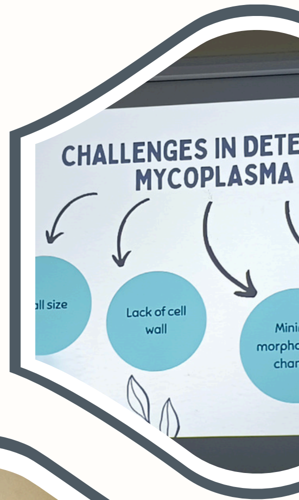
SRI VENKATESWARA COLLEGE
UNIVERSITY OF DELHI

ONE-DAY STUDENT SEMINAR

INNOVATIVE TEACHING

Structuring and Delivering Scientific Presentations

27th February 2025



BY MUSKAN MODI
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B.Sc. (Hons.) Botany

The students of Second Year B.Sc. (Hons) Botany expressed a keen interest in learning how to effectively present scientific concepts. Dr. Manoj Thakur acknowledged this interest and organized a **one day student seminar** as a hands-on learning experience in order to help students develop essential skills in scientific writing, with a strong emphasis on accuracy and precision.

As part of their DSC 4 subject, Microbiology and Plant-Microbe Interactions, the topic chosen for the session was Phytoplasma and Mycoplasma Like Organisms (MLOs). The students were divided into groups, with each group being allotted 10 minutes to present on the assigned topic through a PowerPoint presentation. A preparation period of a few weeks was provided, and the presentations were conducted on 27th February 2025.

Each group of students presented their findings and was asked questions about them. During the session, each group received constructive feedback on their presentation skills. Areas of improvement were also highlighted. This feedback covered aspects such as scientific accuracy, proper citation practices, diagrammatic representation, and the overall structure of the content. The session not only helped students build confidence in presenting scientific concepts but also in reinforcing the importance of meticulous scientific writing and public speaking skills.

This report was submitted to Dr. Manoj Thakur by Muskan Modi (roll no. 1424045)

The report contains a record of each presentation, including the names of group members and geotagged pictures. Individual presentations are attached at the end of this report for reference.

Sir provided the students with the following reference paper to deepen their understanding of the topic-

Namba S. Molecular and biological properties of phytoplasmas. *Proc Jpn Acad Ser B Phys Biol Sci*. 2019 Jul 31;95(7):401–418. doi: 10.2183/pjab.95.028.

Figure 1: Group 1 participants- Notrei and Shweta while presenting.

Group 1 Notrei, Shweta

The first presentation was delivered by Group 1, comprising RM. Notrei and Shweta Maurya, on the topic "History of Mysterious Plant Diseases." They began by introducing the subject and explaining the distinctions and similarities between phytoplasma and mycoplasma. Next, they dived into the various records of plant diseases. They also discussed some major plant diseases caused by phytoplasma, including Aster Yellow Disease, Rice Yellow Dwarf Disease (RYD), and Paulownia Witch's Broom. Through this presentation, students learned that phytoplasma are single-membraned microscopic organisms with the smallest genome, while mycoplasma are parasitic bacteria lacking cell walls. Although they share some features, both groups have distinct characteristics. Understanding these features helps researchers study the various microbial diseases caused in plants. In this presentation, some notable plant diseases and the common symptoms among them were highlighted. The presentation helped in setting up a foundation for understanding the topic, ultimately setting the stage for further discussion.

Figure 1.1: Some of the symptoms of plant diseases caused by phytoplasma and mycoplasma, as shown in the presentation by the students of Group 1.

GROUP 2

Kamakshi, Jiya, Shreya

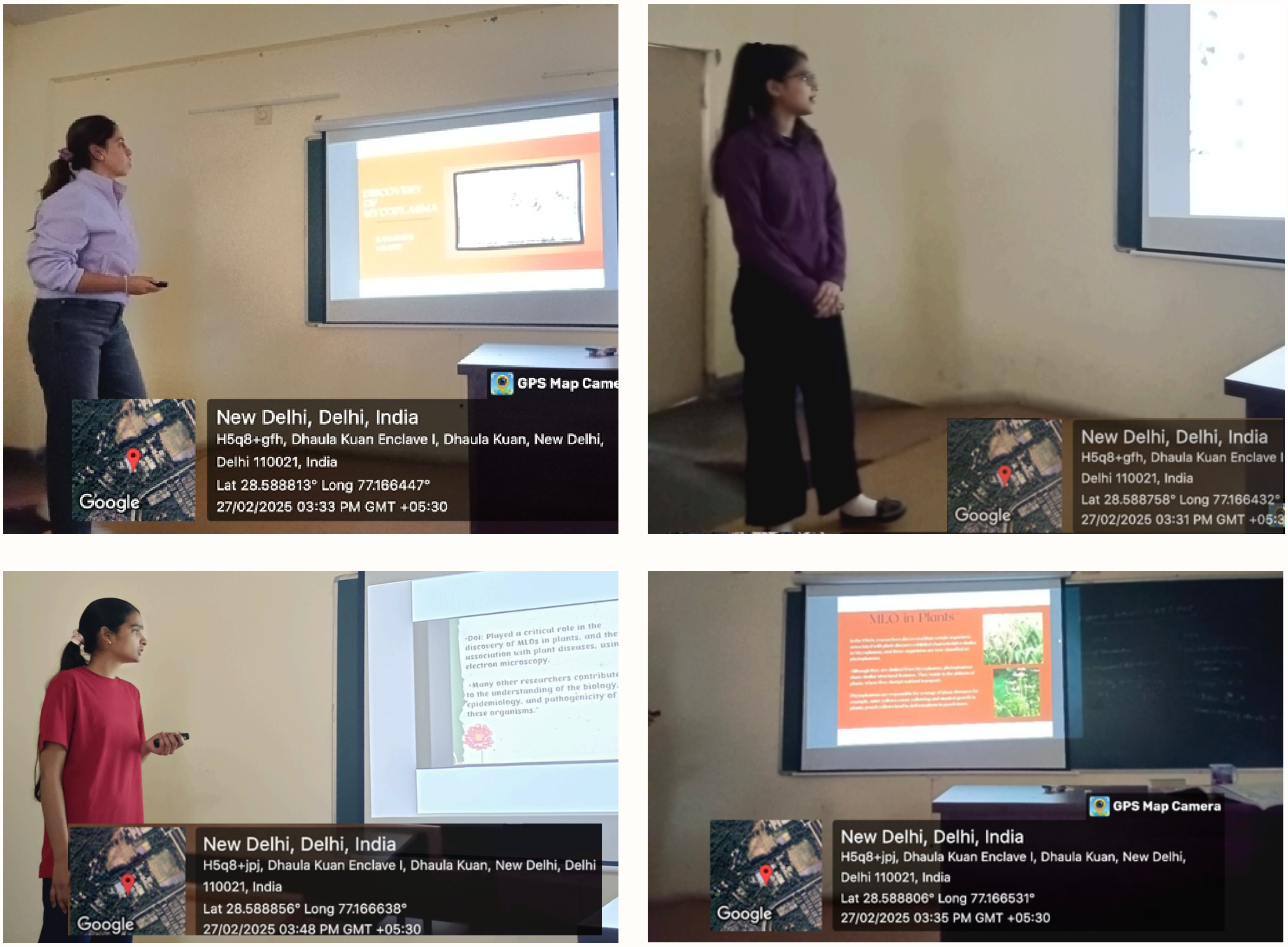
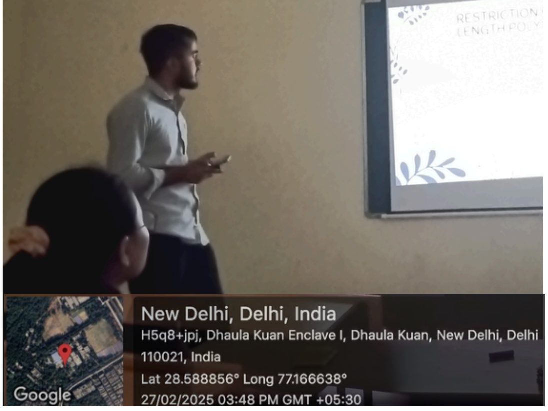
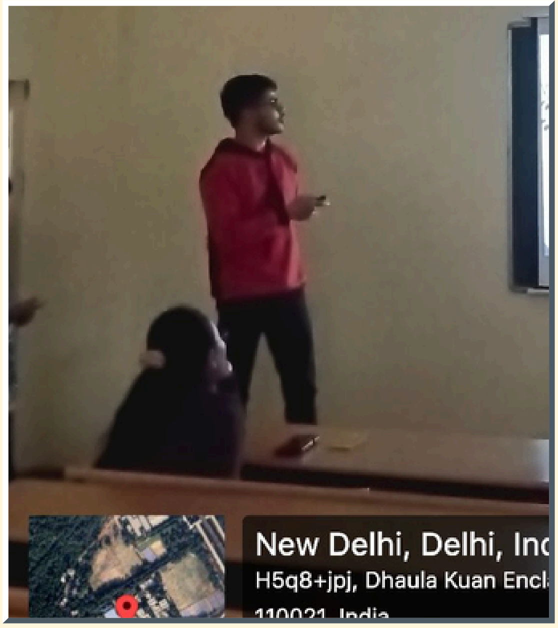


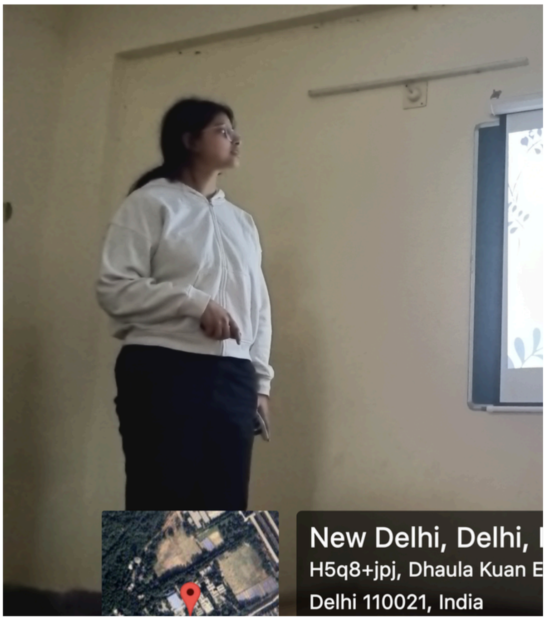
Figure 2: Group 2 participants- Jiya, Kamakshi and Shreya while presenting.

Group 2 members included Kamakshi Dhamija, Jiya Chand, and Shreya Yadav. Their presentation, titled "Discovery of Mycoplasma-like Organisms," shed light on the discovery and identification of MLOs (Mycoplasma-like Organisms). It also covered the challenges in MLO research and the advancements made so far. Additionally, it highlighted the impacts of MLOs on agriculture and human health, emphasizing their role in causing diseases in plants.

MLOs were first discovered in 1898 by Nocard and Roux and were initially classified as PPLOs (Pleuropneumonia-like Organisms). Technologies such as TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy), and cell culturing have played a crucial role in the discovery and study of these organisms and continue to pave the way for further research.







Group 3

Anisha, Kashish, Shivam, Wazir

Group 3 comprised Anisha Tanwar, Kashish Meher, Shivam Poonia, and Wazir Ullah Hussaini. Their presentation, titled "Molecular Detection and Classification of MLOs," explored the diagnosis, identification, and classification of MLOs (Mycoplasma-like Organisms). The significance and role of methods like PCR (Polymerase Chain Reaction), FISH (Fluorescence In Situ Hybridization), and RFLP (Restriction Fragment Length Polymorphism) analysis were discussed by the group members in turns.

During their presentation, a new term was introduced — housekeeping genes. The students were curious to learn the meaning and significance of these genes, and Dr. Manoj Thakur explained that housekeeping genes are those required for the basic cellular maintenance of an organism. They are stably expressed in tissues and are important in understanding evolutionary relationships, thus assisting in classification. The presentation also discussed that since 16S rRNA sequencing is not always sufficient, additional genes such as *SecA*, *GroEL*, and *Tuf* are used.

The significance of gene mapping, genome organization, and chromosome identification was also briefly discussed.

Figure 3: Group 3 participants- Shivam, Wazir, Anisha and Kashish while presenting.

Group 4

Namrata, Ruchi

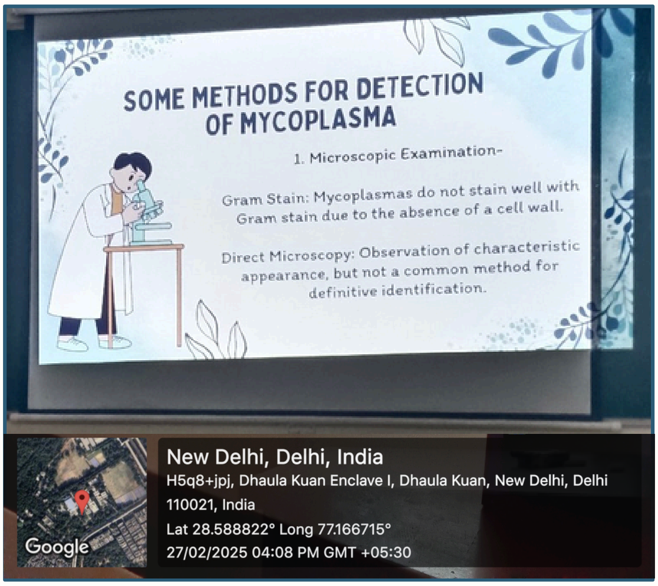


Figure: A slide from the presentation of Group 4.

Figure 4: Group 4 participants- Ruchi and Namrata while presenting.

Group 5 included Namrata Kumari and Ruchi, who were assigned to present on the topic "Detection and Control of Mycoplasma." They first talked about the need to detect mycoplasma in order to combat infections. They then expanded on the challenges associated with mycoplasma detection to emphasize how complex the process is. After establishing this, they discussed various detection methods: microscopic examination, culturing, PCR (Polymerase Chain Reaction), and serological techniques, including ELISA (Enzyme-Linked Immunosorbent Assay), CFT (Complement Fixation Test), and PCR-ELISA. They also talked about control methods and prevention strategies, emphasizing the importance of disinfection and regular screening.

GROUP 5

Divya, Himangshu, Hitesh, Utkarsh

Figure 5.1: Group 5 participant- Divya while presenting.

Group 5 consisted of Divya, Himangshu Medhi, Hitesh Singh Mahara, and Utkarsh Singh, who presented on the topic "Maintenance and Mutation of Phytoplasma." They gave a detailed background on phytoplasma, which included its cell structure, symptoms, transmission, and mode of infection.

Then they talked about the methods of maintenance of phytoplasma, including plant host maintenance and insect vector maintenance (in vivo), as well as tissue culture and cryopreservation (in vitro). Molecular detection methods, including PCR (Polymerase Chain Reaction), qPCR (Quantitative Polymerase Chain Reaction), and FISH (Fluorescence In Situ Hybridization) were also discussed. They then discussed the challenges in phytoplasma maintenance.

The next section of their presentation covered mutations in phytoplasma – types, mechanisms, and effects. Horizontal Gene Transfer (HGT) was discussed in detail and further explained by Dr. Manoj Thakur, who elaborated on its significance. The evolutionary implications of mutations were also discussed. It was concluded that high mutation rates and genomic plasticity drive phytoplasma evolution, enabling them to adapt to new environments and hosts. The presentation ended by emphasizing that phytoplasma coevolve with both plant and insect hosts, leading to the emergence of new strains with variance in virulence.

