

## Original Research Article

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## Studies on Bacterial Canker (*Clavibacter michiganensis* subsp. *michiganensis*) of Tomato (*Solanum lycopersicum*)

Gurvinder Singh\* and Narender K. Bharat

Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry,  
Nauni, Solan (HP)-173 230, India

\*Corresponding author

### ABSTRACT

#### Keywords

*Clavibacter michiganensis* subsp. *michiganensis*, Himachal Pradesh, Tomato, Nutrient glucose broth.

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Bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) causes significant yield losses on tomatoes. It is newly observed bacterial disease in mid hills of Himachal Pradesh, India. Few or less work has, therefore, been done on this disease in the state. This study was conducted to find optimum growth conditions for the bacterium. Under present investigations, five isolates of *Cmm* were thus isolated and maintained in pure cultures. Amongst different nutrient media tested, nutrient glucose broth medium favoured maximum growth of the bacterium. A temperature of 25<sup>0</sup> C and pH from neutral to slightly alkaline was found favourable for the growth of the pathogen.

### Introduction

*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*, is the causal agent of bacterial canker of tomato (*Solanum lycopersicum* (Mill.)). The disease was first described in 1910 in Michigan, USA (Smith, 1910). The disease is widespread and destructive in the main tomato-growing areas, including the USA, Europe, Australia, New Zealand, Africa, Asia and South America. It was found to infect *Lycopersicon* and *Capsicum* species. Sweet pepper (*Capsicum annum*) as well as *Solanum mammosum*, *S. douglassi*, *S. nigrum* and *Nicotiana glutinosa* are also natural hosts. Furthermore a number of other solanaceous hosts are susceptible on

artificial inoculation (Thyr *et al.*, 1975, Fahy and Persely, 1983, Smith *et al.*, 1988).

Bacterial canker is one of the most important bacterial diseases of tomato which become very destructive, if weather conditions are favourable. The disease is devastating due to the systemic movement of bacteria in the vascular tissue (Pine *et al.*, 1955). Yield losses can be severe due to bacterial canker. Estimates of yield loss range from 10% annual regional losses (Hibberd *et al.*, 1992) to 84% for individual growers (Gleason *et al.*, 1993; Strider, 1969). Yield losses attributable to bacterial canker in controlled studies range

from 11 to 99% (Chang *et al.*, 1992; Emmatty and John, 1973; Ricker and Reidel, 1993). As a result of severe yield and economic losses, *C. michiganensis* subsp. *michiganensis* is a quarantine organism in the European Union and in many other countries (de Leon *et al.*, 2008).

In India, the disease was first recorded during 1949 from Nagpur (Jain, 1951). The bacterial canker pathogen (*C. michiganensis* subsp. *michiganensis*) was reported to occur in Karnataka state with an incidence up to 48 per cent (Umesha, 2006). During recent years bacterial canker has also been observed in tomato growing localities in Solan and Sirmour districts of Himachal Pradesh, India (Singh *et al.*, 2015). It is a major disease of out-door tomato as well as under greenhouse conditions and can be very difficult to control under protected cropping.

The present investigations were conducted to isolate the bacterial canker in pure culture, to find out effective nutrient medium, temperature range and pH level for its isolation, growth and other studies.

### **Materials and Methods**

The present investigations were carried out in The Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) during 2012-13. The materials and methods used during the studies are explained here under:

#### **Isolation, Purification and Maintenance of Pathogen Culture**

Field visits were undertaken to the tomato growing localities in Solan and Sirmour districts of Himachal Pradesh. Leaf, fruit and stem samples of tomato plants showing characteristic symptoms of Bacterial canker disease (Fig. 1) were brought to the laboratory

for isolation of the pathogen. Small bits of the diseased tissues taken from affected leaves and fruits along the margin of lesions were cut with the help of sterilized scalpel. The bits were sterilized by dipping in 1 per cent mercuric chloride solution (4% Clorax) for few seconds, washed thrice in sterile water and placed in sterile water drops on a flamed glass slide under aseptic conditions. In order to obtain bacterial ooze, incision was given in the centre of each bit with a sterile blade. Another set of these slides were also examined under the microscope for the presence of bacterial cells. A loopful of suspension was streaked on sterilized nutrient agar plates under aseptic conditions. These Petri plates were incubated at  $25\pm 2^{\circ}\text{C}$  for 48 h and observed for colony formation of the pathogen. Circular, creamy, and creamy to yellowish colony of the bacterium were picked from the Petri plate and Gram staining was performed. The colonies which were Gram +ve were selected. The identification of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) was done on the basis of morphological, physiological and biochemical characters as suggested in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994); Guide to Plant Pathogenic Bacteria (Bradbury, 1986) and Laboratory Guide for Identification of Plant Pathogenic Bacteria (Schaad *et al.*, 2001). Five different isolates of *Cmm* namely, *Cmm1*, *Cmm2*, *Cmm3*, *Cmm4* and *Cmm5* were isolated from different localities in Solan and Sirmour districts. These *Cmm* isolates were purified by streak plate method and maintained on NA at  $4^{\circ}\text{C}$  for further studies.

