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Morphological, physiological and biochemical characteristics of *Xanthomonas campestris* pv. *campestris* by black rot of cabbage

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ABSTRACT

Black rot, the most serious disease of crucifers especially *Brassica oleracea* var. *capitata* (Cabbage) causes huge yield losses. Black rot is a systemic vascular disease. Typical disease symptoms are V-shaped yellow lesions starting from the leaf margins and blackening of veins. The cabbage leaves were tested for *Xanthomonas campestris* pv. *campestris* infection through morphological, physiological and biochemical analysis. The aim of the study was to identify and confirmation of *X. campestris* pv. *campestris* in cabbage. The assay of detected *X. campestris pv. campestris* infections in diseased plants and from bacterial colonies isolated on selective media, and was more sensitive and specific. Among the ten isolates, isolate I_6 produced the maximum growth, lesion size and positive growth in different organic and inorganic sources, different biochemical and morphological analysis.

Key words: Black rot of cabbage, *Xcc*, gram staining, morphological and biochemical analysis

Plant diseases are major constraints in commercial agriculture and pose huge loss to both conventional and organic farming systems. Plant diseases are hard to identify because they are very small. The positive identification of a pathogen often requires specialized equipment, training, and in some cases precise diagnosis in the field is difficult (Nostro *et al*., 2000). Plant pathogens are constantly changing and mutating, resulting in new strains and new challenges to growers (Steven *et al*., 2000). Black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*Xcc*) was one of the wide spread disease of crucifers in many parts of the world (Pammel, 1895). The bacteria most frequently invade the host plant through water pores (hydathodes) at the leaf margins (Babadoost, 1999). *Xcc* symptoms of cabbage leaves first appear as yellow 'V' shaped areas with the open part of the 'V' along edges of the leaf. The diseased areas then become brown and brittle, and the affected leaf veins turn dark brown to black (Seebold *et al.,* 2008). The bacteria cells are gram negative, short rods with rounded ends, with dimensions of 1.17 to 2.07 x 0.54 to 0.99µm, non capsulated, occurring singly, rarely in pairs, and motile with single polar flagellum. The bacterial (*Xcc*) colonies were round with entire margins, slightly raised, shiny, butyrous in consistency and light yellow in colour on nutrient dextrose agar medium (Pammel, 1895). Bhide (1948) also found that on nutrient dextrose agar plates the bacterium produced round colonies with entire margins and those seven day old colonies ranged in diameter from 10 to 12 mm. On agar or gelatin the bacterium produced smooth, moist, shining, round and flat to convex colonies with entire margins. The bacterium grew moderately on nutrient media and in broth showed clouding and a yellow ring without pellicle. The optimum temperature for growth of the organism was from 77° to 86°F (25° to 30°C), the minimum was 41°F (5°C), and the maximum was 96°F (35°C) (Babadoost, 1999). Sharma *et al.,* (2002) studied that the bacterial isolates obtained from seeds showed characteristic morphological, physiological and a positive hypersensitive reaction and thus was identifies to be *X.campestris.* Sain and Gour (2008) revealed that the bacterium produced straw yellow,

smooth, glistening colonies with entire margin and convex elevation on NA medium. The bacterium showed growth between pH 5.0 to 8.0 and 10 to 37°C. Sain *et al.,* (2008) observed some isolates produced smooth, light yellow to coloured colonies while some other produced straw yellow coloured colony with flat to raised configuration on NA medium. In addition the bacterium liquefied gelatin and utilized even plain gelatin; hydrolysed starch and produced hydrogen sulphide in nutrient broth. Also observed that two isolates were produced indole weakly, proteolysed the milk but failed to reduce nitrate in nitrate medium, utilised sodium citrate in Koser's citrate medium, but failed to grow on synthetic asparagine medium, failed to produce gas and acid from any of the carbon compounds grown on inorganic salt medium, utilized inorganic nitrogen, negative to Voge's- Proskauer and Methyl-Red tests and utilized cysteine, lysine, glutamic acid and aspartic acid as sources of nitrogen (Bhide, 1948). Likewise, Chand *et al.,* (1995) as well as Sain and Gour (2001) reported that the liquefied starch, gelatin and casein produced H_2S in tryptone sucrose iron agar media. Media containing sucrose or glucose as the carbon source and various organic nitrogen sources supported optimal *X. campestris* growth and cell yield (Jackson *et al.,* 1998). Rajkumar (2006) reported that *E. carotovora* sub sp. *carotovora* liquefied gelatin, casein and produced H_2S with methyl red, nitrate reductase tests positive and starch hydrolysis negative. Sain and Gour (2008) revealed that the bacteria liquefied starch and casein produced H_2S in tryptone sucrose agar media. The bacterium did not utilize the carbohydrate like arabinose, cellobiose, fructose, galactose, sorbitol, mannose and xylose but derived energy and produced acid from sucrose, trehalose, dextrose, mannitol and poorly from salicin. The study of diseases in plants is an important feature of assessing plant health. A significant problem in the study of bacterial pathogens is the proper identification of the infectious agent. Hence the present study deals with the morphological, physiological identification of *Xanthomonas campestris pv. campestris* from the infected plant of cabbage.

