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Demonstration of Koch's postulates by using plant bacteria

Bacteria are prokaryotic unicellular organisms. About 200 species of bacteria cause diseases in plants.

i). Morphology

Most plant pathogenic bacteria are rod shaped except Streptomyces, which is filamentous. The cell walls of bacteria of most species are enveloped by a viscous, gummy material, which if thin and diffused, is called a slime layer; but if thick, forming a definitive mass around the cell, is called a capsule. Most plant pathogenic bacteria have delicate, threadlike flagella, considerably longer than the cells on which they are produced.

ii). Gram staining reaction

Gram staining reaction differentiates bacteria into gram positive and gram negative types. In this reaction, bacteria fixed on a glass slide are treated with a crystal violet solution for 30 seconds, rinsed gently, treated with iodine solution and rinsed again with water and then alcohol.

Gram positive bacteria retain the violet-iodine stain combination because it forms a complex with certain components of their cell walls and cytoplasm. The rod shaped phytopathogenic bacteria, only the genera *Clavibacter* and *Curtobacterium*, and also *Bacillus* and *Rhodococcus* are gram positive.

Gram negative bacteria have no affinity for the stain combination which is therefore, removed by the alcohol rinse and bacteria remain as nearly invisible as before. *Agrobacterium*, *Erwinia, Pseudomonas, Xanthomonas* and *Xylella* are gram negative.

Name of the disease: Bacterial blight of Rice and the casual organism is *Xanthomonas oryzae* pv. *oryzae*

Bacterial blight of Rice:

- On seedlings, infected leaves turn grayish green and roll up. As the disease progresses, the leaves turn yellow to straw-colored and wilt, leading whole seedlings to dry up and die (also called kresek).
- On young lesions, bacterial ooze resembling a milky dew drop can be observed early in the morning. The bacterial ooze later on dries up and becomes small yellowish beads underneath the leaf.
- On older plants, lesions usually develop as water-soaked to yellow-orange stripes on leaf blades or leaf tips or on mechanically injured parts of leaves. Lesions have a wavy margin and progress toward the leaf base.

To quickly diagnose bacterial blight on leaf:

- cut a young lesion across and place in a transparent glass container with clear water
- after a few minutes, hold the container against light and observe for thick or turbid liquid coming from the cut end of the leaf

Xanthomonas oryzae pv. *oryzae*:

X. oryzae is a rod-shaped, round-ended, Gram-negative species. Individual cells vary in length from approximately 0.7 µm to 2.0 µm and in width from 0.4 µm to 0.7 µm. Cells are motile by means of a single polar flagellum. Colonies on solid media containing glucose are round, convex, mucoid and yellow in colour due to the production of the pigment xanthomonadin. *X. oryzae* is obligately aerobic and does not form spores. Optimal temperature for growth is between 25 and 30 °C.

Materials needed:

- Sterile Water
- Dissecting and compound microscopes
- Dissecting needles and alcohol or sterile (autoclaved) toothpicks
- Bunsen burners or candles
- Paper towels
- Petri dishes
- Plastic containers with healthy rice plant
- Scalpel or shears
- Nutrient agar plates or other bacterial media
- Inoculating Loop etc.

Postulate 1: A specific organism must always be observed in association with the disease.

Procedure:

1. Collected disease specimen from the field
2. Study the characteristics symptoms of the disease specimen
3. Cut a young lesion across and place in a transparent glass container with clear water. After a few minutes, hold the container against light and observe for thick or turbid liquid coming from the cut end of the leaf
4. Prepare a culture plate then, Identify the bacterial colony by using standard catalog

Postulate 2: The organism must be isolated from an infected host and grown in pure culture in the laboratory.

Procedure

1. Isolate the microorganism by using standard method
2. Incubate plates at 30°C for 48 hours and observe plates for growth
3. Choose individual colonies- lightly touch the top of an individual colony with a sterile loop or needle and streak on a fresh agar plate for getting pure culture
4. Identify the bacterial colony by using standard catalog

Postulate 3: When the organism from the pure culture is inoculated into a susceptible host organism, it must cause the disease.

Procedure

1. Select healthy, similar age and species of the plant for inoculation.
2. Inoculate by using standard method with isolated pure culture microorganism
3. After inoculation then keep the plant for incubation at least 10 days and allow to grow plant disease at 30°C under 12-h days

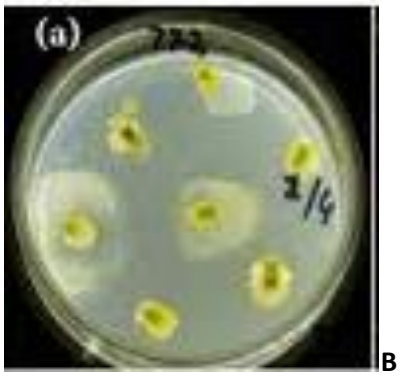
Postulate 4: The infectious organism must be re-isolated from the diseased organism and grown in pure culture.

Procedure

1. Observe the infected area of the plant
2. Aseptically transfer of the microorganism from inoculated plant by using standard method in culture medium and make a data chart for recording the observations
3. Identify the bacterial colony by using standard catalog and compare with previous one.

Remark: This pathogen is absolutely identical with previous one. As a result, it concluded that the disease was Bacterial blight of rice and the casual organism was *Xanthomonas oryzae* pv. *oryzae.*


A



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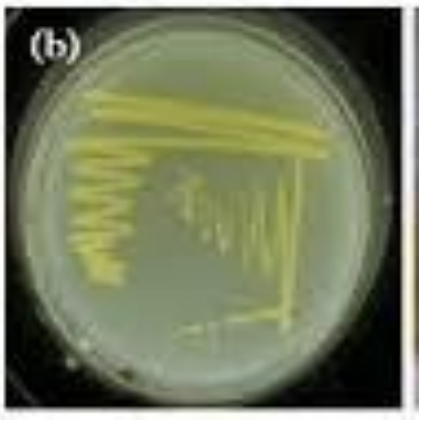

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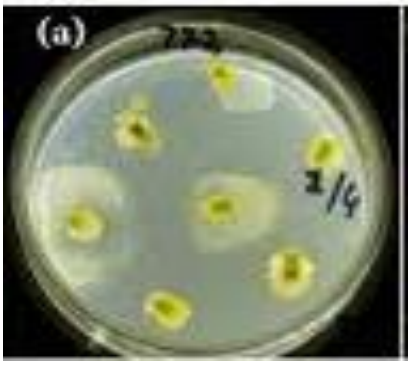

E


F

Figure A: **ASSOCIATION**

Rice plant + *Xanthomonas oryzae* pv. *oryzae*

Figure B-C: **ISOLATION**

Culture the *Xanthomonas oryzae* pv. *oryzae* on media

Pure culture of *Xanthomonas oryzae* pv. *oryzae*

Figure D: **INOCULATION**

Inoculate with isolated *Xanthomonas oryzae* pv. *oryzae*

Figure E-F: **RE-ISOLATION**

Pure culture of *Xanthomonas oryzae* pv. *oryzae*

Identify the bacterial colony by using standard catalog