

"ISOLATION OF *ERWINIA* FROM POTATO TUBER AND BANANA LEAVES AND STUDYING THE EFFECT OF STREPTOMYCIN ON ITS GROWTH"

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Abstract

Plant disease is an impairment of the normal state of a plant that interrupts or modifies its vital functions. Soft rot is one of the diseases caused by Erwinia species. It infects variety of hosts like banana, potato, tomato, pineapple, tobacco etc. A Potato Dextrose Agar medium was used to study the occurrence of soft rot bacteria in potato tuber and banana leaves. The isolated Erwinia species characterized for biochemical and morphological test. The inhibitory effect of Erwinia growth was studied against antibiotic streptomycin at various concentrations. Also we take laboratory application of this inhibitory effect of streptomycin on Tomato seed germination by infection with isolated Erwinia species.

Key words: *Potato, Tomato, Soft rot, Erwinia sps. Streptomycin.*

INTRODUCTION:

Plant diseases are a normal part of nature and one of many ecological factors that help keep the hundreds of thousands of living plants and animals in balance with one another. Plant cells contain special signaling pathways that enhance their defenses against insects, animals and pathogens. In general, a plant becomes diseased when it is continuously disturbed by some causal agent that results in an abnormal physiological process that disrupts the plant's normal structure, growth, function, or other activities. This interference with one or more of a plant's essential physiological or biochemical systems elicits characteristic pathological conditions or symptoms. Various plants are infected by wide ranges of organisms like bacteria, fungi, viruses etc. to wide ranges of plant species and causes disease like bacterial spot tomato, Bacterial soft rot pepper, Virus tomato plant, Virus green bean, Smut on

maize comb, Powdery mildew sunflower, Early blight tomato, *Erwinia* potato tuber rot.

Erwinia is one of the plant pathogen which causes soft rot disease in Potato, Banana, and Tomato etc. *Erwinia* was named after the phytobacteriologist Erwin F. Smith. It is a pathogen that degrade succulent fleshy plant organ such as roots, tubers, stem cuttings and thick leaves. It is also a vascular wilt pathogen, colonizing the xylem and becoming systemic with the plant (M.L. Powelson and J.D. Apple, 1983). This later aspect is the most alarming when vegetative propagation is involved. The pathogen can remain latent in stock plants (ornamentals and bananas) and can thus be spread in cuttings from them. They can be host to the bacteria either by being infected as seed, or from direct inoculation into wounds or natural openings (stomata or lenticels) in mature plants (Elphinstone and Perombelon 1986), which is most common. But, when a plant is infected and the conditions are favorable, the bacteria immediately begin feeding on liquids released from injured cells and start replicating. As they replicate they release more and more pectolytic enzymes that degrade and break down cell walls [3]. And, because of the high turgor pressure within the cells, this maceration effectively causes the cells to explode and die providing more food for the bacteria.

Infections start as tiny watery spots that grow until entire leaves are mushy and collapsing. Stems can also be infected and become mushy and foul smelling and their leaves usually yellow quickly on some plants, a blackened, wet, slimy spot generally starts at the soil line at the base of the plant and progresses into the upper portions of the plant. Rotted areas are usually watery and mushy and have a rotten fishy odor in many cases. The bacteria sometimes form a slimy, gelatinous mass at the base of infected cuttings. In other cases the bacterium is systemic and spreads within the conductive tissues of the plants. Plants wilt, collapse and often die. Due to production of macerating enzymes, infected plants become very mushy and disintegrate especially during the warmer months of the year (A.R Chase).

The bacterium is able to survive in the soil, so that infestation remains between two crops. High humidity and free water favor spread and penetration of the bacteria. Disease development is dependent on high temperatures, generally 25-28°C. The pathogen is ubiquitous.

There are different species of *Erwinia* such as; *E.carotovora*, *E.arodea*, *E.aphidicola* etc. Hosts have been most often reported on bananas, carnations, dahlia, maize etc. It has been naturally found to attack onions, potatoes, tomatoes, tobacco, sweet potato, tulips, pineapple etc. The objectives of present study were to isolate *Erwinia* sps. From Potato tuber and infected Banana plant leaves and studying the

growth inhibitory effect of Streptomycin on isolated *Erwinia* species. Because most of commercialist who engaged in cultivation of such crops faces crop losses because of its infection during hardening condition.

Material and methods:

Collection of sample:

For the isolation of *Erwinia*, potato was collected from local market of Kolhapur. Because Blackleg of potatoes caused by *Erwinia* is a severe field disease leading to the development of an inky black and slimy soft rot of stems. Similarly in banana plant the bacteria mainly act on fleshy storage organs of their host, but they also affect stems and petiole tissue. So, the soft rot symptoms showing leaves of banana were collected from the Seema biotech, Talsande, Warnanagar, Kolhapur.