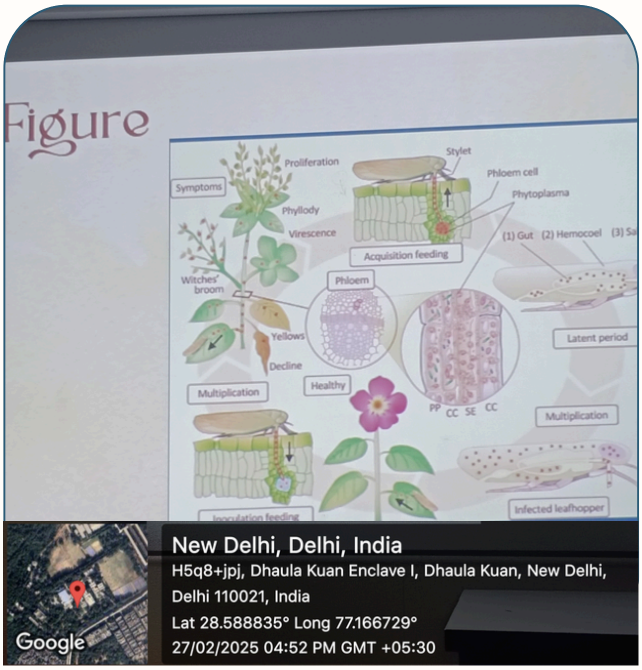
Figure 5.2: Group 5 participants- Himangshu, Hitesh and Wazir while presenting.

GROUP 6

Gunjan, Rohan

Figure 6: Group 6 participants- Rohan and Gunjan while presenting; a slide from their presentation.

Group 6 participants — Gunjan Verma and Rohan Jain, spoke on the topic "Mechanism of Plant and Insect Infection by Phytoplasma." They highlighted the four main processes by which phytoplasma infect plants and insects: transmission, acquisition and inoculation, phloem colonization, and insect related activity. Their presentation sparked an interesting discussion about disease causation and its mechanisms, and how researchers can develop strategies to combat it.



Phytoplasmas are obligate intracellular bacteria that lack many essential genes for normal bacterial functions, including some components of the translation machinery. Their translation system has evolved to be highly specialized due to genome reduction. Their reliance on host-derived molecules makes their translation system unique compared to free-living bacteria.

GROUP 7

Vanshika

Figure 7: Group 7 participant- Vanshika while presenting.

Figure 7.1: A slide from the presentation of Group 7.

The sole member of Group 7, Vanshika Verma, shed light on the topic "Molecular and Biological Properties of Phytoplasmas – Genetic Factors Determining Symptom Development" through a visually rich presentation. She discussed the symptom diversity and economic impacts of phytoplasmas. Understanding these symptoms is highly crucial, as it helps in disease management. Symptoms of phytoplasmas range from yellowing and stunting to floral deformities such as phyllody and virescence.

Dr. Manoj Thakur elaborated on the differences between normal plants and plants with phyllody, showing the class visual examples.

Next, Vanshika discussed effector proteins as the architects of symptoms. In 2009, the first phytoplasma effector protein, TENGU (made up of 38 amino acids), was identified as an inducer of witches' broom. SAP11 is another key effector protein that induces symptoms like leaf yellowing. She concluded the presentation by emphasizing the importance of further research in developing disease-resistant crops.

CONCLUSION

Adrija

Adrija Mitra delivered the concluding remarks on the entire topic. She revisited key points from the students' presentations, mentioning the structure, classification, and effects of phytoplasma and mycoplasma-like organisms (MLOs). She recounted the symptoms and plant diseases caused by these pathogens, along with their detection and control methods.

Phytoplasma continues to pose a significant challenge in agriculture due to its inability to be cultured and its transmission through insects. Nonetheless, ongoing research has resulted in substantial progress in areas such as genomics, detection methods, vector control, and disease management.

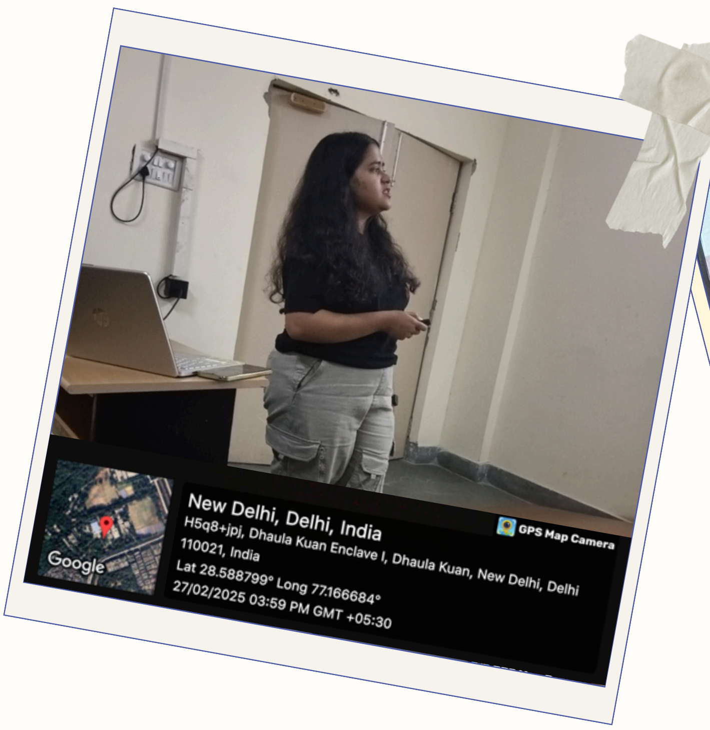


Figure 8: Adrija delivering the concluding remarks; a slide from the presentation.

Figure 9: Students eagerly waiting for Dr. Manoj Thakur's remarks.

After the conclusion of all the presentations, Dr. Manoj Thakur addressed the students and provided feedback on their performance. He highlighted the importance of labeling diagrams, giving proper citations, and maintaining a structured layout. Dr. Thakur also emphasized the need for thorough research and acknowledged the effort required to compile such a vast amount of information. In the end, the students gained valuable lessons in scientific presentation and polished their public speaking skills.

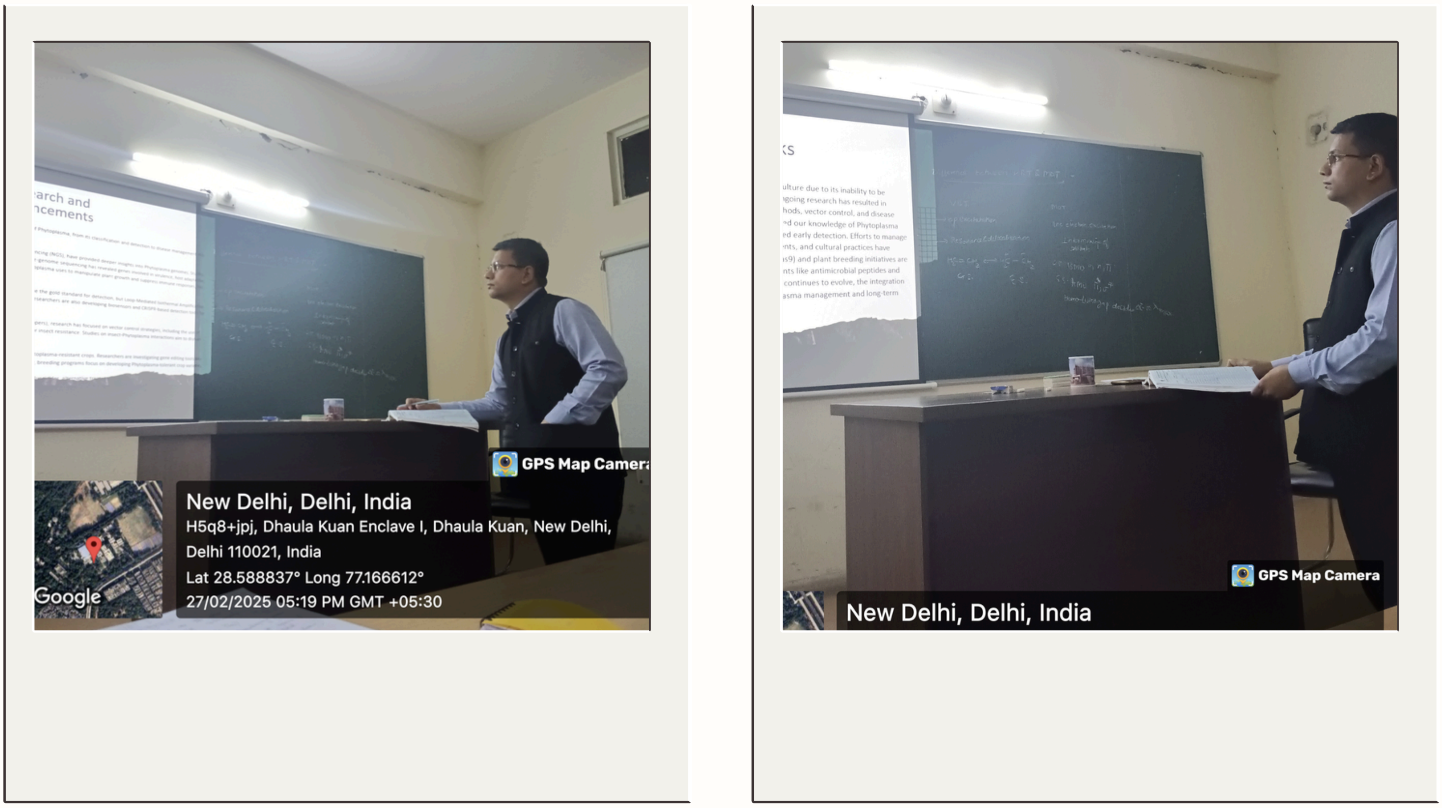


Figure 9.1: Dr. Manoj Thakur giving his final remarks on the presentations and providing feedback.

GROUP 1

PRESENTATION BY BOTANY 1ST YEAR

Submitted by -

- **Paramveer**
- **RM Notrei**
- **Shweta**
- **Riya**

Submitted to -
Dr. Manoj Thakur

HISTORY OF MYSTERIOUS PLANT DISEASES

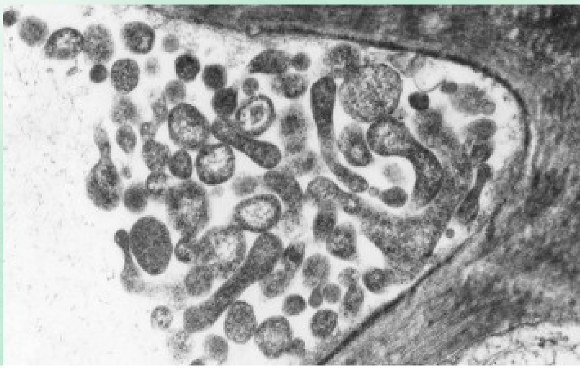
A BRIEF INTRODUCTION ON PHYTOPLASMA

Phytoplasma , initially termed as mycoplasma – like organisms (MLO) ,is an obligate parasite of plants which are actually phloem- inhibiting bacteria and were discovered by a Japanese scientist Doi et al. in 1967.

 They belong to the class mollicutes. They are very minute unicellular prokaryotic organisms that have a size ranging from 200 – 800nm .

 They are pleomorphic since they don’t have a rigid cell wall and is having a single membrane.

 They are known to have the smallest genome.



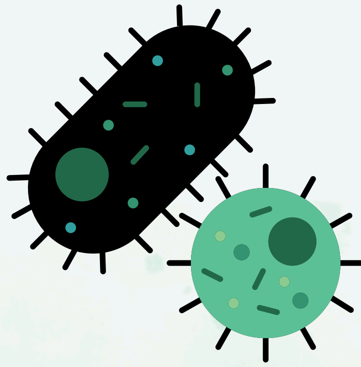
- They live in plant phloem tissues, and their plant- to – plant transmission occurs via insect vectors such as psyllids and planthoppers , grafting and dodder plants.
- It thus infects phloem tissue by moving through the phloem Sap to congregate in mature leaves.
- The symptoms include virescence (loss of normal flower colour) ,phyllody (development of floral parts into leaf – like structures) , proliferation of auxiliary shoots resulting in ‘witches broom’ , sterility of flowers , yellowing and phloem necrosis.

Differences between mycoplasma and phytoplasma

FEATURE	MYCOPLASMA	PHYTOPLASMA
Definition	Mycoplasma is a group of small typically parasitic bacteria that lack cell walls	Phytoplasma is a group of bacteria obligate bacterial parasite of plant phloem tissues.
Size	Ranges between 150-250nm	Ranges between 200-800nm
Transmission	Transmits through various modes	Transmits through insect vectors
Cell membrane	Have a unique cell membrane containing sterols	Has a three layered lipoprotein membrane
Parasitic nature	Parasitic in humans, animals, plants	Parasitic in plant phloem tissues and in some insects

FEATURE	***MYCOPLASMA***	***PHYTOPLASMA***
Alternate name	Also called pleuro-pneumonia like organisms (PPLOs)	Also called mycoplasma like organisms (MLOs)
Culturing ability	Can be grown in an isotonic medium	Cannot be cultured
Example	*Mycoplasma pneumoniae* *Mycoplasma genitalium*	*Candidatus phytoplasma*

Similarities between Mycoplasma and Phytoplasma



- They both are small prokaryotic microorganisms.
- Both bacteria do not have a cell wall.
- Both these groups are pleomorphic.
- These two bacterial groups have both DNA and RNA and also a very small genome.
- Further, they both are parasites.

HISTORY OF MYSTERIOUS PLANT DISEASES

Historical background

•The earliest record of phytoplasma diseases dates back about 1,000 years.

•In Song China, tree peonies infected with phytoplasma exhibited floral virescence (green flowers) and were considered rare

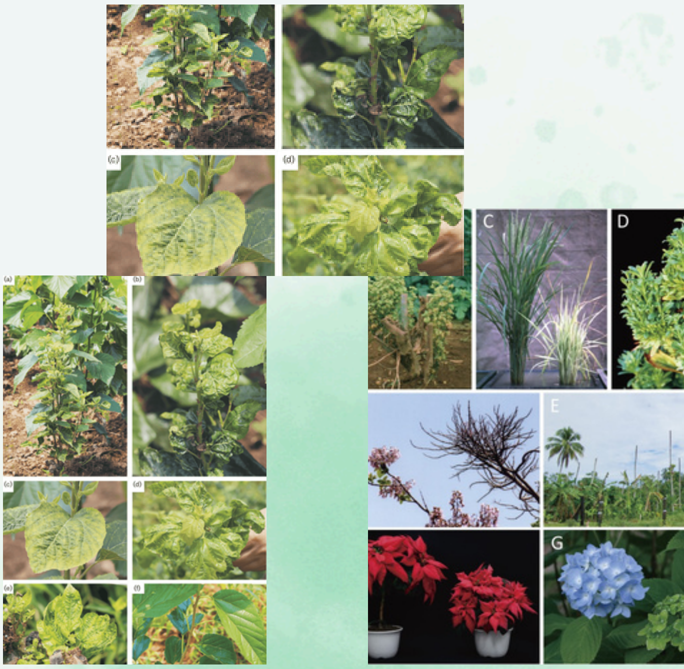
First scientific record in Japan

•Mulberry dwarf disease was first observed around 200 years ago in Japan.

