseases, such as Pear Decline, aster Yellows, and Tomato Big Bud Disease are the predominant forms. In an electron microscope, Phytoplasma is composed of a variety of pleomorphic forms. These include small spherical forms (60–100 nm dia), large globular forms (150–1150 nm dia), and globular and filamentous forms (1–2 m– several million dia). Small round-to-large globular forms are most commonly observed in the late stage of the cell membrane or in the more advanced pathological stages. Thin sections of phytoplasm may be simple round, filamentous, or branched, and may be visible in thick sections of the ultra-microscope section or in serial sections of the sieve element. Phytoplasmic ultrastructures reveal that they are bound together by a 7.5–10 nm trilamellular unit membrane. The cytoplasm contains ribosomes and nuclei in the form of small fibrillar arrays of DNA. All studied phytoplasma have a single immunodeficient protein (with a known function). This protein is thought to be responsible for the insect-phytoplasma interaction.

II. TAXONOMY

The exact nature and taxonomic status of phytoplasmic mollicutes remains uncertain. In 1992, the Sub-Committee on Mollicutes recommended the use of the term "phytoplasma" instead of MLOs for the purpose of describing phytopathogenics. The genus "phytoplasma" was created in 2004 and currently holds the status of Candidatus, which is restricted to bacteria that cannot be cultivated. However, based on the current knowledge their taxonomic position is as follows:

Kingdom	:Prokaryote
Division	:Firmicutes
Class	:Mollicutes
Order	:Acholeplasmatales
Family	:Acholeplasmataceae
Genus	:*Candidatus* phytoplasma

16S ribosomal RNA (RNA) gene sequences have been extensively conserved throughout the phyto-plasma clade, and have thus served as a fundamental molecular tool for the identification of phytoplasmic species, the genotyping of samples, the assignment of taxonomic groups, and the classification of groups and subgroups. Taxonomic groups are derived from differences in fragment sizes generated by the restriction digestion of 16SrRNA gene sequence, referred to as RFLP, or by comparing DNA sequences from 16s/23S spacer regions. Table 1 displays the Phyto-Plasma Groups, Subgroups, and Candidatus species assigned to each group.

Table 1: Phytoplasma 16S Ribosomal RNA RFLP groups and given *Ca*. Phytoplasma Species

Group	No. of Subgroup	*Candidatus* Phytoplasma species
16SrI: aster yellows	22	‘*Ca.* P. asteris’ ‘*Ca*. P. lycopersici’
16SrII: Peanut witches’-broom	6	‘*Ca*. P. aurantifolia’ ‘*Ca*. P. australasia’
16SrIII: X-disease	19	‘*Ca.* P. pruni’
16SrIV: Coconut lethalyellows	3	*

16SrV: Elm yellows	6	'*Ca*. P. ulmi' '*Ca*. P. ziziphi' '*Ca*. P. rubi" '*Ca*. P. balanitae'
16SrVI: Clover proliferation	8	'*Ca*. P. trifolii' '*Ca*. P. sudamericanum'
16SrVII: Ash yellows	3	'*Ca*. P. fraxini'
16SrVIII: Loofahwitches' broom	1	'*Ca*. P. luffae'
16SrIX: Pigeon pea witches' broom group	7	'*Ca*. P. phoenicium
16SrX: Apple proliferation	5	'*Ca*. P. mali' 'Ca. P. prunorum' 'Ca. P. pyri' 'Ca. P. spartii'
16SrXI: Rice yellows dwarf	3	'*Ca*.P. oryzae'
16SrXII: Stolbur group	8	'*Ca*. P. japonicum' '*Ca*. P. solani' '*Ca*. P. australiense' '*Ca*. P. fragariae' '*Ca*. P. convolvuli'
16SrXIII: Mexican periwinkle virescence	2	*
16SrXIV: Bermuda grass white leaf	2	'*Ca*. P. cynodontis'
16SrXV: Hibiscus witches'-broom	2	'*Ca*. P. brasiliense'
16SrXVI: Sugarcane yellows leaf	1	'*Ca*. P. graminis'
16SrXVII: Papaya bunchytop	1	'*Ca*. P. caricae'
16SrXVIII: American potato purple top wilt	1	'*Ca*. P. americanum'
16SrXIX: Chestnut witches' broom	1	'*Ca*. Phytoplasma castaneae'
16SrXX: Rhamnus witches' broom	1	'*Ca*. P. rhamni'
16SrXXI: Pinus phytoplasmas	1	'*Ca*. P. pini'
16SrXXII	2	'*Ca*. P. palmicola'
16SrXXIII	1	*
16SrXXIV	1	*
16SrXXV	1	*
16SrXXVI	1	*
16SrXXVII	1	*
16SrXXVIII	1	*
16SrXXIX: Cassia witches' broom	1	'*Ca*. P. omanense'
16SrXXX: Salt cedar witches' broom	1	'*Ca*. P. tamaricis'
16SrXXXI: Soybean stunt	1	'*Ca*. P. costaricanum'
16SrXXXII:Malaysian periwinkle virescence and phyllody	3	'*Ca*. P. malaysianum'
16SrXXXIII: Allocasuarina muelleriana phytoplasma	1	'*Ca*. P. allocasuarinae'