MATERIALS AND METHODS

Collection of isolates of *Xanthomonas campestris* pv. *campestris* (*Xcc*)

The black rot disease infected cabbage leaves were collected different cabbage growing regions of Tamil Nadu. The leaf bits surface sterilized with 0.1 per cent mercuric chloride and washed in sterile distilled water. These leaf bits were submerged in test tubes each containing 3 ml of sterile distilled water and incubated for 6 h at room temperature (28±2°C) and 10 ml of the diluted bacterial suspension was poured onto nutrient agar plates. After 48 h of incubation, yellowish bacterial growth was appeared on ten isolates and further sub cultured on nutrient agar slants and purified by the dilution plate technique (Waksman, 1952). The morphological characteristics such as cell shape, gram reaction, capsule and spore staining characters of the isolate was studied as described by Aneja, (1993).

Growth in nutrient broth

One ml of the bacterial suspension prepared by using sterile distilled water from the 24 h old actively growing culture of the ten different isolates of the bacterium were added into sterilized conical flasks each containing 50 ml of sterilized nutrient broth and incubated for 72 h at room temperature (28 ± 2°C). The growth was measured in terms of optical density (OD) in Hitachi Spectrophotometer at 565 nm wavelength against nutrient broth blank. Three replications were maintained for each isolate.

Growth in different pH levels

The pH of peptone broth in separate sets of test tubes was adjusted in a pH meter before sterilization using N/10 sodium hydroxide to different levels *viz.,* 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 6.8, 7.0, 7.5, 8.0, 8.5 and 9.0. One ml of the bacterial suspension of each isolate was added into sterilized conical flasks each containing 50 ml of sterilized nutrient broth and incubated for 72 h at room temperature and each isolates were examined. The relative growth was indicated by the extent of turbidity and was measured in terms of OD in a spectrophotometer at 565 nm wave length

Biochemical properties of *Xcc* isolates

The method described by Dowson (1957) was followed for the following tests conducted *viz.,* utilization of carbon compounds, utilization of nitrogen sources, indole production test, Voges proskauer test and methyl red test, casein hydrolysis, starch hydrolysis, H_2S production, gelatin hydrolysis and ammonia production. The 24 h old actively growing slant cultures of each isolate were used in all the experiments.

Utilization of carbon compounds

Seven different carbon sources *viz.,* D-glucose, D-galactose, D-fructose, lactose, sucrose, starch and mannitol were incorporated each at one per cent and starch at 0.1 per cent level into the basal medium (1g ammonium dihydrogen phosphate, 0.2 g potassium chloride, 0.2 g magnesium sulphate in 1000 ml of sterile distilled water and the pH was adjusted to 7.0). Eight ml of the basal medium was added into each test tube and tubes were inoculated with one ml of the inoculum of each bacterial isolates separately and incubated for seven days at room temperature (28±2°C) and examined for growth and production of acid. Bromothymol blue was used as an indicator for acid production (greenish blue at pH 6.2). The uninoculated tubes were served as control.

