Madras Agric. J., 99 (4-6): 338-343, June 2012
https://doi.org/10.29321/MAJ.10.100081

Physiological and Biochemical Variations in Soft Rot Pathogen (*Pectobacterium chrysanthemi)* Isolates of *Aloe vera*

M. Syamala and I. Yesu Raja*
Department of Plant Pathology,
Agricultural College and Research Institute, Madurai – 625 104

All the 15 isolates of *Pectobacterium chrysanthemi* survived up to 37^{0}C and the thermal death point was 38^{0}C. The optimum range of pH for the growth of the isolates was 7.0–7.5.Although all the isolates showed good growth in many carbon sources, salicin supported the maximum growth while ribose was the least favourable. These isolates showed no differences in all the biochemical tests. All the 15 isolates of *P. chrysanthemi* gave positive result to utilization of citrate, nitrate reductase test, catalase test, Oxidation-Fermentation test (O- F test), Gelatin liquefaction, casein hydrolysis, anaerobic growth and sensitively to erythromycin while these showed negative reaction to indole production test, starch hydrolysis, H_2S production, oxidase test methyl red and Voges Proskauer test and arginine dihydrolase reaction.

Key words: *Aloe vera, pectobacterium chrysanthemi*, physiological and biochemical variation.

Aloe vera (L) Burm. is one of the medicinal plants widely used throughout the world (Sofowora, 1984). It is a well known medicinal plant of India and is one of the most demanded crop. It is naturalized throughout the country, more common along the West coast (Robert and Hentry, 2004). Plants of the genus *Aloe vera* belong to old world and are indigenous to Eastern and Southern Africa. The plant is found in the tropics and introduced to India for ornamental and medicinal purpose. *Aloe* genus consists of about 325 species all of which grow in rosette shape (Anselm, 2004). It has been established that the inner gel of the leaf contains most of its beneficial part (Swaminathan and Kochhar, 1992). *Aloe vera* has many medicinal and cosmetic usages and hence has growing demand in the market. The plant is a rich source of amino acids and enzymes (Blumenthal, 2003).The gel of the leaf of *Aloe vera* contains 96 per cent of water and the remaining different elements such as vitamins and minerals (Tyler, 1994). The plant also contains essential oil components (Davis *et al.,* 2000). It is referred to as miracle plant for its numerous uses, particularly in the area of man's health (Hecht, 1981).

Among the diseases affecting this crop, *Phytophthora*, *Pythium* root rot, soft rot, and some slime molds are important. Among these, bacterial soft rot caused by *Pectobacterium chrysanthemi* is economically important as it causes yield loss up to 80 per cent. The disease is serious when abundant moisture is available through irrigation and rain. The symptoms start as water soaked lesions at the base of the leaves. The rotting progress very fast and the whole plant die within two to three days. As rotting progresses, the leaf epidermis bulge out due to gas formation and the leaf content is converted to a slimy mass, which is eventually released.

A bacterium was isolated from the infected tissues. The organism was a non-spore forming, motile, short rod and usually single. It produced circular, convex, small (<1 mm) colonies without pigmentation by 24 h on nutrient agar (Glucose-5g, Peptone-5 g, NaCl-5 g, Beef extract -3 g, Distilled water-1000 ml, Agar agar -18-20 g and pH-7- 7.2). In India, soft rot of *Aloe vera* is wide spread in the areas cultivating this crop. In Tamil Nadu, it is a very serious disease in Madurai, Tuticorin, Tirunelveli and Kanyakumari districts, where the cultivation of cultivar *Aloe barbadensis* occupies major area.

Materials and Methods

Collection of Pectobacterium chrysanthemi isolates

Aloe vera plants showing the typical symptoms of soft rot were collected from the following 15 places of southern Tamil nadu.The diseased specimens collected from different areas were used for isolation of the pathogen.