Isolation of *Erwinia* species: For the isolation two different sources was selected so, two different protocols were conducted as below,

For potato:

Potato tubers were rinsed with water to remove soil and then Punctured ten times with sterile tooth picks, for soft rot pockets (M.L. Powelson and J.D. Apple, 1983) (**Fig:1**). Small sections of tissue dissected from the advancing margin of the soft rot were wrapped in moist paper sealed in two plastic bags and incubated at 24°C for 4 days. The tubers were then examined suspended in 1 ml of sterile water, and aliquot of the suspension were streaked onto PDA medium.

For banana:

Infected plants were uprooted from bags and were washed in tap water followed by sterile water and dried by paper toweling (**Fig: 2**). Surface sterilization was done by the treatment of mercury chloride for 3min and then distilled water wash. Affected tissue (leaves, root) were removed from young lesion by scalpel, tissue was crushed in saline and incubated for 15 min (Vijay J. Pachpute and Pallawi T. kininge, 2012). Aliquot of suspension were streaked onto PDA medium.

Characterization of isolated microorganism

Isolated microorganisms from both the sources was characterized by morphological screening and biochemical tests such as Nitrate reduction, Vogus Proskauer, Sugar test etc (**Fig:3,4,5,6**) according to Bergye's manual of Systematic Bacteriology.

Inhibition test:

The isolated *Erwinia* bacterial sps from both the sources was used for inhibition test. This test is to inhibit the growth of micro-organisms. The introduction of various antimicrobials for treating variety of infection showed the necessity of performing this test. Inhibition test was performed by Inhibiting method and Disc

diffusion method using broad spectrum antibiotic Streptomycin at 50mg, 100mg, 150mg and 200mg concentrations (Fig:7,8,9).

Laboratory application of Streptomycin on *Erwinia species*

After successfully performance of inhibition test, same experiment was applied for laboratory trial with tomato seeds because symptoms observed for potato and banana plants are same for tomato plant; that is yellowing and wilting of the plant [8], browning of vascular tissue, hollowing of pith and soft rotting stem and fruits. And also because of the growth pattern of potato and banana plant (Fig: 10, 11).

Method

Tomato seeds were washed with tap water followed by surface sterilization with Tween- 80, 0.2% HgCl₂ then again by distilled water. Surface sterilized seeds were then sowed in plastic glasses which was filled with soil and allowed for germination. After germination, the roots of the seedlings were dipped in to the isolated bacterial suspension. Infected seedlings were again sowed in the plastic glasses containing soil and observed for the symptoms. After infection of *Erwinia* to the plants growth inhibitory effect of Streptomycin was checked by treatment.

Result and discussion

Infection of bacteria *Erwinia* to potato tuber and banana leaves

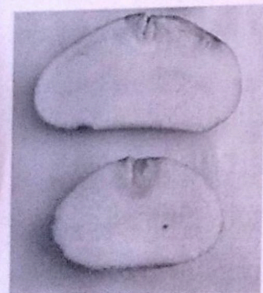
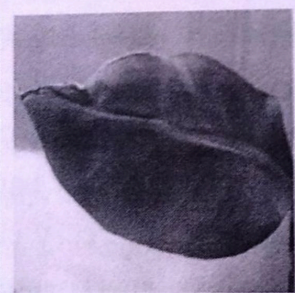


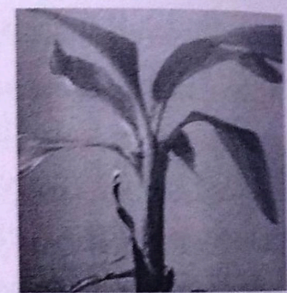
Fig 1: Formation of soft rot pockets on potato



Fig. 2



Infected banana leaves



Banana plant

Colony characteristics of both the isolated *Erwinia* spp.

For potato and banana:

Colony characteristics of well isolated suspected colony of *Erwinia* on PDA agar medium at 28°C for 48 hrs.

Size	Shape	Margin	Elevation	Consistency	Opacity	Color
1.5mm	Circular	Entire	Flat	Sticky	Opaque	White



Fig-3

Fig 3: Isolated colonies of *Erwinia* species from potato tuber

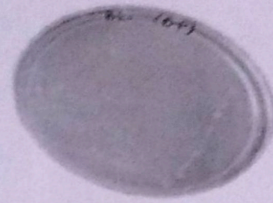


Fig-4

Fig 4: Isolated colonies of *Erwinia* species from infected banana leaves

Gram staining:

Morphologically *Erwinia* is a gram negative and motile organism.

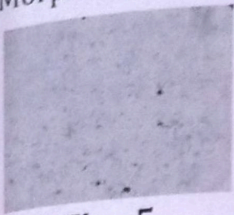


Fig - 5

Fig 5: Gram staining of isolated *Erwinia* species from potato tuber.

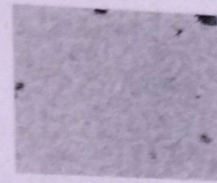


Fig - 6

Fig 6: Gram staining of isolated *Erwinia* species from banana leaves.