•It severely impacted mulberry leaf production, crucial for the silk industry.

•Symptoms - Leaves curl, branches weaken, and plants stunt.

•The National Diet of Japan established the first national research committee in 1897 to study the disease, but it failed after 7 years.



•Scientists discovered that mulberry dwarf disease could be transmitted by insects and grafting.

•Initially, these diseases were misidentified as viral infections due to their unknown pathogens.

Other major phytoplasma diseases

Yellows Diseases (insect-transmissible plant diseases mistaken for viral infections) include:

•Rice yellow dwarf

Rice yellow dwarf disease (RYD) is a phytoplasma disease that causes rice plants to turn pale yellow, stunt, and stop producing grain.It's been a problem for rice farmers in many Asian countries since the 1910s.

For many years after its discovery in 1919, the agent that caused this disease was unknown and was believed to be a virus
It was identified as a phytoplasma in 1967

•Aster yellow disease

Aster yellows is a disease that affects many plants, including vegetables, flowers, and weeds.

Symptoms

•Yellowing of leaves
•Abnormal production of shoots
•Deformed flowers
•Flowers that don't produce seeds
•Unusual smell to the bulb

•Paulownia witches' broom

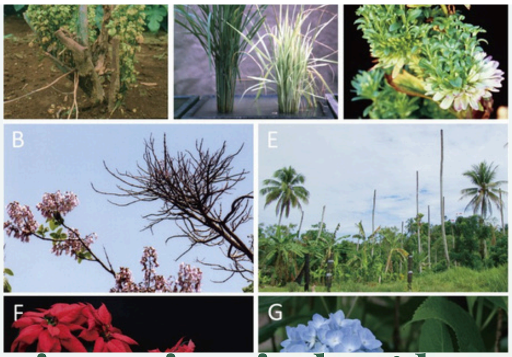
•Paulownia witches' broom (PaWB) is a disease that affects paulownia trees, causing branch and shoot proliferation.

•It's the leading cause of paulownia mortality.

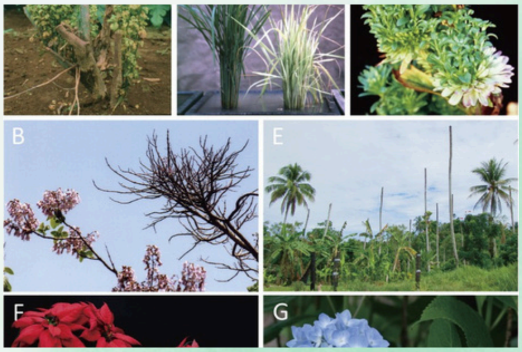
•Transmitted by insect vectors like stink bugs, mirid bugs, and leafhoppers.

•Causes serious economic losses and ecological damage.

Coconut lethal yellowing



Poinsettia witches' broom



Hydrangea phyllody

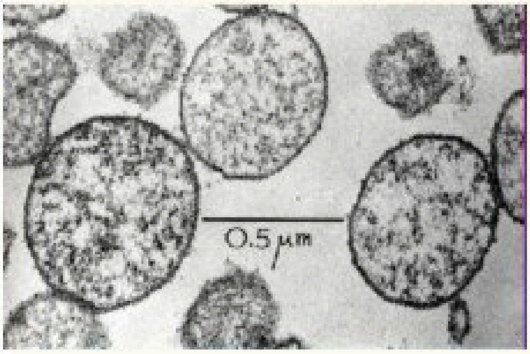
Reference

1. •www.wikipedia.org
2. •difference between.com
3. •www.jstage.jst.go.jp
4. •www.sciencedirect.com

Thank You

GROUP 2

DISCOVERY OF MYCOPLASMA Like Organism

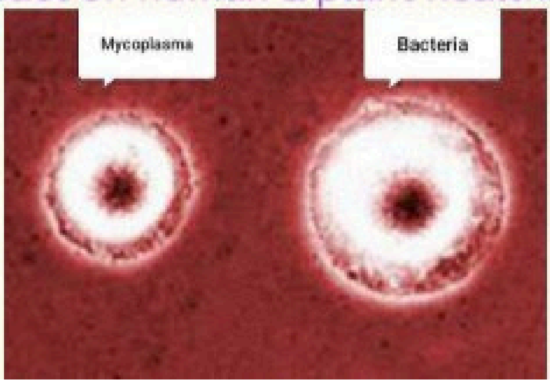


Jiya 1424006
Kamakshi 1424012
Shreya 1424011
Nakita 1424013
Tushar 1424008

Submitted to
Manoj thakur

Introduction

- Mycoplasma like organisms (MLOs) are group of bacteria characteristics similar to mycoplasma , but not exactly the same.
- These organisms are of a great interest in microbiology due to their unique properties and their impact on human & plant health.
- MLOs are known for their small size, lack cell wall and ability to cause diseases.



-jiya chand

What are mycoplasma like organism?

- MLOs are bacteria that resembles mycoplasma , which are the smallest free - living organisms , but may differ in certain genetic and structural aspects.

- *Key features*

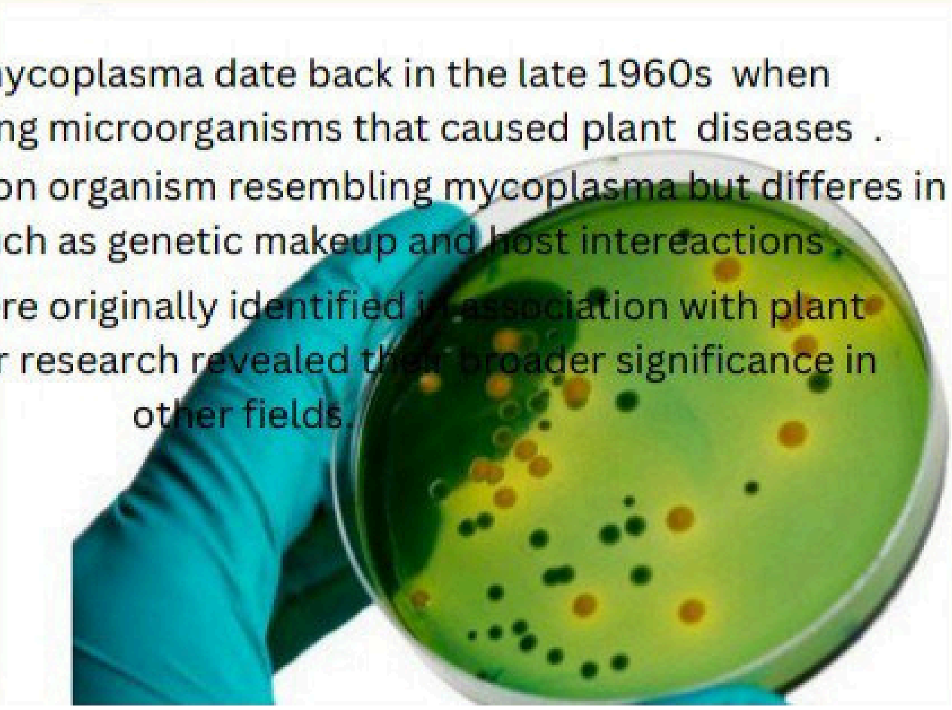
➡ *Lack of a rigid cell wall.*

➡ *Small genome size.*

➡ *Can be pathogenic in (causing disease) in humans, animals & plants.*

A journey through the time..... ⏱

- The discovery of mycoplasma date back in the late 1960s when scientists were studying microorganisms that caused plant diseases .
- Initial studies focus on organism resembling mycoplasma but differes in key aspects , such as genetic makeup and host intereactions .
- These organisms were originally identified in association with plant diseases , but further research revealed their broader significance in other fields.



Tissue culture connection

How mycoplasmas affect tissue cultures

Cell growth

- Mycoplasmas can change cell growth characteristics, inhibit cell metabolism, and disrupt nucleic acid synthesis.

Gene expression

- Mycoplasmas can trigger changes in the expression of many genes in the host cell, including oncogenes, tumor suppressor genes, cytokines, and receptors.

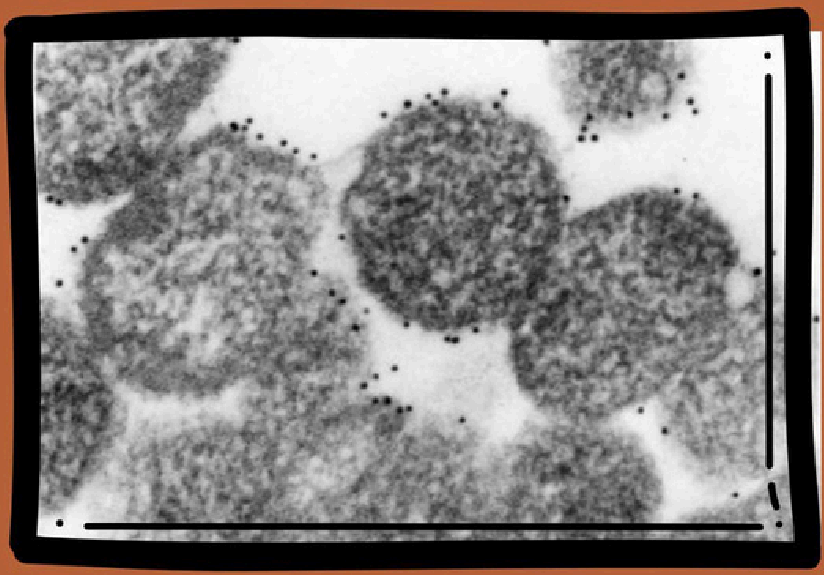
Cell transformation

- Prolonged cultivation of inoculated cells can lead to their irreversible transformation to the extent of malignant degeneration.

-jiya chand

DISCOVERY OF MYCOPLASMA

KAMAKSHI
1424012



MLO in Plants

In the 1960s, researchers discovered that certain organisms associated with plant diseases exhibited characteristics similar to Mycoplasma, and these organisms are now classified as phytoplasmas.

Although they are distinct from Mycoplasma, phytoplasmas share similar structural features. They reside in the phloem of plants, where they disrupt nutrient transport.

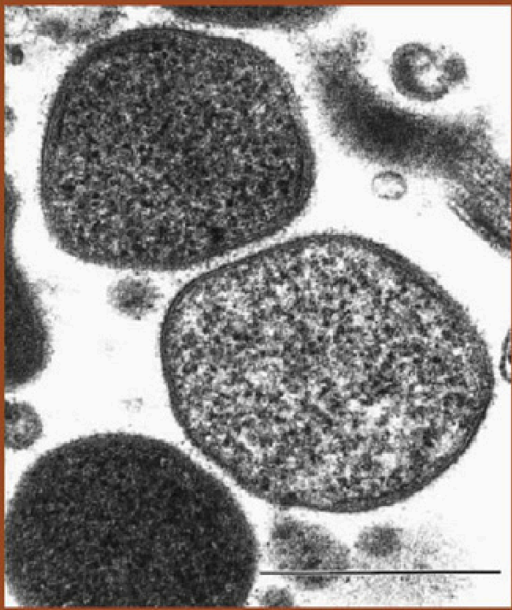
Phytoplasmas are responsible for a range of plant diseases; for example, aster yellows cause yellowing and stunted growth in plants, peach yellows lead to deformations in peach trees.

Electron Microscopy of Mycoplasma

Because Mycoplasma and MLOs are too small to be resolved by light microscopy, electron microscopy is essential for their study.

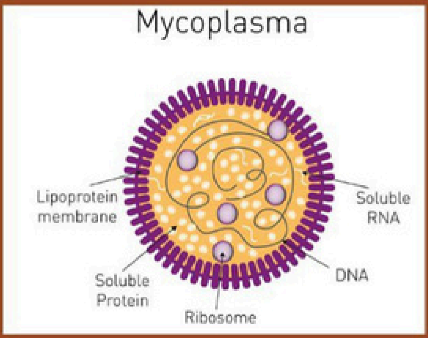
Transmission Electron Microscopy (TEM) provides high-resolution images of their internal structures, which has helped confirm the absence of a cell wall in Mycoplasma and revealed the intracellular location of MLOs within the plant phloem.

In addition, Scanning Electron Microscopy (SEM) is used to study the surface morphology of Mycoplasma, enabling researchers to visualize how these organisms attach to host cells.



Discovery of Mycoplasma

Mycoplasma was first discovered by Nocard and Roux in 1898 while they were studying bovine pleuropneumonia.



Initially, these organisms were classified as Pleuropneumonia-like Organisms (PPLOs) before they were named Mycoplasma.

They are recognized as the smallest self-replicating prokaryotes, measuring between 0.2 and 0.8 micrometers, and they lack a cell wall.

The first human pathogen identified among them was Mycoplasma pneumoniae, discovered in the 1940s, which is known to cause respiratory infections.

Key players in the discovery of MLOs

The journey to understanding Mollicutes, particularly phytoplasmas (formerly MLOs), is marked by the contributions of pioneering scientists. Their work laid the foundation for our current knowledge.

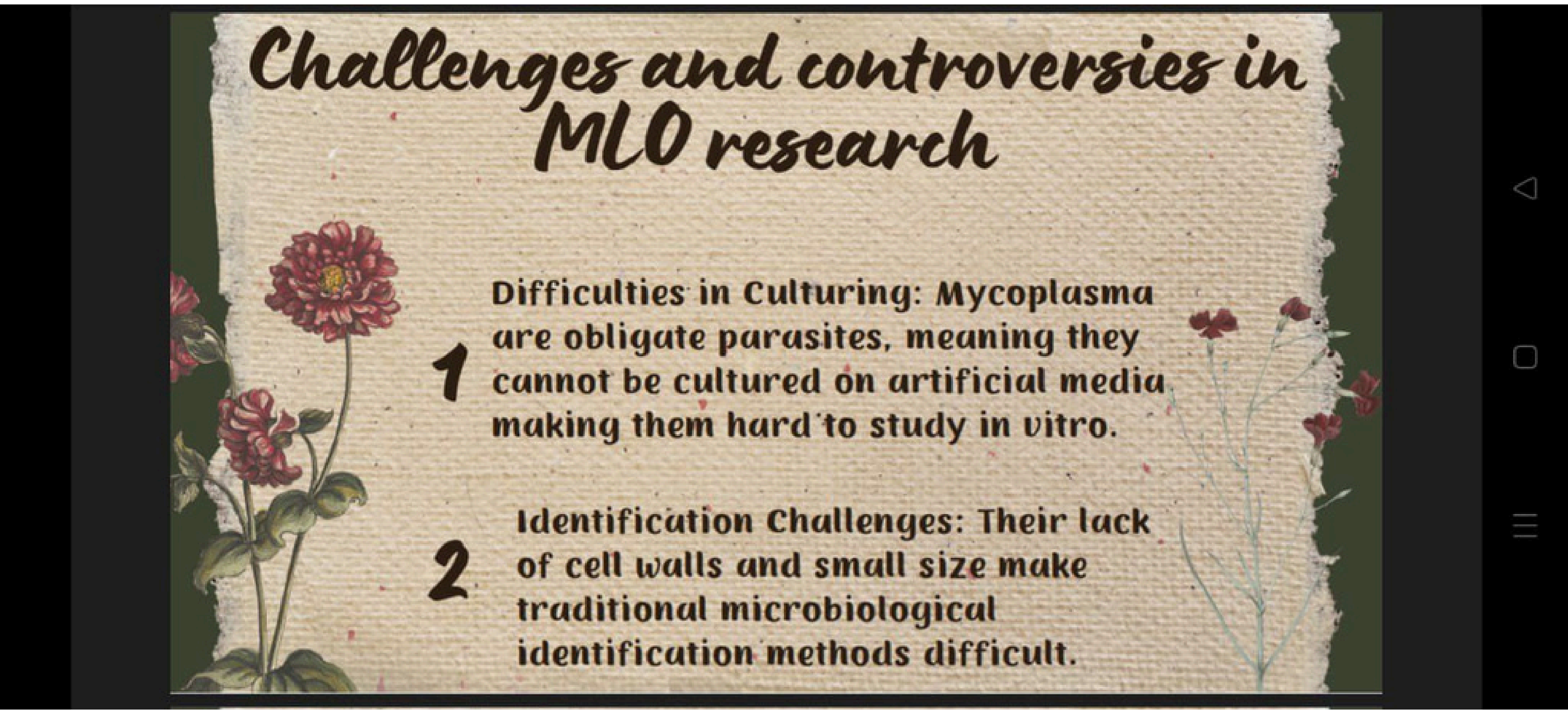
- Dr. D.G. FF Edward: A key figure in the early classification of Mollicutes, particularly those found in animals."