Inorganic nitrogen compounds

Six nitrogen sources *viz.,* ammonium sulphate, ammonium nitrate, ammonium dihydrogen phosphate, calcium nitrate, potassium nitrate and sodium nitrate were incorporated each at 0.1 percent level into the basal medium (consisting of 10 g glucose, 2 g KH_2PO_4, 0.2 g $MgSO_4$ and 0.2 g Kcl in 1000 ml of sterile distilled water). The pH was adjusted to 7.0 and the medium was distributed at 8 ml per test tube and sterilized. Then, 1ml inoculum of each isolate was inoculated into each test tube and incubated at room temperature 28±2°C for seven days and observed for growth. The relative growth was assessed by observing the intensity of turbidity as observed in terms of OD. The uninoculated tubes were served as control.

Indole production

The *Xcc* isolates were grown separately in test tubes containing tryptophane broth (10 g tryptophane and 3 g yeast extract in 1000 ml of sterile distilled water) @ 5 ml / test tube and incubated for 72 h at room temperature (28 ± 2°C). Indole production was tested by inserting into the test tube in such a way that the filter paper strip was pendant just above the medium without touching the medium. Development of pink colour in paper strip indicated positive result (Aneja, 1993).

Starch hydrolysis

Ten ml of peptone sucrose agar medium which already incorporated with 0.2 per cent starch was poured into each sterile petriplate and allowed for solidification. Then each isolates was streaked on this medium separately and incubated for five days. Lugol's iodine solution was added in drops into each petriplate to test the hydrolysis of starch. The development of clear zone around the growth of each isolate in petriplates indicated positive result (Aneja, 1993).

Gelatin liquefication

Gelatin medium (3 g yeast extract, 5 g peptone and 120 g gelatin in 1000 ml of sterile distilled water) was prepared, sterilized and set as cylinder in each test tube. It was vertically stabbed by means of platinum wire dipped in the bacterial suspension as far as the wire would go, incubated for 3-7 days and examined (Aneja, 1993).

H_2S production

Each test tube containing 10 ml of sterilized peptone water was inoculated with a loopful culture of each isolate of *Xcc* under aseptic condition. Then, the filter paper strips pre-soaked in saturated lead acetate solution were inserted in such a way that the filter paper strips were pendant in the tubes just above the medium and examined 72 h after incubation. The papers turned black, if H_2S was produced (Aneja, 1993).

Casein hydrolysis

Test medium containing skimmed milk powder 100 g, peptone 5 g, agar 15 g and pH 7.2 was prepared and equally distributed @ 200 ml into each 250 ml conical flasks and sterilized. Make a single line streak inoculation from each culture into its labeled petriplate across the surface of the medium. A control was maintained without streaking of any isolate and the results were recorded (Aneja, 1993).

Ammonia production

The test tubes containing sterilized peptone water @ 10 ml/test tube were inoculated with a loopful culture of each isolate separately and incubated for 72 h at room temperature. The production of ammonia in each test tube was tested separately by using Nessler's reagent. The formation of a reddish brown precipitate in the test tubes was indication of the positive result (Aneja, 1993).

Voge's proskauer (V.P) test and methyl red (M.R) test

Fumarate medium (5 g sodium chloride, 0.20 g magnesium sulphate, 1 g ammonium dihydrogen phosphate, 1 g dipotassium dihydrogen phosphate, 2.77 g sodium citrate, 5 g glucose, 10 g sodium fumarate in 1000 ml of sterile distilled water) was taken in test tubes (6"X 3/4 ") @ 5 ml /tube, sterilized and inoculated with a loopful culture of each *Xcc* isolates separately and incubated for 48 h until a turbid growth was formed. A pinch of creatine was added into each test tube followed by one ml of 40 per cent KOH. The test tubes were vigorously shaken, kept for 15 min and examined for development of pink colour for V.P test (positive). For M.R test 2-3 drops of methyl red (0.1 g methyl red dissolved in 300 ml of 95 per cent ethanol and made up to 500 ml with sterile distilled water) were added into each test tubes containing glucose fumarate broth cultures previously incubated at room temperature for 48 h. The development of red colour indicated positive result (Aneja, 1993).

RESULTS AND DISCUSSION

The study of plant pathogens are an important feature of assessing plant health. A significant problem in the study of bacterial pathogens of the plants is the proper identification of the infectious agent. But the isolation and identification of plant disease causative organisms was successfully done by the plant researchers. Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (Mandavia *et al*., 1999). *Xanthomonas* is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. Cabbage leaves showing the typical black rot disease symptoms were collected from different cabbage growing areas of Tamil Nadu with a view to identify the variability of the pathogen, if any. The pathogen was isolated from diseased specimen separately, purified and the isolates were maintained on Nutrient Agar (NA) medium at room temperature for further use.