Isolates	Locations	Districts
I_1	Kelekalangal	Tirunelveli
I_2	Tenkasi	"
I_3	Kadambur	"
I_4	Kayattar	Tuticorin
I_5	Duraisamypuram	"
I_6	Muthulapuram	"

*Corresponding author email: yesupatho@yahoo.co.in

I_7	Nagarkoil	Kanyakumari
I_8	Krishnankoil	Virudhunagar
I_9	Melur	Madurai
I_{10}	Palamedu	"
I_{11}	AC&RI (Madurai)	"
I_{12}	TNAU	Coimbatore
I_{13}	Paramakudi	Ramanathapuram
I_{14}	HC&RI , Periyakulam	Theni
I_{15}	Gandhigram	Dindugal

Isolation of pathogen

The soft rot bacterium *P.Chrysanthemi* was isolated from the diseased tissues of plants collected during the survey. The infected soft portion was surface sterilized with 80 per cent ethanol. By using sterilized inoculation needle punctures were made on the decayed tissue and streaks were made gently onto the *Erwinia* selective medium (Poly galacturonic acid-5.0 g, $NaNO_3$-1.0 g, K_2KPO_4 -4.0 g, $MgSO_4$.$6H_2O$-0.2 g, sodium heptadecyl sulphate-0.1ml, NaOH (1N)-17 ml, Beef extract-5g, Agar agar-20 g, Distilled water-1000 ml and pH -7.0) in Petri plate. The plates were incubated at lab temperature 28^0C. After 48 h of incubation, the cream white bacterial growth was purified by the dilution plate technique (Waksman, 1952). The isolate was maintained on the *Erwinia* selective medium slants by paraffin method (Aneja, 1993).

Identification of bacterial isolates

The bacterial isolates were identified by observing the morphological, cultural, physiological and biochemical characteristics. The identity of the bacterium was confirmed by Microbial Type Culture Collection and Gene Bank (MTCC) at the Microbial Institute of Technology, Chandigarh, India.

Effect of temperature on growth

Bacterial cultures were grown in mineral salt liquid medium (K_2HPO_4-16.266 g $L^{-1,}$ KH_2PO_4 -0.899 g L^{-1}, $(NH_4)_2SO_4$- 1.2 g.L^{-1}, $MgSO_4$.$6H_2O$ - 0.818 g.L^{-1} CaCl2 -0.075 g.L^{-1},Polygalacturonic acid (Potassium salt, Sigma) -4.0 g.L-1, distilled water-1000 ml and pH-8.0) in 250 ml Erlenmeyer flasks under gyrotory agitation (180 rpm) in a thermostatic water bath at various temperatures: *viz.,* 5 to 38^0C. The volume of the medium was 10 per cent of the total flask volume. Batch precultures and cultures were made in the same conditions. The starting optical density (OD) of the cultures was 0.05. For each strain, three independent cultures were made.Growth was monitored by OD measurements at 580 nm. The final OD value was recorded after 48 h.

Effect of pH on growth

The pH of peptone broth in test tubes was adjusted in a pH meter before sterilization using N/ 10 hydrochloric acid or N/10 sodium hydroxide as the case might be to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 11.0. The test tubes were separately inoculated with the actively growing culture of *P. chrysanthemi* isolates, incubated at room temperature and the growth was measured after 72h in terms of OD in a Hitachi Spectrophotometer at 585 nm wavelength against peptone water blank.

Growth in NaCl

The bacterial isolates were inoculated in to the test tubes containing nutrient broth supplemented with 2.5, 5.0, 7.0, 8.5 and 10.0 per cent concentration sodium chloride and observed daily for the growth up to seven days.

Anaerobic growth

The bacterial cultures of the isolates were inoculated into the tubes containing sterilized glucose broth and incubated in an anaerobic jar. Alternatively, the broth was overlaid with sterile mineral oil and incubated at 24°C for observing the bacterial growth (Hugh and Leifson, 1953).

Biochemical properties of isolates

The method described by Dowson (1957) was followed for the following test conducted *viz.* utilization of carbon compounds, indole production test, methyl red and Voges Proskauer tests, utilization of citrate, caesin hydrolysis ,starch hydrolysis, nitrate reductase test, H_2S production, oxidase test, catalase test , oxidation – fermentation test (0 – F test), gelatin hydrolysis, arginine dihydrolase reaction and sensitivity to erythromycin and anaerobic growth. A 24h old actively growing slant culture was used in all experiments.