•Doi: Played a critical role in the discovery of MLOs in plants, and their association with plant diseases, using electron microscopy.

•Many other researchers contributed to the understanding of the biology, epidemiology, and pathogenicity of these organisms."

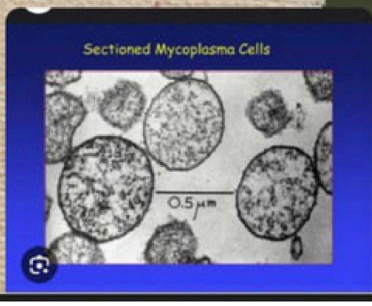
Mycoplasma

Mycoplasma haemofelis


Challenges and controversies in MLO research
1
Difficulties in Culturing: Mycoplasma are obligate parasites, meaning they cannot be cultured on artificial media making them hard to study in vitro.
2
Identification Challenges: Their lack of cell walls and small size make traditional microbiological identification methods difficult.

3 Taxonomic Debates: The classification and nomenclature of mycoplasma have been subject to ongoing revisions and debates.

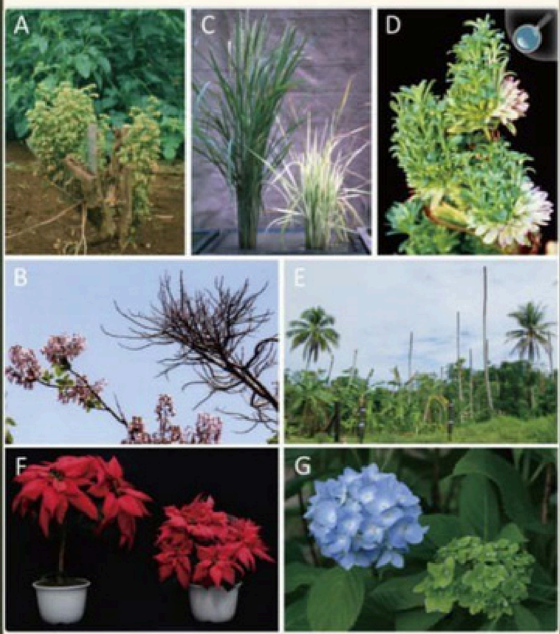
4 Controversies: The exact mechanisms of pathogenesis and transmission are still areas of active research and debate.



Impact of MLOs on agriculture and human health

Mycoplasmas have a profound impact on both agriculture and potentially, human health.

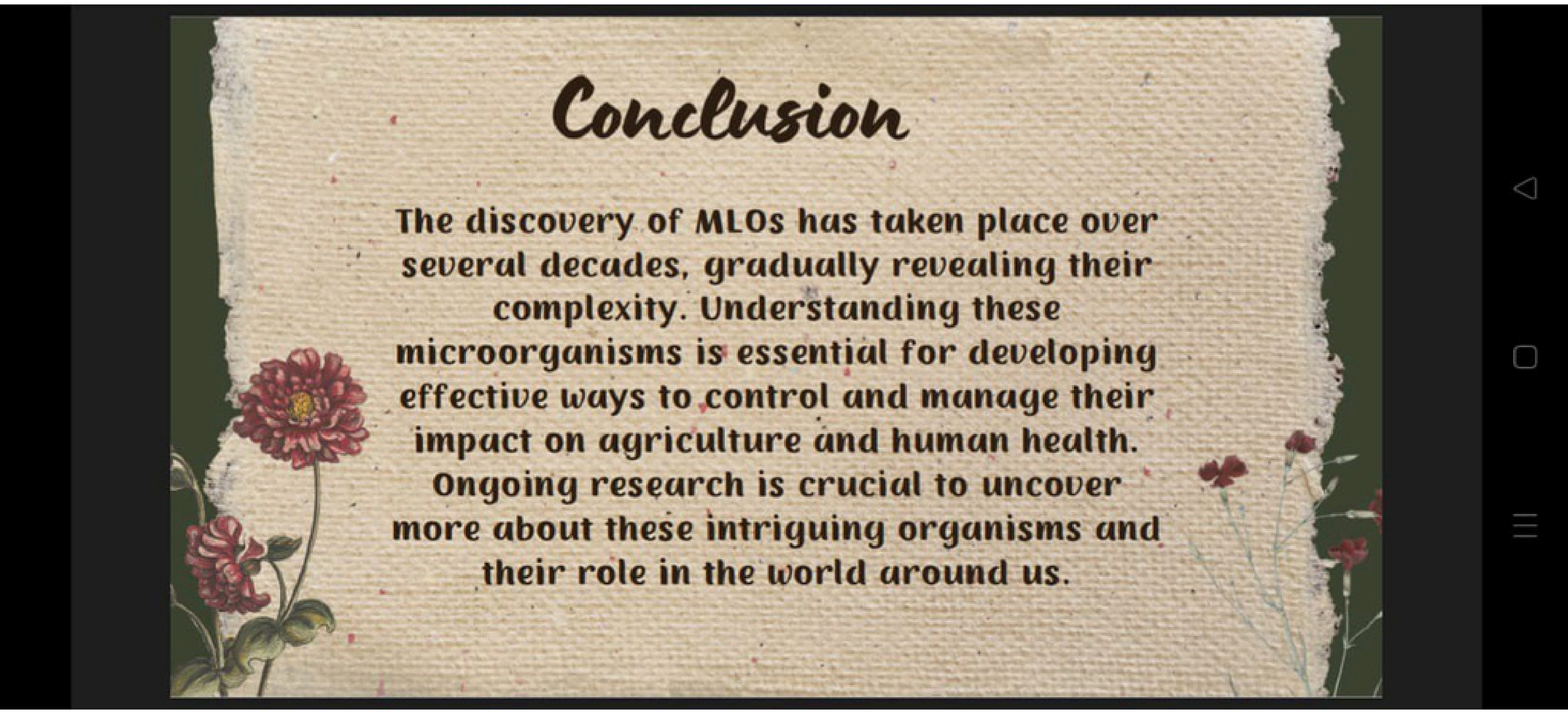
•Agricultural Impact: They cause a wide range of plant diseases, leading to significant crop losses and economic damage. Examples include aster yellows, citrus greening, and coconut lethal yellowing,etc.

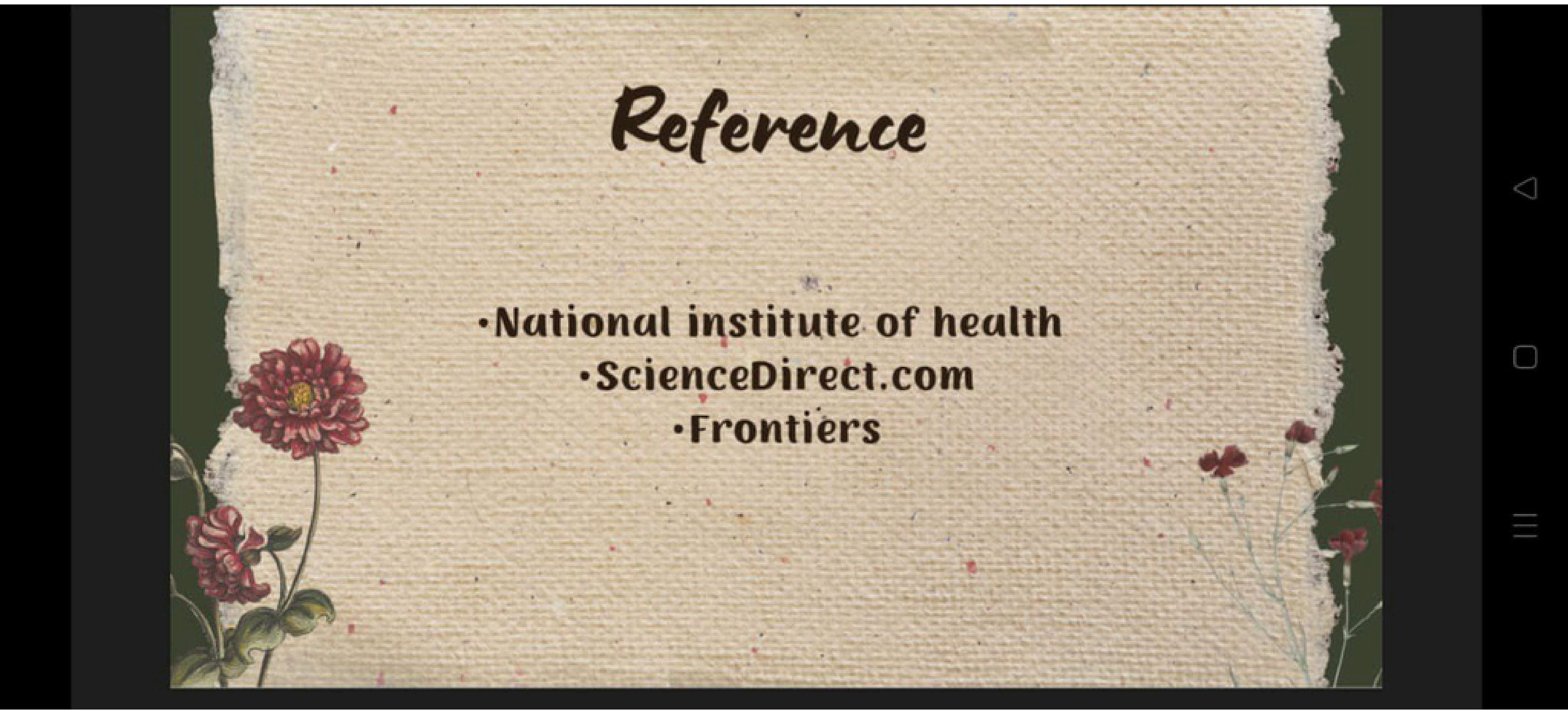


Various symptoms caused by yellows diseases. (A) Mulberry dwarf. (B) Paulownia witches' broom. (C) Rice yellow dwarf. (D) Aster yellows. (E) Coconut lethal yellowing. (F) Poinsettia witches' broom. (G) Hydrangea phyllody. (B, C, F, G) right side: infected plants; left side: healthy plants. The photographs were kindly provided by Drs. Akira Shirata (A), Norio Nishimura (B), and Akira Shinkai (C, D).

- Human Health Potential: While primarily known as plant pathogens, there is growing interest in the potential role of Mollicutes in human infections. Some species are known to infect humans, and research continues to explore their potential pathogenicity.

- The understanding of MLOs is crucial for developing strategies to mitigate their impact.


Conclusion
The discovery of MLOs has taken place over several decades, gradually revealing their complexity. Understanding these microorganisms is essential for developing effective ways to control and manage their impact on agriculture and human health. Ongoing research is crucial to uncover more about these intriguing organisms and their role in the world around us.


Reference
•National institute of health
•ScienceDirect.com
•Frontiers

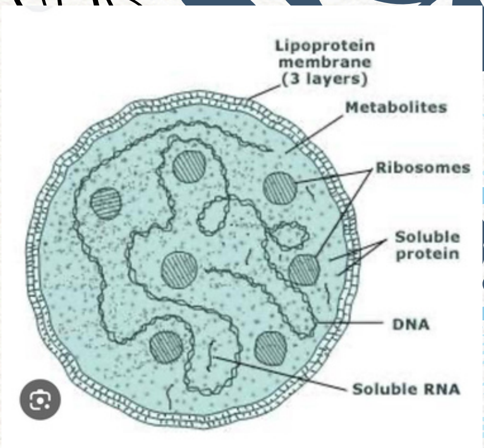
GROUP 3

MOLECULAR DETECTION & CLASSIFICATION OF MLO

by- Wazir Ullah Hussaini (1424017)
Shivam Poonia(1424014)
kashish Meher (1424016)
Anisha Tanwar(1424015)

INTRODUCTION

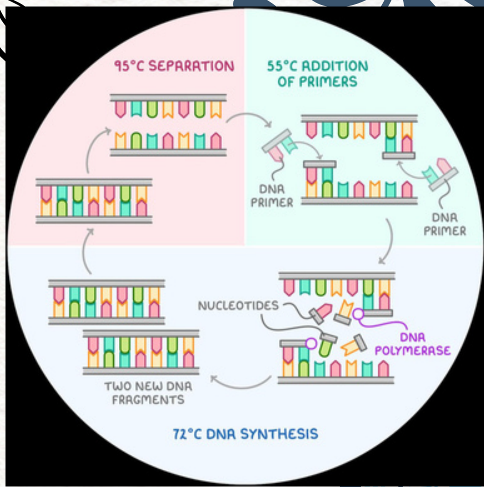
Molecular detection of Mycoplasma-like organisms (MLO) plays a crucial role in the accurate diagnosis, classification, and identification of these pathogens. MLOs are a diverse group of microorganisms that are notoriously challenging to detect and study using conventional microbiological methods. This difficulty arises from several factors, most notably their small size, which often places them below the detection limit of traditional light microscopy, and their lack of a rigid cell wall, which renders them resistant to common bacteriological techniques that rely on cell wall-targeting antibiotics, staining methods, or culturing in conventional media.



POLYMERASE CHAIN REACTION

PCR is one of the most commonly used molecular methods. It involves amplifying specific DNA sequences unique to MLOs. PCR primers are designed to target conserved regions of the 16S rRNA gene or other specific genes, enabling the identification and differentiation of MLO species.