Biochemical test: For potato and banana

TEST	OBSERVATION	RESULT	
		FOR POTATO	FOR BANANA
Nitrate reduction	Formation of pink colour	positive	Positive
Vogus proskars	Formation of pink colour	positive	Negative
Glucose	Colour changes from yellow to orange red and acid, gas formation	positive	Positive
Xylose	Colour changes from yellow to orange red and acid, gas formation	positive	Positive
Fructose	No colour change and no acid, gas formation	positive	Positive

INHIBITION TEST:

For potato:

1ST method: It was observed that after 48 hrs of incubation, growth of bacteria was observed at concentration 50mg and little at 100mg but no growth was observed at concentration 150mg and 200mg of streptomycin.

The present study deals with the isolation of Bacteria *Erwinia* from infected banana leaves and from potato tubers and its identification based on biochemical testing's with this inhibiting property of *Erwinia* was studied and applied on tomato plant. The reaction of obtained results after incubation of potato tuber was similar to that observed in the case of natural infection, it gives unpleasant odour. Obradovic and Arsenijevic (1997), reported similar results with lesion development. Infected banana plant was collected from Green house. Obtained Biochemical test results were performed and compared with the Bergye's Manual of Systematic Bacteriology and found that nitrate reduction test showed positive results for both the isolated spp. From potato tuber and infected plant but Vogus Proskauer's test shows positive result only to isolated species from potato tuber and negative results to the isolated spp from infected banana leaves. Sugar test; such as glucose, Xylose and Fructose showed positive results for both the isolated spp.

After identification of *Erwinia* the inhibition of this bacterium was studied by the action of streptomycin as it is broad spectrum antibiotic, previous results shows sensitiveness to the streptomycin for some strains and even resistant to streptomycin. But here, sensitiveness of streptomycin to the *Erwinia* was obtained. Antimicrobial activity of isolated *Erwinia* species was checked against antibiotic streptomycin and it was found there was growth of the bacteria *Erwinia* at concentration 50mg and 100mg for both the isolated spp and best results were obtained at concentration 150mg and 200mg. Hence, *Erwinia* species was sensitive to streptomycin. After getting successful results; Tomato seeds were treated with *Erwinia* as the symptoms obtained were similar with previous result obtained in 2007 [8]. Successful infection results were obtained and then treated with streptomycin and it was observed that plant was cured.

REFERENCES

- 1) A.A. Hajhamed; Wafaa M. Abd El- Sayed; A. Abou El-Yazied and N.Y. Abd El- Ghaffar; Suppression of bacterial soft rot disease of potato
- 2) A.R. Chase on *Erwinia* Blight of Foliage plants indoors.
- 3) Alpheus Kgabo Matlala on Detection of *Erwinia* spp on potatoes.
- 4) Bergye's manual of Systematic Bacteriology
- 5) Data sheets on Quarantine Pests *Erwinia chrysantheni* prepared by CABI and EPPO for the EU under contract 90/309003
- 6) Fernandez-Borrero, O.; Lopez-Duque, S. (1970) [Soft rot of the pseudostem of banana (*Musa paradisiaca*) caused by *Erwinia paradisiaca* n. sp.]. *Cenicafe* 1, 3-44.
- 7) Fiori, M. and Schinaffino, A. 2004. Bacterial stem rot in green house pepper (*Capsicum annuum* L.) in sardinia (Italy); Occurance of *Erwinia carotovora* subsp. *carotovora*. *J. phytopathology* 152: 28-23
- 8) Hibar, k., Daami-Remadi, M., and El Mahjoub, M in 2007; First report of *Pectobacterium carotovorum* subsp. *Carotovorum* on Tomato plants in Tunisia.

- 9) M.C.M Perombelon and J.M. VAN DER WOLF on methods for the detection and quantification of *Erwinia carotovora* on potato.
- 10) M.L. Powelson and J.D. Apple 1983. Soil and Seed Tubers as Sources of Inoculum of *Erwinia carotovora* pv. *Carotovora* for Stem Soft Rot of Potatoes
- 11) Maher e., kelman, a; and de boer,s ;h.1981. Infestation of potato tubers by *erwinia carotovora* from soil
- 12) Margaret Barth, Thomas R. Hankinson, Hong Shuang, and Frederick Brridt; Microbiological Spoilage of fruits and vegetables
- 13) Momol M.T., Aldwinckle H.S., 2000. Geneticdiversity and host range of *Erwinia amylovora*.In: Vanneste J.L, (ed.). Fire Blight, The Diseaseand its Causative Agent *Erwinia amylovora*, pp.55-72. CABI Publishing, Wallingford, UK.
- 14) Perombilon, m.c.m. 1979. Factors affecting the accuracy of tuber incubation test for the detection of contamination of potato stacks by *Erwinia carotovora*
- 15) Schatz a., bugie e., Waksman s.a., 1944.streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proceeding of the society for experimental biology and medicine 55: 66-69.
- 16) Vanneste J.L., 2000. What is fire blight? Who is *Erwinia amylovora*? How to control it? In: Vanneste J.L.(ed.). Fire Blight, The Disease and its Causative Agent *Erwinia amylovora*. pp. 1-5.CABI Publishing, Wallingford, UK.
- 17) Vijay J. Pachpute and Pallawi T. kininge 2012. Effect of copper sulphate and Streptomycin of isolated strains of *Pectobacterium* spp. from banana plants