*-No type species name

III. DISEASE CYCLE

Phytoplasma are transmitted by circulatory and long-term transmission by leafhoppers feeding on phloem of infected plants (Figure. 2) such as Cicadellidae, planthoppers, and Psyllids. Phytoplasma can be acquired from the host plant by an insect consuming phloem of the infected plant. The source plant can influence leafhopper acquisition from the host plant. The inability of vectors to acquire from a specific plant species may be caused by plant metabolites that interfere with insect feeding. Alternatively, the insect's feeding behaviour may be dependent on the host plant's titre. Phytoplasmas can pass through the insect's gut wall and multiple in the haemolymph. They then enter the salivary glands and multiply further.

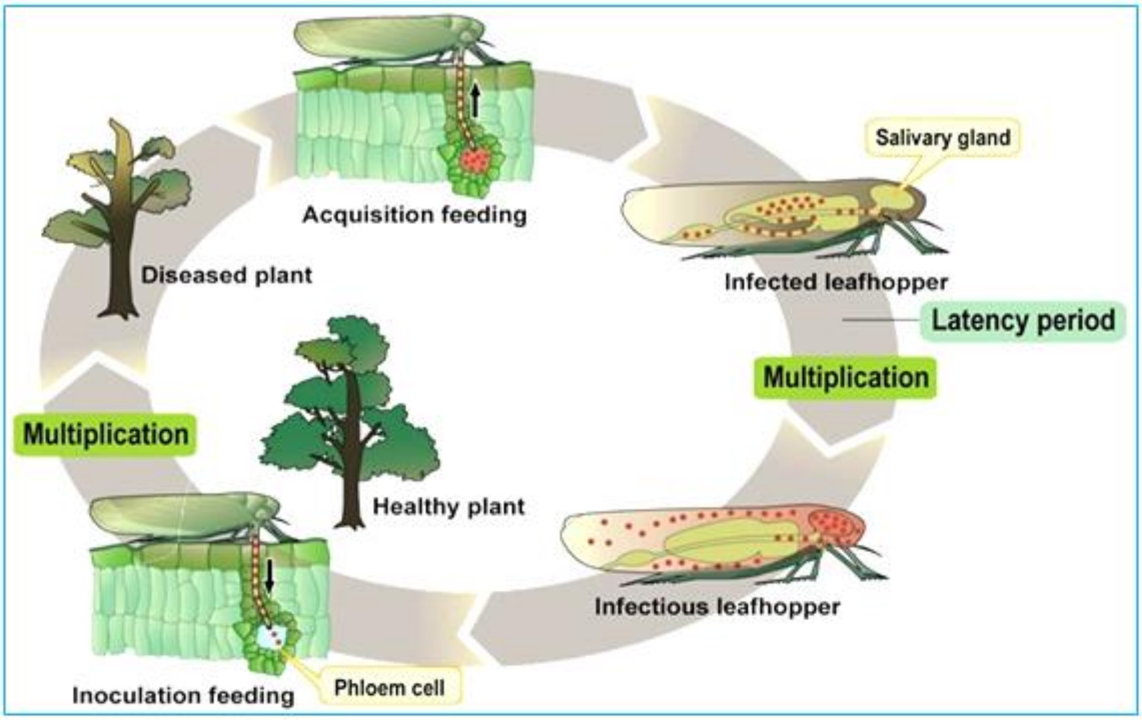

Figure 2: Disease Cycle of Phytoplasmas

The mechanisms underlying phytoplasma migration through insect membranes are poorly understood. Surface adhesions could be involved in phytoplasma transmission. The presence of receptors indicates that the phytoplasma contains genetic material required for insect vector penetration. Insect vectors are capable of transmitting the Phytoplasma only after infection of the insect's salivary glands, thus creating a latent period of 2-6 weeks between acquisition and transmission. Once an insect vector is infected, it is inoculated for life. Once the latency period has passed, the insect is able to spread the Phytoplasmas to a plant host through feeding on the phloem tissue of the plant host. The Phytoplatasma is transferred from the insect to the phloem through saliva, which is necessary for mouthpart lubricating during feeding. The transmission efficiency of Phytoplatmas can be influenced by the insect's sex and life cycle, temperature, age of the host plant, and type of host plant. As phytoplatasmas multiply in the plant host phloem tissue prior to causing symptoms, a latent period of infection exists between insect vector transmission and symptom expression (Figure. 2). Phytoplasmas multiply in 'infected plant phloem tissue' and can spread across the entire plant, including roots. Phytoplasmas can also be transferred from host plant to host plant by cleft grafting, dodder (parasitic vine, Cuscuta spp.), or vegetative propagation such as cuttings or rhizomes. Phytoplasmas have been found in the embryo tissues of coconut palms with lethal yellowing disease, raising the possibility of phytoplasma seed transfer.