Morphological and cultural characteristics of *Xcc* isolates

In the present investigation, all the ten isolates of *Xcc,* the inciting of cabbage black rot disease collected from different places of Tamil Nadu was identified as gram-negative, rod shaped bacteria and each having a single motile polar flagellum and brought into pure culture were similar in their morphological characteristics (gram negative rods) as described by Dye and Lelliott (1974). The colonies of all the ten isolates were circular form, convex in elevation, filiform in nutrient agar and opaque. The colony dia varied from 2.5 to 4.0 mm. The isolates I_6 and I_7 were produced biggest colonies of 4.0 and 3.8 mm respectively while I_{10} produced smallest (2.5 mm) colony. The colony dia of the I_1, I_2, I_3, I_4, I_5, I_8 and I_9 was 3.7, 3.5, 3.1, 3.6, 2.7, 2.9 and 3.2 mm, respectively. Likewise, Bhide (1948) reported that the size of seven day old colonies of *Xcc* ranged from 10 to 12 mm in dia. on nutrient agar and Sain *et al.,* (2008) observed that some isolates *Xcc* produced smooth, light yellow to coloured colonies while some other produced straw yellow coloured colony with flat to raised configuration on NA medium. The texture of the colony produced by the isolates I_1, I_3, I_7, I_8 and I_{10} was smooth whereas that of I_2, I_4, I_5, I_6 and I_9 was less smooth. The consistency of the colony of I_2, I_4, I_5, I_6 and I_9 was more slimy and less glistening while that of I_1, 1_3, I_7, I_8 and I_{10} was slimy and glistening. The colony margin was entire in the five isolates except I_1, I_4, I_5, I_6 and I_{10} where it was lobate. The colony colour of I_5, I_6 and I_9 was deep yellow and that of I_2, I_3, I_4 and I_8 was yellow and it was pale yellow in I_1, I_7 and 1_{10}. The isolates I_2, I_3, I_7, I_9 and I_{10} had profuse growth while I_1, I_4, I_5, I_6 and I_8 had moderate growth (Table 1). Such variations in the cultural characteristic of *Xcc* isolates were also described by Bhide (1948). Verma (1986) reported that the variation in the colour of colony of *Xanthomonas campestris pv. malvacearum* isolates positively correlated with virulence. He described that the deep in colour of the colony (deep yellow) indicate the most virulent isolate whereas pale yellow colour indicate the least virulent isolate.

Growth of *Xcc* isolates at different pH levels

The growth of isolate I_6 at different pH levels recorded the maximum mean growth of 0.40 OD value at 565 nm while I_{10} recorded the minimum mean growth (0.22). The isolates I_3 and I_5 were statistically on par with each other. The other six isolates *viz.,* I_1, I_2, I_4, I_7, I_8 and I_9 were significantly different from each other in making growth at different pH levels. Among the different pH levels tested, all the isolates were grown well at pH 6.8. The pH levels 6.5 and 7.0 were ranked next

best in supporting maximum growth of all the isolates. All the ten isolates were failed to grow at pH levels of 4.0, 4.5, 8.5 and 9.0 (Table 2).

Table 1. Cultural characteristics of isolates of *Xanthomonas campestris* pv. *campestris* (*Xcc*)

Isolates	Texture	Margin	Consistency	Colour	Growth	Growth of *Xcc* in NA medium[2 DAI (mm)]*
I_1	Smooth	Lobate	Slimy, glistening	Pale yellow	Moderate	3.7
I_2	Less smooth	Entire	More slimy, less glistening	Yellow	Profuse	3.5
I_3	Smooth	Entire	Slimy, glistening	Yellow	Profuse	3.1
I_4	Less smooth	Lobate	More slimy, less glistening	Yellow	Moderate	3.6
I_5	Less smooth	Lobate	More slimy, less glistening	Deep yellow	Moderate	2.7
I_6	Less Smooth	Lobate	More slimy, less glistening	Deep yellow	Moderate	4.0
I_7	Smooth	Entire	Slimy, glistening	Pale yellow	Profuse	3.8
I_8	Smooth	Entire	Slimy, glistening	Yellow	Moderate	2.9
I_9	Less smooth	Entire	More slimy, less glistening	Deep yellow	Profuse	3.2
I_{10}	Smooth	Lobate	Slimy, glistening	Pale yellow	Profuse	2.5
CD (P=0.05)						**0.18**