Real-time PCR can be used for quantification and monitoring the presence of MLOs with high sensitivity and specificity

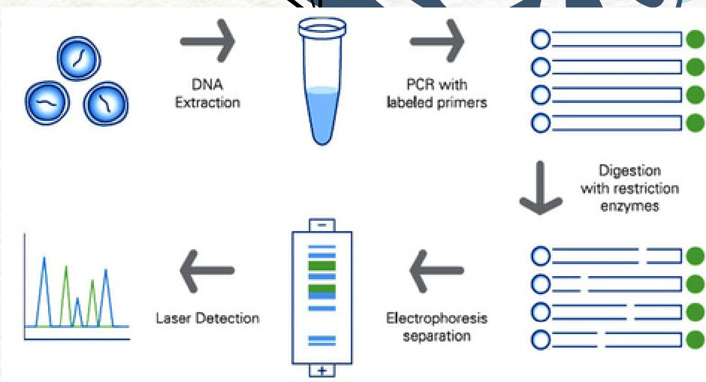


FLUORESCENT IN SITU HYBRIDIZATION

Fluorescent In Situ Hybridization (FISH) is a molecular technique used to detect specific DNA or RNA sequences in chromosomes, cells, or tissues using fluorescently labeled probes. The process involves denaturation, hybridization, washing, and fluorescence microscopy for visualization. FISH is widely used in genetics, cancer diagnosis, microbial identification, and plant cytogenetics. In botany, it helps in gene mapping, genome organization, and chromosome identification.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Restriction Fragment Length Polymorphism (RFLP) is a molecular technique used to detect genetic variation by identifying differences in DNA fragment lengths. It involves the digestion of DNA samples with restriction enzymes that cut the DNA at specific sequences. The resulting fragments are then separated by gel electrophoresis. Variations in the fragment lengths, caused by mutations or polymorphisms in the DNA, can be observed. RFLP is used in genetic mapping, disease gene identification, and forensic analysis. It is a powerful method for studying genetic diversity, though it is time-consuming and requires high-quality DNA samples.



Classification

Classification of Mycoplasma-Like Organisms (MLOs / Phytoplasmas)

Mycoplasma-like organisms (now classified as Phytoplasmas) are a group of cell-wall-less bacteria belonging to the class Mollicutes. They are classified based on molecular phylogenetics, primarily using 16S rRNA gene sequences.

TAXONOMIC

The term Candidatus is used because phytoplasmas cannot be cultured in vitro, preventing formal classification as a bacterial species.

Level	Classification
Domain	Bacteria
Phylum	Mycoplasmatota (Tenericutes)
Class	Mollicutes
Order	Acholeplasmatales
Family	'Candidatus Phytoplasmataceae'
Genus	'Candidatus Phytoplasma'

GENE SEQUENCING

Classification Based on 16S rRNA Gene Sequencing

Phytoplasmas are grouped into ribosomal (16Sr) groups and subgroups based on similarity in their 16S rRNA sequences.

Major 16Sr Groups (Examples):

Each group is further divided into subgroups (e.g., 16SrI-A, 16SrI-B, etc.) based on finer genetic variations.

16Sr Group	Example Phytoplasma	Host Plants Affected
16SrI	'Ca. Phytoplasma asteris'	Aster yellows, apple proliferation
16SrII	'Ca. Phytoplasma australasia'	Peanut witches' broom
16SrIII	'Ca. Phytoplasma pruni'	Peach X-disease
16SrV	'Ca. Phytoplasma ulmi'	Elm yellows
16SrIX	'Ca. Phytoplasma phoenicium'	Almond witches' broom
16SrXII	'Ca. Phytoplasma solani'	Grapevine yellows

HOUSEKEEPING GENES

Classification Based on Housekeeping Genes

Since 16S rRNA gene sequences may not always provide sufficient resolution, additional genes are used:

SecA (Signal Recognition Protein Gene)

GroEL (Chaperonin Protein Gene)

Tuf (Elongation Factor Tu Gene)

These genes help in refining classification and understanding evolutionary relationships

FUNCTIONAL

Phytoplasmas can also be classified based on:
1. Host Range - Some infect a broad range of plants (e.g., Aster yellows phytoplasma), while others are host-specific.
Aster yellows phytoplasma is a microscopic, single-celled organism that causes aster yellows disease in plants. It's a type of bacterium that lacks a cell wall.
2. Vector Transmission - Spread by different insect vectors such as leafhoppers, psyllids, and planthoppers.
3. Geographical Distribution - Some phytoplasmas are restricted to specific regions due to vector availability and climate conditions.

WHOLE GENOME SEQUENCING

Classification Using Whole Genome Sequencing (WGS)

Mycoplasma classification based on whole genome sequencing primarily relies on comparing the genetic sequence of different Mycoplasma strains, allowing researchers to identify distinct species and subtypes by analyzing the degree of similarity between their genomes, particularly focusing on key genetic markers and overall genome structure, often utilizing methods like Average Nucleotide Identity (ANI) to determine taxonomic relationship

Examples of Mycoplasma classification using whole genome sequencing:

Mycoplasma pneumoniae:

Different subtypes of M. pneumoniae have been identified based on variations in the P1 adhesin gene, which is crucial for its pathogenicity.

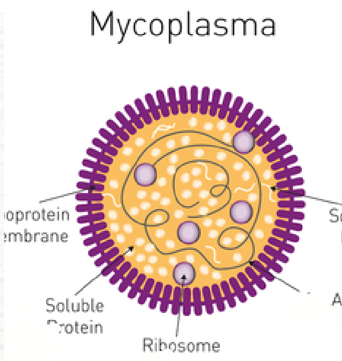
CONCLUSION

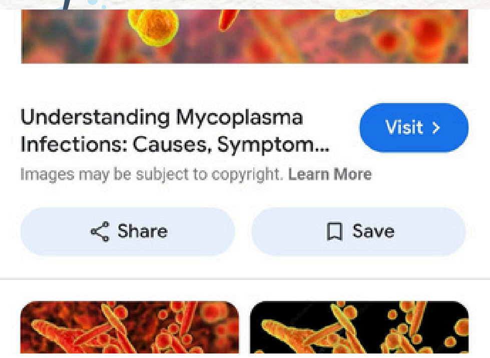
Molecular detection and classification of Mycoplasma-like organisms (MLOs) are vital for overcoming the challenges posed by traditional diagnostic methods. Techniques like PCR, DNA sequencing, and real-time PCR provide high sensitivity, specificity, and rapid identification of MLOs, even in low concentrations or when culture is impractical. These methods enable accurate species and strain classification, essential for understanding pathogenicity, monitoring outbreaks, and assessing antimicrobial resistance. Molecular tools also enhance epidemiological studies and improve disease management in human, veterinary, and agricultural contexts. Overall, molecular detection is crucial for advancing diagnostics and controlling diseases caused by MLOs.

THANK YOU

GROUP-4

MYCOPLASMA : DETECTION AND CONTROL

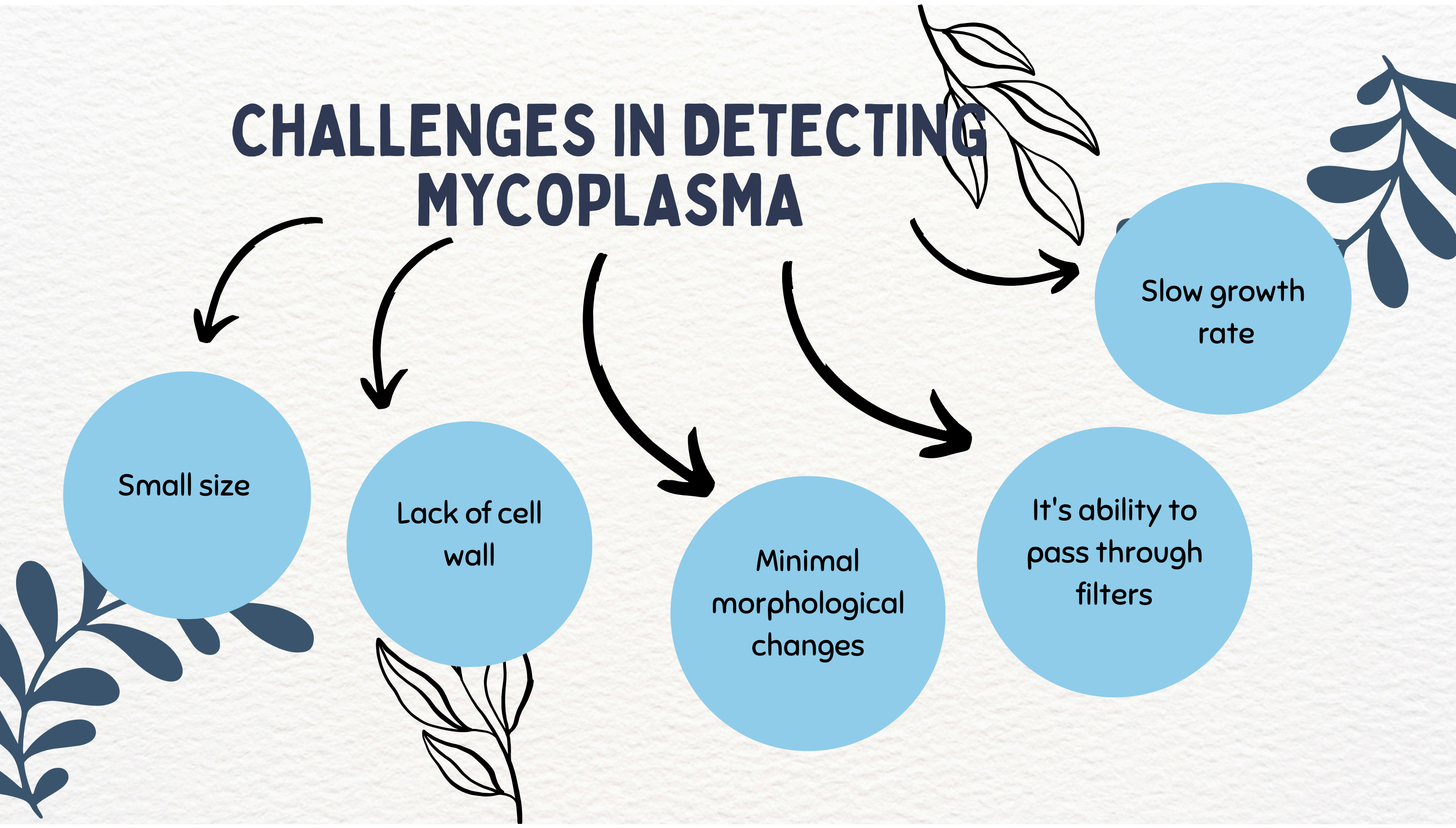




Submitted to - Dr. Manoj Thakur
Submitted by - Namrata (1424019) and Ruchi (1424020)

NEEDS FOR DETECTION

√Diagnosis and treatment of infections.
√Prevent spread of disease.
√Economic losses prevention in animal agriculture and human healthcare.
√Develop new treatments and vaccines.
√Follow health and safety rules.
√Public health protection, especially in vulnerable populations.
√Animal health monitoring and disease management.
√Ensure food safety.


CHALLENGES IN DETECTING MYCOPLASMA
Small size
Lack of cell wall
Minimal morphological changes
It's ability to pass through filters
Slow growth rate

2. Culture Techniques-

Selective Media: Mycoplasmas require special culture media (e.g., Eaton's agar) due to their unique nutritional requirements.

Incubation: Mycoplasma colonies may take several days to weeks to appear.

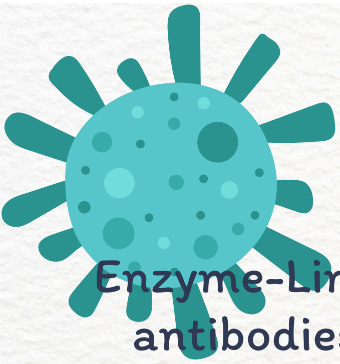
Limitations: Slow growth and difficulty in distinguishing them from other microorganisms.

3. PCR (Polymerase Chain Reaction)-

Method: PCR can detect Mycoplasma DNA directly from clinical samples (e.g., sputum, urine, swabs).

Application: This method can identify specific Mycoplasma species, even in the presence of other microorganisms.

Advantages: Highly sensitive and specific.



4. Serological Methods-

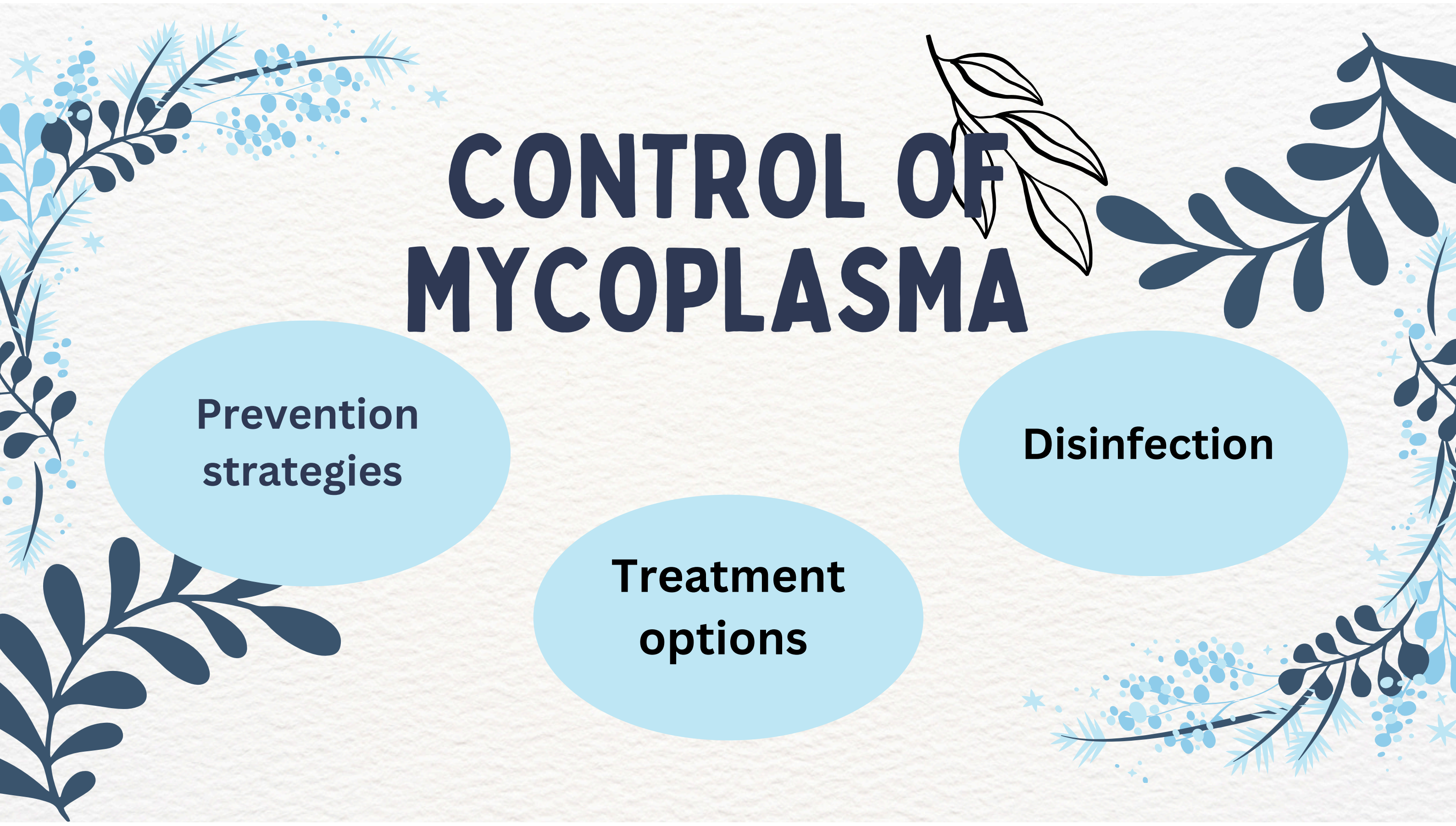
Enzyme-Linked Immunosorbent Assay (ELISA): Detects antibodies against Mycoplasma species in the blood.

