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Isolation and Identification of *Xanthomonas campestris* from Cruciferous plants

[1]Anubha Sharma, [2]Anshul Thakur, [3]Shreya Jain
[1]Research Intern, [2]Senior Research Associate, [3]CEO & Principal Investigator
[1 2 3]Department of Microbiology
[1 2 3]Acentric Biotech & Research Laboratory, Mohali, India

Abstract: The genus *Xanthomonas* is amongst the most common families of plant which are associated with bacterial diseases. The gram-negative and phytopathogenic bacteria, *Xanthomonas campestris* causes black rot in all cruciferous and brassicas plants such as *Brassica oleracea var. botrytis*, *Brassica oleracea var. capitata* or *Raphanus sativus* and is recognized as one of the most devastating cruciferous diseases globally. This bacterium's seed-borne stage is a key mechanism of viability and long-distance transmission. The bacterial cells cause infection on leaf edges of the plants via stomata, resulting in circular lesions or via hydathodes, resulting in V-shaped lesions. *Xanthomonas campestris* is used as a xanthan gum producer in large-scale industrial procedures. In this study, a selective media was prepared for isolation of bacteria, gram staining along with different biochemical tests were performed for identification purposes.

IndexTerms: ***Xanthomonas, Xanthomonas campestris*, xanthan gum, PSPA media**

I. INTRODUCTION

Xanthomonas:

Xanthomonas is a genus of Proteobacteria which s gram-negative, aerobic and rod-shaped belonging to the gamma division that is predominantly made up of plant diseases of significant economic value[1]. *Xanthomonas* species usually infect a variety of plants, causing diseases such as spots on fruit and leaves, blights on leaves, and vascular wilts. Even though the xanthomonads have a broad host range, particular strains are usually limited to certain host plants[2]. Many commercially significant crops are infected by members of the genus *Xanthomonas*. Few pathogens damaging main food crops in third-world locations are *Xanthomonas axonopodis* on cassava, *Xanthomonas oryzae* on rice, *Xanthomonas campestris*, which results in black rot of crucifers and, bacterial spots which is caused on tomato and pepper are caused by *Xanthomonas vesicatoria*[3]. Xanthomonas species are commonly propagated by diseased material of plants like cuttings, seeds, including mechanical ways like pruning shears that are contaminated. Irrigation water and rain splashes are the most common ways for bacteria to spread to developing plant tissues.

Xanthomonas campestris:

The cells of this species of bacteria are identified as straight, short rods that are not vibrioid. The most common cruciferous crop disease is known as black rot whose causative agent is *Xanthomonas campestris pv. campestris*[4]. *Brassica oleracea var. capitata, Lactuca sativa, Raphanus sativus, Brassica jucea,* and *Brassica oleracea var. botrytis* are few among the crucifers that are vulnerable. Black rot is amongst the most devastating crucifer vascular disease, and it is found across the temperate and subtropical regions of the world where normal temperatures range between 24 and 30 degrees Celsius[5]. These pathogens can live in soil for around 6 weeks and in debris of plants up to two years in temperate areas. Plant debris and soil are the only key inoculum agents in the tropical areas, where consecutive harvests of crucifers are cultivated without proper rotation crop[2]. Presence of blackening of vascular tissues and yellow V-shaped lesions can be seen on edges of the host leaves as the symptoms. The industrial bacterium responsible for a biopolymer known as xanthan gum synthesis is *Xanthomonas campestris*. This biopolymer is commonly utilized as a thickening in industrial applications because of its good aqueous rheological characteristics[6].As a thickening agent in many domains of utilization, xanthan gum is a significant factor in biotechnological investigations using *Xanthomonas campestris pv. campestris*. The primary goals in this paper were to isolate and identify strains of *Xanthomonas campestris pv. campestris* which were producing black-rot symptoms in cruciferous plants.

Figure 2: Samples of *Lactuca sativa*

Xanthan gum:
Xanthan gum is a natural polysaccharide that is non-toxic and an essential biopolymer which is widely used in pharmaceutical industries. It is widely used in cosmetics and in toiletries as it is non-sensitive and does not irritate the skin or the eyes. Particularly in the food sector, xanthan gum is manufactured on a wide scale as an emulsifying, thickening, stabilizing and gelling ingredient. It is used in various sectors such as bakery, dairy, pet food, oil industry, agriculture, cleaners and many more[7].

II. MATERIALS AND METHODS

Isolation of Xanthomonas campestris from leaves of cruciferous vegetables:
Leaves of cruciferous vegetables such as *Lactuca sativa*, and *Brassica oleracea var. capitata* having V-shaped black and yellow lesions were collected from local vegetable markets of Sector 15 and Sector 26 in Chandigarh, India. Collected samples were washed in the washing area and small pieces of infected leaves was done; leaves were surface sterilized for one minute with 70% ethyl alcohol and then pieces were washed three times with sterile water. On a sterilized glass slide, the infected leaf pieces were crushed to get bacterial suspension. This resulting suspension was then spreaded onto a Potato Sucrose Peptone Agar (PSPA) plate, pH was maintained at (7.0 +-0.2) and incubated for 48 hours at room temperature and examined. Selective isolated colonies were identified by the presence of yellow, mucoid, sliminess and were streaked on Potato Sucrose Peptone Agar which was incubated for 48 hours at room temperature for maintenance and further characterization.

Identification of bacteria:
In bacteriology, Gram staining is a significant differential stain which was discovered by Christian Gram who was a Danish Bacteriologist. Gram staining is essential for phenotypic characterization of microorganisms. The staining process distinguishes bacterial species based on their structure of cell wall. Gram-positive bacteria are distinguished by a thick peptidoglycan coating that stains blue and Gram-negative bacteria are distinguished by a thin peptidoglycan coating that stains pink. After the incubation period was up, strains of bacteria were put onto the sterilized glass slide and stained. Bacterial culture was examined under the microscope and it appeared red colour, very small rod-shaped bacteria that means Gram negative bacteria.

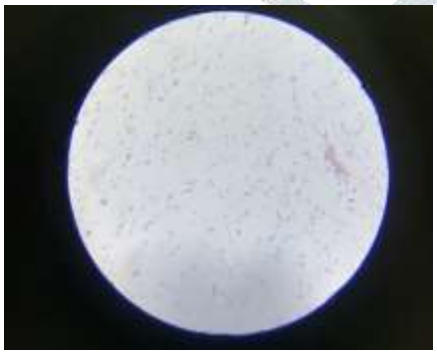

Figure 3: Small rod, Gram negative

Identification of bacteria by Biochemical Tests:
Bacterial identification is primarily dependent on distinctions in their biochemical activities. It is done to minimise the time it takes to identify microorganisms, cut expenditures, and assure or improve the accuracy of identifying the unknown bacteria Several biochemical tests were carried out in order to identify the *Xanthomonas campestris* bacterium. These tests were carried out on bacteria that could not be distinguished based on their morphological characteristics.

Biochemical Tests	*Xanthomonas campestris*
Catalase Test	Positive
MR Test	Negative
VP Test	Negative
Urease Test	Positive
Citrate Test	Negative
Indole Test	Negative
Starch Agar Test	Negative
Nitrate Reduction Test	Negative
Mannitol Hydrolysis Test	Negative

Table 1: Results of Biochemical Tests

III. RESULTS

Following the bacterial isolation procedure, the bacteria was isolated from the leaves of cruciferous vegetables and Gram staining and biochemical tests were further carried out, the bacteria was identified as Xanthomonas campestris on PSPA media.

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