*Mean of three replications; DAI-Days after inoculation

Table 2. Effect of different pH levels on the growth of isolates of *Xcc*

Isolates	OD value at 565 nm*												Mean (OD value)
	pH level												
	4.0	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**6.8**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**	
I_1	0.00	0.00	0.40	0.47	0.52	0.68	0.71	0.65	0.43	0.34	0.00	0.00	**0.35**
I_2	0.00	0.00	0.41	0.45	0.46	0.50	0.59	0.54	0.41	0.30	0.00	0.00	**0.31**
I_3	0.00	0.00	0.31	0.32	0.43	0.48	0.61	0.51	0.31	0.21	0.00	0.00	**0.27**
I_4	0.00	0.00	0.43	0.49	0.52	0.55	0.67	0.57	0.44	0.25	0.00	0.00	**0.33**
I_5	0.00	0.00	0.30	0.40	0.37	0.42	0.53	0.47	0.39	0.18	0.00	0.00	**0.26**
I_6	0.00	0.00	0.45	0.54	0.57	0.71	0.78	0.72	0.54	0.43	0.00	0.00	**0.40**
I_7	0.00	0.00	0.42	0.53	0.54	0.67	0.73	0.65	0.47	0.37	0.00	0.00	**0.37**
I_8	0.00	0.00	0.29	0.37	0.40	0.45	0.56	0.52	0.41	0.22	0.00	0.00	**0.27**
I_9	0.00	0.00	0.28	0.31	0.39	0.46	0.51	0.45	0.30	0.23	0.00	0.00	**0.24**
I_{10}	0.00	0.00	0.24	0.28	0.34	0.37	0.50	0.40	0.28	0.20	0.00	0.00	**0.22**
Mean	**0.00**	**0.00**	**0.35**	**0.42**	**0.45**	**0.53**	**0.62**	**0.55**	**0.40**	**0.27**	**0.00**	**0.00**	-

*Mean of three replications, **CD (P=0.05)**; **Isolates - 0.01; pH levels - 0.01; Isolates × pH levels - 0.02**

Table 3. Effect of carbon compounds on the growth of isolates of *Xcc*

Isolates	OD value at 565 nm*							Mean*
	D-Glucose	D-Galactose	D-Fructose	Lactose	Sucrose	Starch	Mannitol	
I_1	0.66	0.65	0.63	0.54	0.61	0.33	0.27	**0.53**
I_2	0.45	0.57	0.47	0.43	0.54	0.43	0.18	**0.44**
I_3	0.56	0.52	0.58	0.47	0.60	0.46	0.25	**0.49**
I_4	0.61	0.57	0.36	0.50	0.63	0.60	0.31	**0.51**
I_5	0.53	0.45	0.46	0.29	0.62	0.32	0.12	**0.40**
I_6	0.70	0.64	0.66	0.52	0.69	0.61	0.28	**0.59**
I_7	0.66	0.51	0.59	0.55	0.65	0.62	0.32	**0.56**
I_8	0.60	0.58	0.56	0.50	0.54	0.31	0.21	**0.47**
I_9	0.47	0.54	0.50	0.46	0.52	0.45	0.18	**0.45**
I_{10}	0.54	0.41	0.42	0.29	0.56	0.29	0.12	**0.38**
Mean*	**0.58**	**0.54**	**0.52**	**0.46**	**0.60**	**0.44**	**0.22**	-

*Mean of three replications; CD (P=0.05) ; Isolates - 0.01; Carbon compounds- 0.01; Isolates × Carbon compounds- 0.03

Table 4. Effect of carbon sources on acid production in the medium by the isolates of *Xcc*

Isolates	D-Glucose	D- Galactose	Lactose	Maltose	Sucrose	Succinic acid	Arabinose
I_1	S	S	M	S	M	S	M
I_2	S	M	S	M	S	S	S
I_3	M	M	M	M	S	M	S
I_4	S	S	M	S	M	S	M
I_5	S	M	S	M	M	N	S
I_6	M	S	S	M	S	N	M
I_7	S	M	M	S	M	M	S
I_8	S	M	S	S	S	S	S
I_9	M	M	S	M	S	S	M
I_{10}	S	S	M	S	S	M	S