Complement Fixation Test (CFT): Detects antibodies against M. pneumoniae but is less commonly used due to variability.

Limitations: May not distinguish between current and past infections.

5. PCR-ELISA-

It is a hybrid technique combining PCR and ELISA, allowing both detection of Mycoplasma DNA and antibodies in a single test.

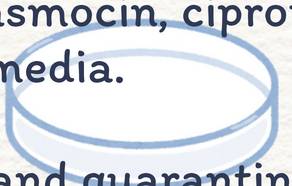

CONTROL OF MYCOPLASMA
Prevention strategies
Treatment options
Disinfection

PREVENTION STRATEGIES

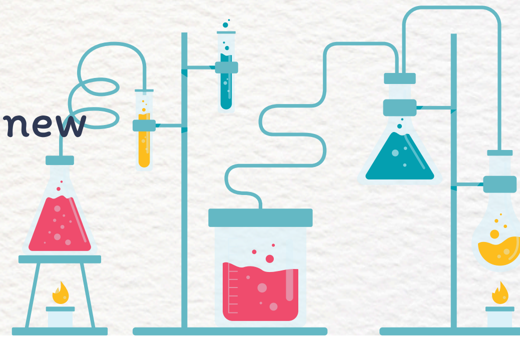
1. <u>In</u> <u>Laboratories</u>

- Regular screening of cell cultures using PCR or enzymatic assays.

- Use of antibiotics (e.g., Plasmocin, ciprofloxacin) in culture media.



- Strict aseptic techniques and quarantine of new cell lines.



2. In Agriculture

- Biosecurity measures (isolate infected livestock).
- Vaccination (e.g., live attenuated vaccines for *M. bovis*).

3. In Humans:

- Hygiene practices to prevent respiratory/STI transmission.
- Screening and treatment of sexual partners for *M. genitalium*.

TREATMENT OPTIONS

Antibiotics

- Macrolides (azithromycin), tetracyclines (doxycycline), and fluoroquinolones (moxifloxacin).

- Rising resistance in M. genitalium (up to 50% macrolide resistance in some regions).

DISINFECTION

- Effective against mycoplasma in environments:
- 70% ethanol, quaternary ammonium compounds, and UV light.
- Autoclaving (121°C for 15-20 minutes).

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THANK YOU

GROUP-5

PHYTOPLASMA

It's maintenance and mutation

Divya (1424025)
Himangshu (1424021)
Hitesh(1424026)
Neel(1424022)

Submitted to Dr. Manoj

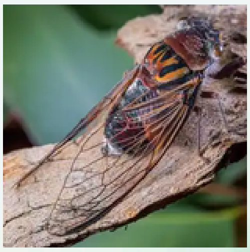
Introduction of Phytoplasma

Phytoplasmas are a group of bacteria-like organisms that are obligate plant pathogens. They belong to the class Mollicutes, which is characterized by
lack of a cell wall, making them different from most other bacteria.
Phytoplasmas primarily infect plants and can cause a variety of plant diseases.

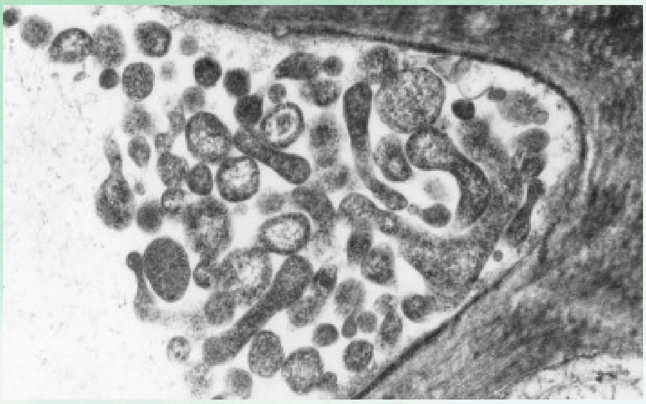
- They are also transmitted by insect vectors, most commonly leafhoppers , planthoppers, and cicadas.

Planthoppe


Leafhopper


Cicada

Yoji Doi and his colleagues first discovered phytoplasma in 1967. They were initially called mycoplasma-like organisms (MLOs) because they looked similar to mycoplasma



EM of Phytoplasma

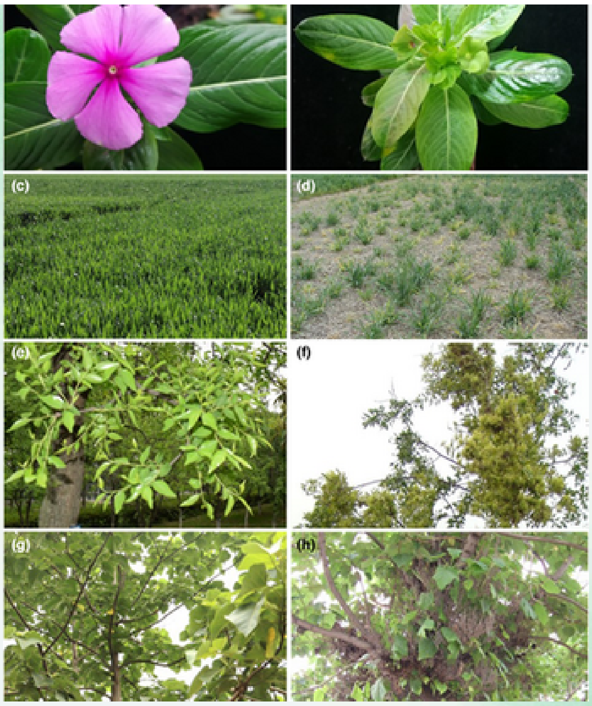
Shape: they are typically pleomorphic, meaning they lack a fixed shape and can appear as various forms, including coccoid (spherical), filamentous, or elongated.

Cell Structure: Phytoplasmas are cell-wall-deficient, a characteristic that places them in the Mollicutes class. This feature makes them flexible and able to adapt to various plant and insect c

- **Infection Mechanism: Phytoplasmas infect plant tissues, particularly the phloem, which is responsible for transporting nutrients and sugars. They enter the phloem tissue through insect vectors or sometimes via grafting between plants.**

- **Symptom : The infection of plants by phytoplasmas can lead to a range of symptoms, such as yellowing of leaves, stunting, flowering abnormalities, and death of plant tissues. This is because the phytoplasmas disrupt the plant's normal growth and nutrient** flow.

- **Transmission: Phytoplasmas are transmitted by insect vectors, which feed on infected plants and acquire the pathogen. The insect vectors then transmit the pathogen to healthy plants when they feed. This cycle of transmission allows phytoplasmas to spread rapidly across crops .**



Symptoms of phytoplasma infection in several plant species. (a) healthy periwinkle; (b) phyllody symptoms in periwinkle; (c) healthy wheat; (d) wheat blue dwarf disease in field; (e) healthy jujube; (f) jujube witches' broom disease; (g) healthy Paulownia; and (h) Paulownia witches' broom disease.

Maintenance Of Phytoplasma

1. Plant Host Maintenance (In Vivo)

Selection of Host Plants

infect various plants, but some species are more suitable for long-term maintenance.

Common host plants used for phytoplasma maintenance include:

Periwinkle (*Catharanthus roseus*)
Highly susceptible and widely used for experimental studies.

Tomato (*Solanum lycopersicum*) -
Some phytoplasmas can be maintained tomato plants.

2. Insect Vector Maintenance (In Vivo)

- Phytoplasmas rely on sap-feeding insects for transmission, mainly from the order Hemiptera.

Common Insect Vectors:
Leafhoppers (Cicadellidae)
Planthoppers (Fulgoridae)
Psyllids (Psyllidae)

Leafhopper

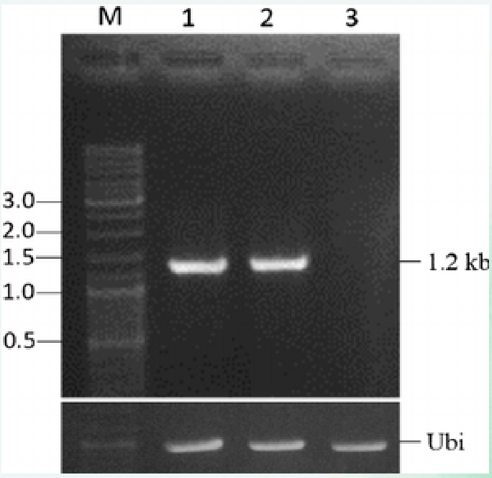
Methods of Maintenance Using Insects:

1. **Rearing Insect Vectors:**
Insects are collected from infected fields or bred in controlled conditions.
They are fed on infected plants to acquire phytoplasma.
After an incubation period, infected insects are transferred to healthy plants.

2. **Vector Transmission:**
Healthy insects are allowed to feed on infected plants.
After an incubation period (latency period), the insects are transferred to healthy plants to spread the phytoplasma.

3. **Monitoring Infection in Insects:**

PCR (Polymeras Chain Reaction) is used to confirm phytoplasma presence in insect vectors.
Insect dissections can be performed to analyze salivary glands, midguts, and other tissues for phytopla localization.



Detection of phytoplsma by nested PCR

3. In Vitro Maintenance Methods

Since phytoplasmas cannot be cultured in artificial media, alternative techniques like plant tissue culture and cryopreservation are used.

Tissue Culture Maintenance

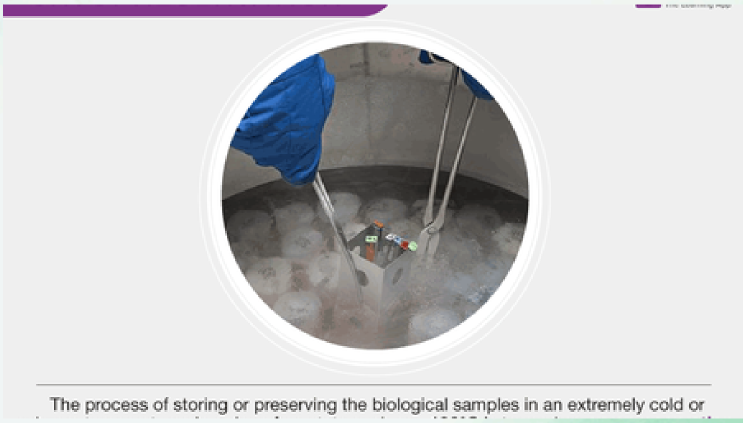
- Infected plant tissues (such as stems, leaves, or callus tissues) can be grown in a sterile medium.
- Used for short-term maintenance of phytoplasma in the absence of a whole plant.

Cryopreservation

• Plant tissues infected with phytoplasmas are stored at ultra-low temperatures (-196°C) using liquid nitrogen.

• This allow for long-term storage of phytoplasma-infected material.

• Upon revival, the infected tissues are grafted onto healthy plants for further study.



4. Molecular Detection & Monitoring of Phytoplasma

Since phytoplasmas are difficult to detect visually, molecular techniques are essential for confirming their presence in maintained plants or insects.

1. **Polymerase Chain Reaction (PCR)**

Universal primers (e.g., 16S rRNA gene primers) are used to detect phytoplasma DNA.

Nested PCR can increase sensitivity.

2. **Quantitative PCR (qPCR)**

Allows precise quantification of phytoplasma load in plant or insect samples.

3. **Loop-Mediated Isothermal Amplification (LAMP)**

A rapid and sensitive technique for field detection.

4. **Fluorescence In Situ Hybridization (FISH)**

Uses fluorescent probes to visualize phytoplasmas within plant phloem or insect tissues.

5. Electron Microscopy

Provides visual confirmation of phytoplasma presence in phloem sieve tubes.

Challenges in Phytoplasma Maintenance

- Host Specificity: Some phytoplasmas require specific plant hosts or insect vectors.
  - Vector Rearing Complexity: Insect vectors require controlled conditions and regular feeding cycles.
  - Loss of Virulence: Over time, phytoplasma populations may decline in maintained plants.
  - Contamination Risk: Insect vectors can acquire secondary infections, affecting phytoplasma maintenance.

Mutation of Phytoplasma

Mutations refer to alterations in the DNA sequence of an organism. These changes can arise naturally or as a result of external influences such as radiation, chemicals, or viruses. Depending on their nature, mutations can impact an organism's characteristics and may be harmful, beneficial, or neutral.

Types of Mutation in Phytoplasma

1. Point mutations – single nucleotide changes.

2.Insertion/Deletion mutations – adding or removing DNA segments

3.Rearrangement mutations – altering the gene order within the genome

4. Duplication mutations - affecting host virulence and adaption

Mechanisms of Mutation in Phytoplasma

A. <u>Genome Reduction and Rearrangement</u>

Phytoplasmas have small genomes (530–1350 kb), much smaller than other bacteria, due to the loss of non-essential genes.

They rely on their hosts for survival, leading to further gene losses over time.Genome rearrangement occurs through large-scaled deletions, insertions, and duplications.

<u>B. Point Mutations and Small-Scale Changes</u>

Mutations occur in genes encoding effector proteins, leading to variations in disease symptoms.

Single nucleotide polymorphisms (SNPs) contribute to differences in virulence and host adaptation.

Insertions and deletions (Indels) can modify protein functions, impacting phytoplasma-host interactions.

C. Variable Membrane Proteins (VMPs) and Antigenic Variation

VMPs are highly mutable surface proteins that help phytoplasmas evade plant immune responses.

Frequent gene duplications and recombination events create antigenic diversity.

This adaptation mechanism allows phytoplasmas to infect different host plants and insect vectors.

D. Horizontal Gene Transfer (HGT)

Phytoplasmas acquire genes from plants, other bacteria, or even insect vectors through horizontal gene transfer.

This process enables them to develop new virulence factors and enhance their survival strategies.

Evidence of integrated plant-derived genes in phytoplasma genomes suggests ongoing co-evolution with hosts.

Effects of Mutation in Phytoplasma

B. Antibiotic Resistance and Adaptation

Phytoplasmas lack many essential metabolic pathways but mutations in ribosomal proteins confer resistance to certain antibiotics (e.g., tetracyclines).
This makes disease control difficult, as traditional antibiotic treatments may become less effective.

C. Insect Vector Adaptation

Phytoplasmas rely on insects for transmission, and mutations in surface proteins affect insect-vector interactions.

Some mutations increase phytoplasma colonization efficiency in specific insect species, expanding their host range

Evolutionary Implications of Phytoplasma Mutations

High mutation rates and genome plasticity drive phytoplasma evolution, allowing them to adapt to new environments and hosts.

Loss of DNA repair genes increases genomic instability, accelerating evolution through genetic drift.

Phytoplasmas are coevolving with both plant and insect hosts, leading to the emergence of new strains with varied virulence

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Thank You

GROUP 6

MECHANISM TO INFECT PLANTS AND INSECTS BY PHYTOPLASMA .

Submitted to: Dr Manoj Thakur .
Presented By: Gunjan Verma – 1424041.