#### **Effect of different liquid media on the growth of different isolates of bacterial canker pathogen**

Six different nutrient media namely, Nutrient broth (NB), Nutrient glucose broth (NGB),

Nutrient yeast extract broth (NYEB), Yeast extract nutrient broth (YENB), Yeast extract sucrose peptone broth (YESPB), and Yeast extract dextrose calcium carbonate broth (YEDCCB) were used. Flasks containing 50 ml of different media were sterilized in autoclave at 15 lbs. /inch<sup>2</sup> for 20 minutes. For each broth medium five different replications were taken and were inoculated with the bacterium. One loopful of the bacterial culture was inoculated in each of the flasks replication wise and incubated at the most effective temperature for the growth of bacterium in BOD incubator cum orbital shaker at 140 rpm for 48 hours. After 2 days of incubation the bacterial growth was seen in the media and the optical density (OD) of the culture was calculated by using Spectrophotometer at 540 nm.

#### **Effect of different temperature regimes on the growth of different isolates of bacterial canker pathogen**

To study the effect of different temperature regimes viz., 10, 15, 20, 25, 30, 35 and 40°C on growth of the bacterium, 150 ml 'Erlenmeyer' flasks, each containing 50 ml of sterilized NB (pH 7.0) were autoclaved. After that inoculated with a loopful of 48 hours old bacterial culture inside a laminar air flow chamber and incubated at different temperature regimes for 48 hours along with uninoculated flasks containing nutrient broth and supplemented with 1.5 ml of sterile distilled water to serve as check. Each treatment was replicated thrice. The OD value of each replication was observed by using Spectrophotometer at 540 nm.

#### **Effect of different pH Levels on the growth of different isolates of bacterial canker pathogen**

The effect of different pH levels viz., 4, 5, 6, 7 and 8 was observed on growth of the

bacterium under laboratory condition. 150 ml 'Erlenmeyer' flasks, each containing 50 ml of NB were subjected to adjustment of desired pH levels of the medium by adding N/10 HCl or N/10 NaOH. The desired pH levels in acidic and alkaline ranges, respectively and recorded with the help of digital pH meter. After sterilization at 15 lbs. /Inc<sup>2</sup> pressure for 20 minutes, each of the flasks were inoculated with a loopful of 48 hours old bacterial culture and incubated at 25<sup>0</sup> C along with the uninoculated flasks supplemented with equal volume of sterile distilled water in place of inoculum, to serve as check. After 2 days of incubation the growth of bacterial culture appeared in broth. The OD value of each replication was calculated by using Spectrophotometer at 540 nm.

#### **Results and Discussion**

##### **Effect of different liquid media on the growth of different isolates of bacterial canker pathogen**

From the data (Table 1) it is evident that highest growth in terms of optical density (1.86) was recorded in nutrient glucose broth medium followed by Yeast extract dextrose calcium carbonate broth medium (1.85) and yeast extract sucrose peptone broth medium (1.82). Lowest optical density was recorded in nutrient broth medium. Amongst various isolates, *Cmm3* and *Cmm4* exhibited maximum OD value of 1.90 and 1.92 in yeast extract dextrose calcium carbonate broth medium. The growth of bacterial canker pathogen isolates was better in nutrient glucose broth medium followed by yeast extract dextrose calcium carbonate broth medium. Nutrient glucose broth medium has been found better under present investigations. Smith *et al.*, (1988) also reported that optimal growth is achieved in nutrient glucose agar where colonies are 1 mm in diameter after 3-5days and 2-3 mm

after 7–8 days in culture. Various workers have evaluated different culture media for isolation of bacterial canker pathogen and have used nutrient glucose yeast extract

medium (NGYEM) for storing various isolates of the pathogen (Ftayeh *et al.*, 2011). However, they modified the medium later on for better growth of the bacterium.

**Fig.1** Characteristic symptoms of Bacterial canker disease of tomato on a) leaves, b) fruit, c) stem, and d) dead tomato plants due to bacterial canker



a.



b.



c.



d.