S = Slight; M = Moderate; N = No production

Table 5. Effect of inorganic nitrogen sources on the growth of isolates of *Xcc*

Isolates	OD value at 565 nm*						
	Ammonium sulphate	Ammonium nitrate	Ammonium dihydrogen phosphate	Calcium nitrate	Potassium nitrate	Sodium nitrate	Mean (OD value)
I_1	0.50	0.42	0.49	0.52	0.39	0.30	**0.44**
I_2	0.43	0.47	0.44	0.32	0.32	0.31	**0.38**
I_3	0.46	0.38	0.39	0.21	0.31	0.34	**0.35**
I_4	0.43	0.45	0.63	0.29	0.33	0.44	**0.43**
I_5	0.37	0.28	0.34	0.24	0.29	0.27	**0.30**
I_6	0.62	0.51	0.67	0.55	0.47	0.45	**0.55**
I_7	0.57	0.39	0.59	0.54	0.43	0.42	**0.49**
I_8	0.37	0.31	0.47	0.25	0.29	0.34	**0.34**
I_9	0.41	0.35	0.45	0.30	0.40	0.29	**0.37**
I_{10}	0.34	0.34	0.35	0.29	0.22	0.28	**0.30**
Mean	**0.45**	**0.39**	**0.48**	**0.35**	**0.35**	**0.34**	**-**

*Mean of three replications; **CD(P=0.05);** Isolates - 0.01; Inorganic nitrogen sources- 0.01; Isolates × Inorganic nitrogen sources- 0.02

Table 6. Biochemical characteristics of isolates of the *Xcc*

Isolates	Indole production	Starch hydrolysis	Gelatin liquefaction	H_2S production	Ammonia production	Casein hydrolysis	Voge's Proskauer	Methyl red
I_1	-	+	+	+	-	-	-	-
I_2	-	-	+	+	-	+	-	-
I_3	-	+	+	-	-	-	-	-
I_4	-	+	+	+	-	-	-	-
I_5	-	+	+	+	-	-	-	-
I_6	-	+	+	+	-	+	-	-
I_7	-	-	+	+	-	-	-	-
I_8	-	+	+	+	-	-	-	-
I_9	-	+	+	-	-	-	-	-
I_{10}	-	+	+	+	-	-	-	-

+ = Positive result; - = Negative result

Singh and Verma (1980) also reported similar findings and the results also indicated that some isolates, however were tolerant both to acidic and alkaline pH. The pH 6.8 was the optimum for the bacterial growth observed by Bandyopadhyay and Chattopadhyay (1986).