IV. SIGN AND SYMPTOMS OF PHYTOPLASMA'S DISEASES

Plants that have been infected with Phytoplasma show a variety of distinct and non-distinctive symptoms, indicating a significant alteration in the equilibrium of plant metabolism. Symptoms of Phytoplasma infection may range from yellowish, chlorotic, or bronzed foliage to stunting (reducing the internode and leaf size) and virescence (developing green flowers and a decrease in normal pigments). Secondary auxiliary buds may proliferate, leading to a witches' broom effect, and internodes may elongate into slender shoots. Secondary roots, big buds, and enlarged stipules may also proliferate. Inhibition of flowering and other flower abnormalities may be present, as well as abnormal fruits and seeds. Off-season growth may also be observed, as well as brown discoloration in phloem tissue. Symptoms may vary depending on the type of phytoplasm, the host plant, the age of the plant, the stage of the disease, and environmental conditions.

1. **Phyllody:** It appears during the blooming season and is most common in sesamum. Floral structures morph into green leafy structures. The sepals transform into leaf-like structures, while the corolla, stamens, and carpels turn green and leafy. The ovary is also deformed, with an elongated structure. The leaves are shortened, the plant is stunted, and the branching is abnormal, resulting in an unrecognizable plant.

2. **Spikes:** Spike disease is a plant pathogen that affects sandal trees of all ages. It can be divided into two distinct species: the rosette variety and the pendular variety. The rosette variety is more prevalent and is commonly referred to as Rosette spike or, more commonly, spike disease. This type of spike is characterized by the entire shoot of the tree, which appears to have four rows of spikes on top of each other. Additionally, the plant's overall size has been significantly reduced little leaf.

Plate: A) Sesamum phyllody B) Sugarcane grassy shoots C) Brinjal little leaf

3. **Little Leaf:** Little leaf is widespread in brinjal. The plants' leaves and nodes have shrunk dramatically, giving them a bushy appearance. Many auxiliary buds are stimulated to develop into short branches with small leaves.

4. **Grassy Shoot Disease:** It is a serious disease of the sugarcane. At the base of the affected plants, numerous, thin tillers appear, identifying it as such. The clump has a crowded, grass-like appearance as a result of premature and excessive tillering. Tillers have thin, grass-like leaves that are pale yellow and narrow. The clumps that are affected are stunted and have different levels of chlorophyll loss, from complete green to white. Phytoplasma infection causes foliar yellowing and reddening, small leaves, vein clearing, vein enlargement, vein necrosis, leaf roll, leaf curl, premature defoliation, undersized fruits, poor terminal growth, sparse foliage, dieback, stunting of overall plant growth, and decline in woody plants. It is unlikely that phytoplasmas consumed nutrients, caused deficiency in plants, and displayed all of these symptoms. In rare cases, phytoplasma-infected plants donot exhibit any symptoms during their lifetime.

V. TRANSMISSION OF PHYTOPLASMA

Phytoplasma transmission from plant to plant occurs primarily through the feeding activity of inoculative vector insects, vegetative propagation of infected plant material, and graft inoculation. Phytoplasma is primarily spread by phloem-feeding insects, lives in phloem sieve tubes, and persistently colonizes its hosts. The geographical distribution and impact of phytoplasma diseases are determined by the phytoplasma's host range as well as the feeding behavior of the insect vector. Insect vectors can acquire multiple phytoplasma species/strains by feeding on multiple-infected source plants or sequentially feeding on different plants infected with different phytoplasmas. Variations in vector specificity range from extremely low, when a particular phytoplasma can be transmitted, primarily by polyphagous leafhopper species, to extremely high, when phytoplasmas are transmitted by only one or two vectors. Hemipteran insects, particularly phloem-feeding leafhoppers (Cicadellidae) and psyllids, spread phytoplasma in nature through persistent, self-replicating vectors. Phytoplasmas travel through the intestinal wall of their natural insect hosts, circulate in the hemolymph, and multiply in various tissues, including the salivary glands, where they combine with saliva to infect plants. Phytoplasmas can spread through propagation materials, which can result in their long-distance dispersal and introduction into areas where they have not yet been discovered. Recent studies on the discovery of phytoplasma in alfalfa, canola, corn, tomato, and oilseed rape plants' seed and seedling progeny suggest that certain plant-host phytoplasma are transmitted through seeds. Pathosystems may exist. Furthermore, all phytoplasmas can be experimentally transmitted by plant parasitic dodder (Cuscuta spp.) and by grafting infected plant material onto healthy plants.

VI. CONCLUSIONS

It is very challenging to distinguish naturally occurring variants of this significant group of pathogens and comprehend their epidemiology and interactions with the host because none of the PLOs have been cultured on synthetic media and persistant types of insect vector relationships. However, molecular biology has significantly increased our knowledge of plant pathogenic mollicutes over the past ten years. Transgenosis can now be used to treat mollicute-induced disease, and various methodologies can now be used to study how mollicutes interact with their plant and insect hosts.

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