**Table.1** Effect of different liquid media on growth (optical density) of various isolates of bacterial canker pathogen of tomato

Nutrient medium	Optical density of isolates of bacterial pathogen					Mean
	<i>Cmm1</i>	<i>Cmm2</i>	<i>Cmm3</i>	<i>Cmm4</i>	<i>Cmm5</i>	
Nutrient broth medium	1.71	1.73	1.73	1.76	1.70	1.73
Yeast extract nutrient broth medium	1.75	1.79	1.88	1.73	1.69	1.77
Nutrient glucose broth medium	1.85	1.82	1.87	1.89	1.86	1.86
Nutrient yeast extract broth medium	1.79	1.80	1.82	1.75	1.70	1.77
Yeast extract sucrose peptone broth medium	1.87	1.88	1.80	1.85	1.69	1.82
Yeast extract dextrose calcium carbonate broth medium	1.85	1.80	1.90	1.92	1.79	1.85
<b>Mean</b>	1.80	1.81	1.83	1.82	1.74	

C.D. (0.05); Isolates = 0.01; Media = 0.01; Isolate × Media = 0.02

**Table.2** Effect of different temperature regimes on the growth (optical density) of various isolates of bacterial canker pathogen of tomato

Temperature (°C)	Optical density of isolates of bacterial pathogen					Mean
	<i>Cmm1</i>	<i>Cmm2</i>	<i>Cmm3</i>	<i>Cmm4</i>	<i>Cmm5</i>	
<b>10</b>	0.52	0.48	0.96	0.99	0.42	0.67
<b>15</b>	1.18	1.19	1.10	1.03	0.72	1.04
<b>20</b>	1.25	1.26	1.29	1.23	1.24	1.25
<b>25</b>	1.39	1.44	1.59	1.47	1.46	1.47
<b>30</b>	1.24	1.40	1.52	1.39	1.32	1.37
<b>35</b>	0.79	1.21	0.88	0.92	0.94	0.95
<b>40</b>	0.26	0.87	0.53	0.63	0.66	0.59
<b>Mean Isolates</b>	0.95	1.12	1.12	1.09	0.97	

C.D. (0.05); Isolates = 0.01; Temperature = 0.01; Isolate × Temperature = 0.03

**Table.3** Effect of different pH levels on the growth (optical density) of various isolates of bacterial canker pathogen of tomato

pH Level	Optical density of isolates of bacterial pathogen					Mean
	<i>Cmm1</i>	<i>Cmm2</i>	<i>Cmm3</i>	<i>Cmm4</i>	<i>Cmm5</i>	
<b>4</b>	0.07	0.10	0.07	0.07	0.05	0.07
<b>5</b>	0.90	0.93	0.92	1.07	1.19	1.00
<b>6</b>	1.30	1.28	1.26	1.30	1.32	1.29
<b>7</b>	1.30	1.36	1.44	1.30	1.39	1.36
<b>8</b>	1.28	1.43	1.50	1.33	1.29	1.36
<b>Mean</b>	0.97	1.02	1.04	1.01	1.05	

C.D. (0.05); Isolates = 0.01; pH level = 0.01; Isolate × pH level = 0.02

**Effect of different temperature regimes on the growth of different isolates of bacterial canker pathogen**

The effect of different temperature ranges on the growth w.r.t. the optical density of the culture of bacterial canker pathogen was observed significant (Table 2). Amongst

different temperature regimes tested, maximum growth of bacterial isolates was observed at 25<sup>0</sup>C. The bacterial growth on the temperatures below and above 25<sup>0</sup>C declined which suggested that 25<sup>0</sup> C is the optimum temperature for the growth of bacterial canker pathogen. Eichenlaub *et al.*, (2006) observed that *Clavibacter michiganensis* subsp.

*michiganensis* grows optimally at 25°C. The optimum temperature range for bacterial canker of tomato has also been observed between 25- 30°C (Anon., 2013; Sen *et al.*, 2015).

### **Effect of different pH levels on the growth of different isolates of bacterial canker pathogen**

It is evident from the data in table 3 that the bacterial canker pathogen grew to a variable extent at all the pH levels tested. Amongst the various pH levels, maximum growth w.r.t. optical density was recorded at pH 8.0 followed by pH 7.0. The growth at pH 6.0 and 5.0 were next best in the order and did not differ significantly with each other. However, the growth w.r.t. OD value at pH 4.0 was minimum differing significantly from rest of the pH levels. Therefore, neutral to slightly alkaline pH proved better for growth of bacterial canker pathogen. Huang and Tu (1999) have observed neutral to slightly high pH to be better for growth of bacterial canker pathogen of tomato. Eichenlaub *et al.*, (2006) observed that optimum pH for growth of this bacterium is between 7 and 8. Hence, the results of present investigation corroborate the findings of such earlier studies.

It can be concluded that the Bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*) pathogen grew better on nutrient glucose broth medium at temperature range of 25-30°C with a pH level between neutral to slightly alkaline.

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