Utilization of carbon compounds

The growth of all the ten isolates was maximum in sucrose by recording mean OD value of 0.60 at 565 nm. It was followed by D-glucose, D-galactose, D-fructose, lactose and starch. The least utilized carbon source was mannitol by recoding OD value of 0.22. Among the ten isolates, I_6 recorded the maximum mean growth (OD 0.59) by growing in all the seven carbon sources tested. The isolates *viz.,* I_1, I_2, I_3, I_4, I_5, I_7, I_8 and I_9 (0.53, 0.44, 0.49, 0.51, 0.40, 0.56, 0.47, and 0.45) were statistically significantly different from each other in utilizing different carbon sources. The isolate I_{10} recorded the least growth (OD 0.38) in utilizing these carbon sources (Table 3). Media containing sucrose or glucose as the carbon source and various organic nitrogen sources supported optimal *X. campestris* growth and cell yield (Jackson *et al.,* 1998). Sain and Gour (2008) reported that the bacterium did not utilize the carbohydrate like arabinose, cellobiose, fructose, galactose, sorbitol, mannose and xylose but derived energy and produced acid from sucrose, trehalose, dextrose, mannitol and poorly from salicin. In support of acid production, among the seven carbon sources tested, the D-glucose supported slight production of acid in the medium by grown over by the seven isolates *viz.,* I_1, I_2, I_4, I_5, 1_7, I_8 and I_{10} and moderate acid production by 1_3, I_6 and I_9. The six isolates *viz.,* I_2, I_3, I_5, I_7, I_8 and I_9 were produced moderate acid in the medium by utilizing D-galactose and I_1, I_4, I_6 and I_{10} were produced only slight acid. By utilizing lactose five isolates *viz*., I_1, I_3, I_4, I_7 and I_{10} were produced moderate acid while rest of the five isolates were produced only slight acid. The sucrose favoured only slight production of acid by I_2, I_3, I_6, I_8, I_9 and I_{10} isolates and was supported moderate production of acid by the rest of the four isolates. The isolates I_3, I_7 and I_{10} showed moderate acid production in succinic acid while I_5 and I_6 were failed to produce acid and the other isolates I_1, I_2, I_4, I_8 and I_9 were showed only slight acid production in the same. Arabinose supported moderate acid production of four isolates *viz.,* I_1, I_4, I_6 and I_9 while the rest of the isolates were produced only slight amount of acid in the medium. A comparison of acid production by all the ten isolates of *Xcc* by utilizing different carbon sources revealed that five isolates *viz.,* I_1, I_2, I_4, I_8 and I_{10} were overall produced slight amount of acid in the medium. Three isolates *viz.,* 1_3, I_6 and I_9 were overall produced moderate acid from majority of the carbon sources. Two isolates *viz.,* I_5 and I_6 were failed to produce acid in the medium containing succinic acid (Table 4). In similar acid production by *Xcc* isolates from glucose, galactose, maltose, cellobiose, arabinose and mannose was also reported by earlier workers (Buchanan and Gibbons, 1974; Dye and Lelliott, 1974).

Utilization of inorganic nitrogen compounds growth

The isolate I_6 in utilizing the six nitrogen sources was significantly different from all the other nine isolates by recording the maximum mean growth of 0.55 OD value at 565 nm. The isolates I_7 and I_1 were ranked next best and they were statistically different from each other. The isolate I_5 and I_{10} were showed the least utilization of all the inorganic nitrogen sources. Among the six different inorganic nitrogen sources tested, ammonium dihydrogen phosphate was significantly superior over all the other isolates by supporting the maximum (OD 0.48) growth of all the ten isolates and it was significantly different from other sources. The least supporting inorganic nitrogen source for the growth of ten isolates was sodium nitrate (Table 5). Verma (1986) reported similar results in the utilization of these ammonical forms of nitrogen sources. Media containing sucrose or glucose as the carbon source and various organic nitrogen sources supported optimal *X. campestris* growth and cell yield (Jackson *et al.,* 1998).

Biochemical characteristics

All the *Xcc* isolates (except I_2 and I_7) were showed positive result to starch hydrolysis, gelatin liquefaction and H_2S production, but showed negative result to Indole production, ammonia production, voge's -proskauer test and methyl red test (Table 6). The above mentioned positive and negative result of the experiment confirms all the ten isolates of *Xcc* belong to bacterium group only. Ability of the isolates to liquefies gelatin, H_2S production and proteolysis of milk were reported by Dye and Lelliott (1974). Sain and Gour (2008) reported that the bacteria liquefied starch and casein produced H_2S in tryptone sucrose agar media.

CONCLUSION

Black rot is considered the most important disease of cabbage because *X.campestris* pv. c*ampestris* infections may not become apparent until the warm summer months, the pathogen spreads rapidly, and losses due to the disease may exceed 50% in warm, wet climates. The identified *X.campestris* pv. *campestris* bacteria cells are gram negative, short rods with rounded ends, with dimensions of 1.17 to 2.07 x 0.54 to 0.99µm, non-capsulated, occurring singly, rarely in pairs, and motile with single polar flagellum. The bacterium produced round colonies ranged in diameter from 10 to 12 mm. The optimum temperature for growth was from 25o to 30oC, the minimum was 5oC, and the maximum was 35oC. However, infected hosts are symptomless below 18°C. The bacterial isolates are showed positive reaction to the biochemical tests viz., starch hydrolysis, gelatin liquefaction and H2S production, but showed negative result to Indole production, ammonia production, voge's -proskauer test and methyl red test. Based on the experiments confirmed the presence of *X.campestris* pv. *campestris* as causative pathogen for black rot of cabbage in the infected leaves of cabbage. These results also indicate that the cabbage plants grown in different regions are highly infected by the bacterium *X.campestris* pv. *campestris*.

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