INTRODUCTION

- PHYTOPLASMAS ARE BACTERIAL PATHOGENS THAT CAUSE DISEASES IN PLANTS AND INSECTS. THEY ARE TRANSMITTED BY INSECT VECTORS SUCH AS LEAFHOPPERS, PLANTHOPPERS.

- THE MECHANISM OF PHYTOPLASMA INFECTION INVOLVES THE ATTACHMENT OF THE BACTERIA TO THE INSECT VECTOR'S GUT EPITHELIUM AND EVENTUAL TRANSMISSION TO THE PLANT HOST.

- The mechanism by which phytoplasmas infect plants and insects .

- IT INVOLVES A COMPLEX RELATIONSHIP BETWEEN THE PATHOGEN, THE HOST PLANT, AND THE INSECT VECTOR.

- It done mainly four process.

1. Transmission to plants.
2. Activities within insects .
3. Acquisition and inoculation .
4. Phloem colonization.

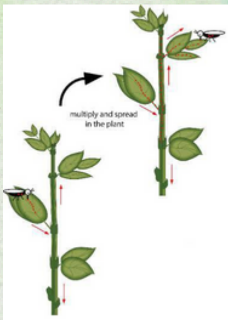
Transmission to plants.

- Phytoplasmas are primarily transmitted to plants by insect vectors, such as ,leafhoppers, planthoppers, psyllids.

- These insects acquire the phytoplasmas while feeding on infected plants and then transmit them to healthy plants when they feed again.

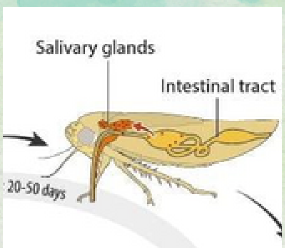
- Different phytoplasma strains are transmitted by specific insect vectors. This means that a particular insect species may only be able to transmit certain phytoplasma strains.

Understanding the transmission process of phytoplasmas by insect vectors crucial for developing effective strategies to manage phytoplasma diseases and protect plant health.

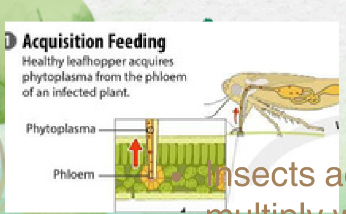


Activities within insects

- PHYTOPLASMA ENTERS INSECTS DURING FEEDING ON INFECTED PLANTS.
- In insects, phytoplasmas establish systemic infection by spreading from the gut to hemocoel, then to the salivary gland, each of which presents a barrier to insect transmissibility .
- IT COLONIZES AND REPLICATES WITHIN THE INSECT'S GUT. PHYTOPLASMA MIGRATES FROM THE GUT TO THE INSECT'S SALIVARY GLANDS.

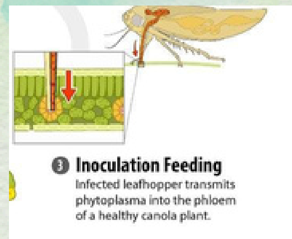


Acquisition and inoculation



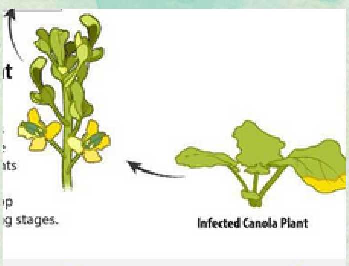
- Insects acquire phytoplasmas by feeding on infected plants. The phytoplasmas multiply within the insect's body and are then transmitted to healthy plants through saliva during feeding.

- phytoplasmas are initially injected into the phloem sieve tubes and then propagate within and spread from phloem tissues of the infected leaf to the main stem, root, and leaves via the bipolar movement of the phloem fluid at night and in the day.

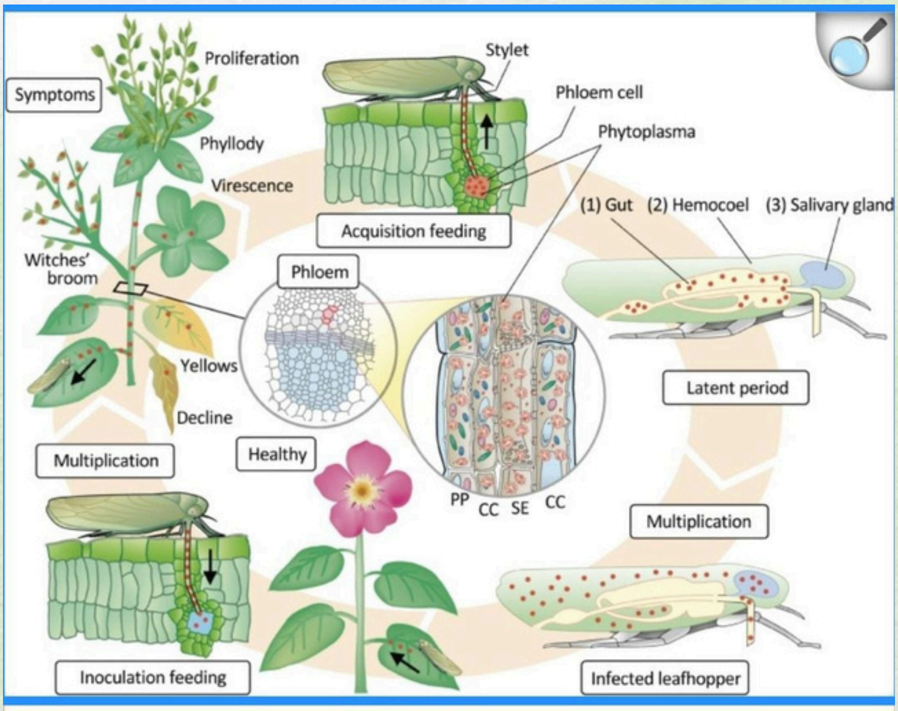


Phloem colonization

- It Primarily Reside In The Phloem Tissue Of Infected Plants. When A Susceptible Plant Is Infected, The Phytoplasma Colonizes The Phloem Sieve Elements, Where It Multiplies And Spreads Throughout The Plant's Vascular System. As The Phytoplasma Proliferates, It Disrupts The Normal Functioning Of The Phloem, Leading To A Range Of Symptoms Such As Yellowing, Stunting, Witches Brooms, And Phyllody.



Figure



Symptoms
Proliferation
Phyllody
Virescence
Witches' broom
Yellows
Decline
Stylet
Phloem cell
Phytoplasma
Acquisition feeding
Phloem
(1) Gut
(2) Hemocoel
(3) Salivary gland
Latent period
Multiplication
Healthy
PP
CC
SE
CC
Multiplication
Inoculation feeding
Infected leafhopper

- specialized protein translation system in phytoplasma

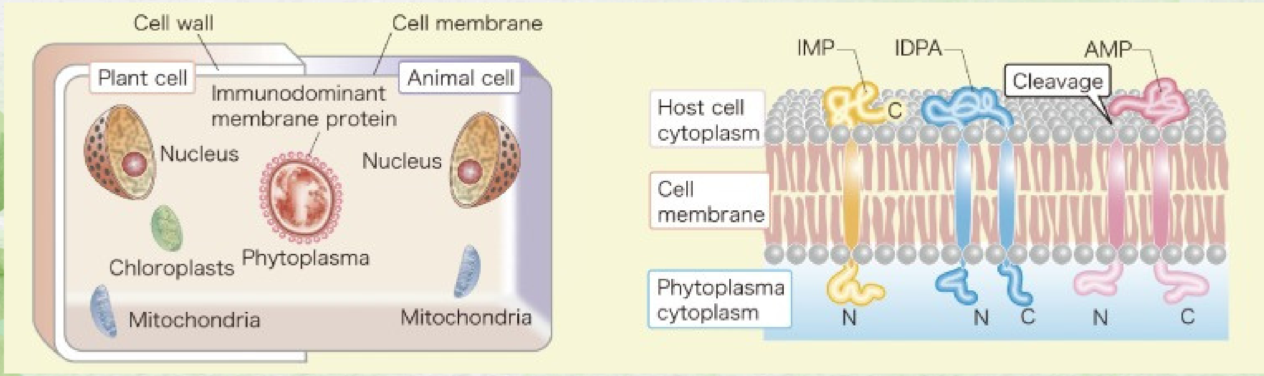
In phytoplasma, the specialized protein translation system has some unique characteristics, and AMP (adenosine monophosphate) and IMP (inosine monophosphate) may play roles in adaptation and translation efficiency.
Phytoplasmas are obligate intracellular bacteria that lack many essential genes for normal bacterial functions, including some
components of the translation machinery. Their translation system has evolved to be highly specialized due to genome reduction.

1)tRNA and Codon UsagePhytoplasmas have an unusual codon usage and often lack certain tRNA genes, relying on the host’s translation machinery to compensate.

2)They may use non-standard decoding strategies, sometimes involving modified nucleotides like inosine (IMP) in tRNAs, which allows for wobble base pairing and expanded codon recognition.AMP, IMP, and Nucleotide MetabolismIMP (inosine monophosphate) is a key intermediate in purine metabolism and may be involved in nucleotide recycling for RNA synthesis.AMP (adenosine monophosphate) plays a role in energy balance and may influence translation through interactions with regulatory proteins like AMP-activated protein kinase (AMPK) or other metabolic pathways.

3)Host DependenceDue to their minimalist genome, phytoplasmas rely on their plant or insect hosts for many essential molecules, including some amino acids and nucleotides.This host dependence could influence how their translation system functions under nutrient-limited conditions.

Phytoplasmas have a highly specialized and streamlined translation system, possibly incorporating IMP for flexible codon decoding and AMP for energy regulation. Their reliance on host-derived molecules makes their translation system unique compared to free-living bacteria.



- The immunodominant membrane proteins of phytoplasmas and their localization. Most of the phytoplasma cell surface is covered with three membrane proteins termed immunodominant membrane proteins (IMP, AMP, and IDPA (immunodominant membrane protein A)), which are thought to be involved in interactions with both the insect and plant hosts. This figure was reproduced with modifications based on the original literature.

Phtyoplasmas have broad host range which included plant host and vector(insect host)

- Plant Hosts:

- Phytoplasmas infect a wide variety of plants, including fruit trees, vegetables, cereals, ornamentals, and wild plants. They cause diseases like:
- Yellowing and Witches’ Broom (e.g., in coconut, peanuts, and legumes)
- Stolbur disease (in tomatoes and potatoes)
- Aster yellows (in many ornamental and crop plants)
- Lethal Yellowing (in palms)

- **Insect Vectors (Insect Hosts):**

- Phytoplasmas are transmitted mainly by sap-sucking insects, especially leafhoppers (Cicadellidae), planthoppers (Fulgoromorpha), and psyllids (Psylloidea). These insects acquire phytoplasmas while feeding on infected plants and later transmit them to healthy plants.
- Since phytoplasmas lack cell walls, they rely on these hosts for survival and reproduction, making them highly adaptable pathogens

CONCLUSION

- Phytoplasmas are unique, cell-wall-less bacteria that infect plants, causing various diseases that lead to significant agricultural losses. They are transmitted primarily by insect vectors, such as leafhoppers, and can cause symptoms like yellowing, stunting, and abnormal growth patterns in plants. Due to their small genome and inability to be cultured in artificial media, studying phytoplasmas remains a challenge.

- Efforts to manage phytoplasma-related diseases focus on controlling insect vectors, removing infected plants, and breeding resistant crop varieties. Advances in molecular biology have improved detection and identification methods, leading to better disease management strategies. However, further research is needed to develop effective treatments and understand the intricate interactions between phytoplasmas, their hosts, and insect vectors.

Reference

- https://pmc.ncbi.nlm.nih.gov/articles/PMC6766451/#:~:text=Phytoplasma%2Dinfected%20plants%20exhibit%20phloem,flowers)%2C%20floral%20virescence%3B%20abnormal
- ARTICLE BY : Shigetou NAMBA


THANK
YOU

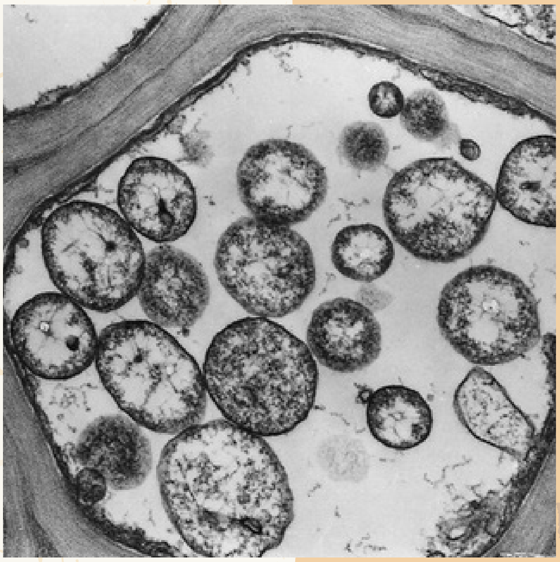
GROUP 7

MOLECULAR AND BIOLOGICAL PROPERTIES OF PHYTOPLASMAS

GENETIC FACTORS DETERMINING SYMPTOM DEVELOPMENT

PHYTOPLASMAS: THE INVISIBLE PATHOGEN

- Phytoplasmas: Cell wall-less bacteria, obligate parasites of plant phloem.
- Transmitted by insect vectors, leading to systemic infections.
- Cause a wide array of plant diseases.



TEM OF PHYTOPLASMA

PHYTOPLASMA INFECTED PLANTS SHOWING CHARACTERISTIC SYMPTOMS

SYMPTOM DIVERSITY AND ECONOMIC IMPACT

- Symptoms range from yellowing and stunting to floral abnormalities (phyllody, virescence).
- Significant economic impact on agriculture and horticulture worldwide.
- Understanding symptom development is crucial for disease management.

EFFECTOR PROTEINS: THE SYMPTOM ARCHITECTS

Many plant-pathogenic bacteria secrete proteins, termed effectors, into the cytoplasm of host cells for successful colonization. However, the phytoplasma genome does not contain any known effector-like genes.

In 2009, the first phytoplasma effector protein, TENGU, a secreted peptide of 38 amino acids, was identified as an inducer of witches' broom.

TENGU is conserved among various phytoplasma strains. Following secretion from the phytoplasma cell, TENGU is cleaved *in planta* to a peptide of 12 amino acids, which is then transported to the shoot apical meristem, wherein it inhibits the signaling pathway of the plant hormone auxin and induces witches' broom symptoms.

VARIABLE MEMBRANE PROTEINS (VMPS) AND SURFACE PROTEINS

- VMPS EXHIBIT HIGH GENETIC DIVERSITY, CONTRIBUTING TO HOST SPECIFICITY.
- INVOLVED IN INSECT VECTOR INTERACTIONS AND IMMUNE EVASION.
- SURFACE PROTEINS PLAY A CRUCIAL ROLE IN INITIAL HOST CONTACT.

SAP11 : A KEY EFFECTOR PROTEIN

Secreted Protein

SAP11 is a secreted protein. It manipulates plant development.

Targets Transcription Factors

It targets plant transcription factors. This leads to degradation or altered localization

Symptom Induction

It induces symptoms like leaf yellowing.

HORIZONAL GENE TRANSFER(HGT)

- Conjugation
- Transduction
- Transformation

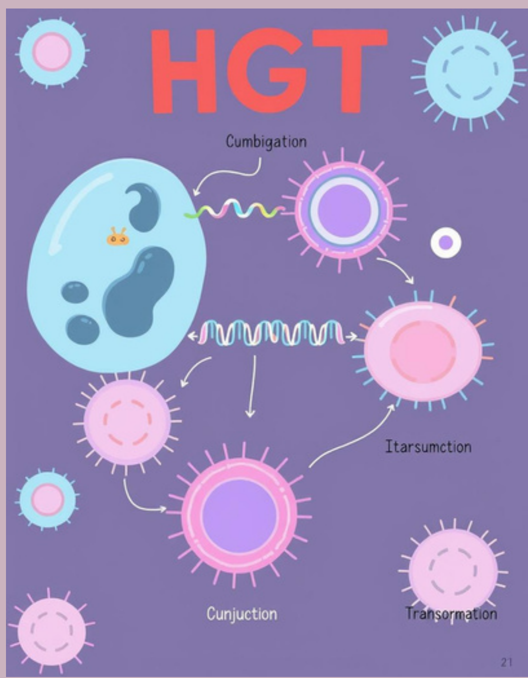


diagram illustrating HGT mechanisms (conjugation, transduction, transformation), bacterial cells exchanging genetic material

IMPACT ON PLANT PHYSIOLOGY

Hormone Imbalance

- Phytoplasmas disrupt plant hormone balance.

Altered Metabolism

- They alter carbohydrate metabolism. Photosynthesis is reduced.

Compromised Defenses

- Plant defenses are compromised. Plants are more susceptible.

CONCLUSION AND FUTURE DIRECTIONS

KEY GENETIC FACTORS

- VMPs, effectors, and HGT are important.

FUTURE RESEARCH AND DISEASE-RESISTANT CROPS

- Identify novel effectors and their actions.
- Develop resistant crops based on virulence factors.

REFERENCES:

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Google Scholar
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PRESENTED BY:

- *Vanshika Verma*

THANKYOU

GROUP 8
CONCLUSION

MOLECULAR AND BIOLOGICAL PROPERTIES OF PHYTOPLASMA

A conclusion

Submitted to : Dr. Manoj Thakur
Presented by - Adrija Mitra (1424027)

WHAT IS PHYTOPLASMA

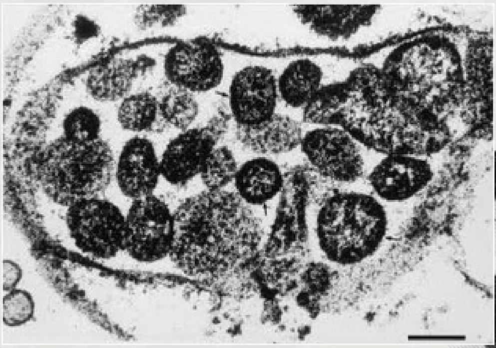


Fig. TEM of Phytoplasma

Phytoplasma are certainly a fascinating group of plant pathogens. They are a group of bacteria belonging to the class Mollicutes , known for lacking a cell wall and thus, is morphologically pleiomorphic. They are obligate intracellular parasites and hence, can only survive and reproduce inside living cells, specifically within the phloem tissue of plants.

History

Long before their formal discovery, diseases caused by phytoplasmas were observed and recorded. One of the earliest examples is the mulberry dwarf disease in Japan which has records going back centuries. These diseases were often believed to be caused by viruses because the causal agents couldn't be cultured and were too minuscule to be seen with regular microscope.

The pivotal moment came in 1967 when Japanese scientists Yoji Doi, Makoto Teranaka, Kenji Yora, and Hiroyuki Asuyama make a breakthrough discovery by using electron microscopy on ultra thin sections of plants with yellows diseases. They observed small, pleiomorphic bodies in the phloem tissue. These bodies resembled mycoplasma rather than viruses. Their paper published in the Annals of the Phytopathological Society of Japan was revolutionary in plant pathology.

Phytoplasma was originally called Mycoplasma-Like Organisms (MLOs) because of their structural and biological similarities to mycoplasmas, a group of bacteria that lack cell walls.

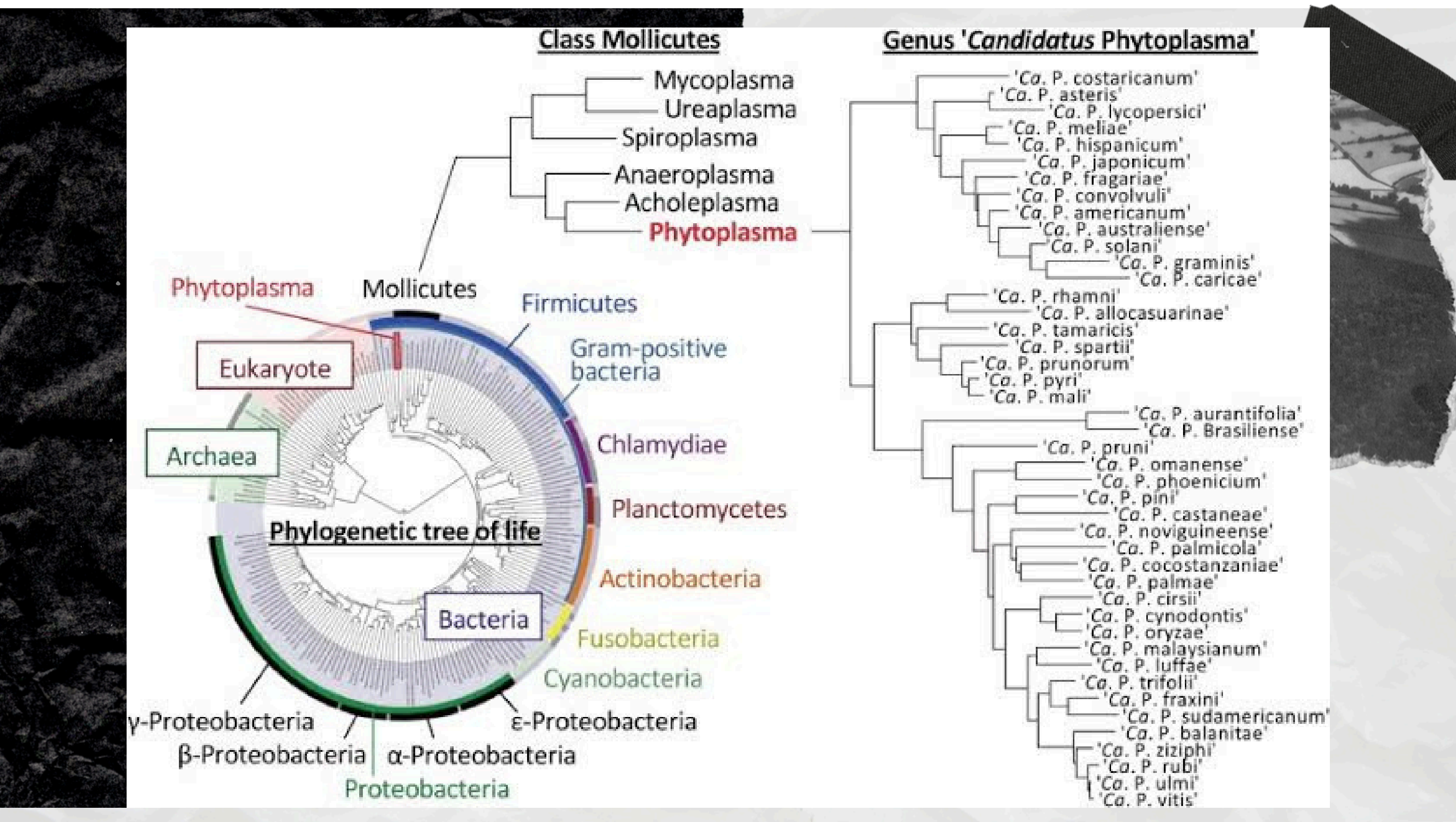
In 1992, DNA sequencing of the 16S rRNA gene showed that MLOs were genetically distinct from mycoplasmas. In 1994, the International Committee on Systematics of Prokaryotes (ICSP) officially renamed them Phytoplasma to better reflect their plant-specific nature (Phyto- meaning plant).

CLASSIFICATION OF PHYTOPLASMA

Before 1967, plant diseases like aster yellows and witches' broom were observed, but their causal agents remained unknown. Scientists initially suspected viruses or unidentified microorganisms. In 1967, Doi et al. discovered microscopic, pleomorphic, wall-less bacteria in diseased plants using electron microscopy. These organisms resembled mycoplasmas, which are known to infect animals, and were therefore named Mycoplasma-Like Organisms (MLOs).

However, it wasn't until 1992 that DNA sequencing of the 16S rRNA gene revealed that MLOs were genetically distinct from mycoplasmas, leading to their classification as a unique bacterial group within Mollicutes. In 1994, the International Committee on Systematics of Prokaryotes (ICSP) officially renamed them Phytoplasma to reflect their plant-specific nature (Phyto- meaning plant).

Since the 2000s, whole-genome sequencing has improved Phytoplasma classification, allowing scientists to group them into various strains and subgroups. Because Phytoplasma remains unculturable, classification is based primarily on genetic markers like 16S rRNA gene sequences. Currently, Phytoplasma belongs to the Acholeplasmataceae family within the order Acholeplasmatales, class Mollicutes, and is designated under the genus Candidatus Phytoplasma due to its unculturable status. Ongoing advancements in genomics and molecular phylogenetics continue to refine our understanding of Phytoplasma classification.


Class Mollicutes
Mycoplasma
Ureaplasma
Spiroplasma
Anaeroplasma
Acholeplasma
Phytoplasma
Genus 'Candidatus Phytoplasma'
'Ca. P. costaricanum'
'Ca. P. asteris'
'Ca. P. lycopersici'
'Ca. P. meliae'
'Ca. P. hispanicum'
'Ca. P. japonicum'
'Ca. P. fragariae'
'Ca. P. convolvuli'
'Ca. P. americanum'
'Ca. P. australiense'
'Ca. P. solani'
'Ca. P. graminis'
'Ca. P. caricae'
'Ca. P. rhamni'
'Ca. P. allocasuarinae'
'Ca. P. tamaricis'
'Ca. P. spartii'
'Ca. P. prunorum'
'Ca. P. pyri'
'Ca. P. mali'
'Ca. P. aurantifolia'
'Ca. P. Brasiliense'
'Ca. P. pruni'
'Ca. P. omanense'
'Ca. P. phoenicium'
'Ca. P. pini'
'Ca. P. castaneae'
'Ca. P. noviguineense'
'Ca. P. palmicola'
'Ca. P. cocostanzaniae'
'Ca. P. palmae'
'Ca. P. cirsii'
'Ca. P. cynodontis'
'Ca. P. oryzae'
'Ca. P. malaysianum'
'Ca. P. luffae'
'Ca. P. trifolii'
'Ca. P. fraxini'
'Ca. P. sudamericanum'
'Ca. P. balanitae'
'Ca. P. ziziphi'
'Ca. P. rubi'
'Ca. P. ulmi'
'Ca. P. vitis'
Phytoplasma
Mollicutes
Firmicutes
Eukaryote
Gram-positive bacteria
Archaea
Chlamydiae
Planctomycetes
Phylogenetic tree of life
Actinobacteria
Bacteria
Fusobacteria
Cyanobacteria
γ-Proteobacteria
ε-Proteobacteria
β-Proteobacteria
α-Proteobacteria
Proteobacteria

Symptoms caused by Phytoplasma

Fig. Symptoms of phytoplasma diseases on some ornamental species: (a) Chrysanthemum phyllody associated with stolbur phytoplasmas; (b) leaf and flower malformation/discoloration in rose; (c) yellows disease of Hibiscus; (d, e, f) Catharanthus little leaf, phyllody and virescence diseases; (g) flower virescence of lily; (h) fasciation of lily; (i) witches' broom disease of lily; (j) aster yellows in Callistephus chinensis (aster); (k) flower virescence and malformation of Gladiolus; (l) multiple sprouts in gladiolus corms; (m) hydrangea virescence; (n) virescence and malformation in ranunculus flowers

Phytoplasma detection is challenging since these bacteria cannot be cultured in artificial media. Symptom-based diagnosis is useful but not definitive. Electron microscopy (EM)can visualize Phytoplasma in plant phloem but lacks strain differentiation and sensitivity.

Molecular methods like PCR (Polymerase Chain Reaction) are the most reliable, targeting the 16S rRNA gene for precise identification. Variants like Nested PCR (higher sensitivity) and qPCR (quantitative detection) are commonly used. LAMP (Loop-Mediated Isothermal Amplification) is a rapid, field-friendly alternative.

Serological methods like ELISA and Western Blotting are less effective due to Phytoplasma's low protein content. For advanced research, Next-Generation Sequencing (NGS) provides whole-genome insights but is expensive and impractical for routine use. Overall, PCR and LAMP remain the most effective detection methods for Phytoplasma.

Control methods

Phytoplasma management relies on vector control, host resistance, alternative treatments, and early detection. Since sap-sucking insects like leafhoppers transmit the disease, controlling vectors through insecticides, biological control (e.g., parasitoid wasps), and cultural practices (e.g., removing infected plants, crop rotation) is essential.

Developing resistant crop varieties through breeding and genetic engineering (CRISPR) offers long-term protection. Alternative treatments like antimicrobial peptides, beneficial microbes, and plant immune boosters (e.g., salicylic acid) are being explored. Early detection using PCR and LAMP tests allows for timely intervention, while quarantine measures prevent disease spread. An integrated approach combining these strategies is key to effective Phytoplasma control.

Research and advancements

Over the years, significant research has enhanced our understanding of Phytoplasma, from its classification and detection to disease management and host interactions.

1. Genomic and Molecular Studies

Advancements in DNA sequencing, particularly Next-Generation Sequencing (NGS), have provided deeper insights into Phytoplasma genomes. Studies of the 16S rRNA gene have helped classify different strains, while whole-genome sequencing has revealed genes involved in virulence, host adaptation, and insect transmission. The discovery of effector proteins, which Phytoplasma uses to manipulate plant growth and suppress immune responses, has been a major breakthrough in understanding its pathogenicity.

2. Improved Detection Techniques

Traditional methods like PCR (Polymerase Chain Reaction) continue to be the gold standard for detection, but Loop-Mediated Isothermal Amplification (LAMP) is gaining popularity due to its rapid and field-friendly nature. Researchers are also developing biosensors and CRISPR-based detection tools for more precise and quicker diagnostics.

3. Insect Vector Control and Disease Management

Since Phytoplasmas are transmitted by sap-sucking insects (e.g., leafhoppers), research has focused on vector control strategies, including the use of biological control agents, RNA interference (RNAi), and plant breeding for insect resistance. Studies on insect-Phytoplasma interactions aim to disrupt the transmission cycle and reduce disease spread.

4. Host Resistance and Genetic Engineering

Advancements in plant biotechnology are exploring ways to develop Phytoplasma-resistant crops. Researchers are investigating gene editing tools like CRISPR-Cas9 to modify plant genes involved in susceptibility. Additionally, breeding programs focus on developing Phytoplasma-tolerant crop varieties.

5. Alternative Treatment Strategies

Since Phytoplasma cannot be controlled with conventional antibiotics or fungicides, alternative treatments are being studied. These include antimicrobial peptides, endophytic bacteria (beneficial microbes), and plant immune boostersthat enhance plant resistance.

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