Utilization of carbon compounds

Fourteen carbon sources *viz.,* adonitol, arabinose, cellobiose, dulcitol, D- mannose, ribose, inositol, lactose, maltose, mannitol, rhamnose, salicin, sorbitol, and xylose were separately incorporated at one per cent level and starch at 0.2 per cent level into the basal medium consisting of 1. 0g ammonium dihydrogen phosphate, 0.2g potassium chloride, 0.2 g magnesium sulphate and 1lit. distilled water and adjusted to pH 7.0. Durham's fermentation tube was filled with the medium, dropped into a test tube each containing eight ml of the medium in the inverted position without air bubble and sterilized. The test tubes were inoculated with the bacterial isolates, incubated for seven days at room temperature of 28 ± 2^0C and examined for growth, acid and gas production. Bromothymol blue was used as an indicator for acid production (greenish blue at pH 7.0; greenish yellow at pH 6.4; yellow at 6.2). Uninoculated tubes served as control. Three replications were maintained for each carbon source.

Indole production test

The test medium containing tryptone, 10 g; L-tryptophan, 19 g and distilled water, 1000 ml, was prepared and sterilized. It was dispensed in sterilized Petri plates and test organisms were inoculated. After two days, 0.5 ml of Kovacs' indole reagent was added and the result was observed (Aneja, 1993).

Methyl red test and Voges Proskauer (VP)tests

The broth containing peptone, 7 g; dextrose, 5g; potassium phosphate, 5 g and distilled water 1000 ml was prepared, dispensed in test tube and autoclaved at 15 lb pressure for 15 minutes. The test pathogens were inoculated two test tubes and one control was maintained and incubated at 35^{o}C for 48 h. Then five drops of methyl red was added and colour change was observed after five minutes. Twelve drops of V-P reagent-I and 2-3 drops of V-P reagent II were added on another set of test tubes and incubated for 15-30 minutes. Observation was made and tabulated (Aneja, 1993).

Utilization of citrate

The bacterial culture was streaked on to the surface of Simmon's citrate agar (Magnesium sulphate-0.2 g, Ammonium dihydrogen phosphate-2.0 g, Dipotassium hydrogen phosphate-2.0 g, Sodium citrate-5.0 g, Sodium chloride- 0.08 g, Bromothymol (Potassium salt, Sigma)-15.0 g, Agar agar-20 g, Distilled water-1000 ml) slant. Appearance of blue colour indicated the utilization of citrate. Original green colour indicated citrate non utilization.

Caesin hydrolysis

Test medium containing skimmed milk powder 100 g, peptone 5 g, agar 15 g and pH 7.2 was prepared and distributed in 250 ml conical flasks. Sterilized medium was poured in Petri dishes and single line streak of the bacterium was made on to the medium. A control was maintained without plate streak and the results were recorded (Aneja, 1993).

Starch hydrolysis

The isolates were streaked onto peptone sucrose agar medium incorporated with 0.2 per cent starch in Petri dishes and incubated for five days. Lugol's Iodine solution was used for testing the hydrolysis of starch. Production of clear zone around the growth indicated positive result.

Nitrate reductase test

The test medium contained KNO_3, 1 g; peptone, 5 g; yeast extract, 3 g and distilled water, 1 litre. The pH was adjusted to 7-7.2 by addition of 40 % NaOH. The medium was dispensed to test tube and autoclaved at 121^{o}C for 15 minutes. The culture of *P.chrysanthemi* was inoculated and incubated at 27^{o}C. The tube was examined for evidence of gas production (Schaad, 1992).

Hydrogen sulphide production

Test medium containing peptone, 30.0 g; beef extract, 3.0 g; Ferrous ammonium sulphate, 0.2 g ; Sodium thiosulphate, 0.025 g ; Agar, 3.0 g and distilled water, 1000 ml were used. The ingredients were dissolved and dispensed in 18 x 150 mm culture tubes and autoclaved at 121^{o}C for 15 minutes. The test organisms were inoculated into each tube, incubated at 35-36^{o}C for 48 h and observations were made (Schaad, 1992).

Oxidase test

The 24 h old bacterial cultures were spot inoculated on oxidase disc and change in colour of the disc from white to purple or blue was observed.

Catalase test

Smears of 24 h old bacterial cultures were prepared on clean slide and covered with a few drops of three per cent hydrogen peroxide. Formation effervescence indicated the presence of catalase in the culture.

Oxidation -Fermentation test (0 – F test)

The medium used contained 2.00 g peptone, 5.00 g sodium chloride, 0.30 g dipotassium hydrogen phosphate, 0.03 g bromothymol blue, 10.00 g glucose, 3.00 g agar agar and one l. of distilled water. The pH was adjusted to 7.1 and the medium was sterilized at 1.05 kg cm^{-2}. Two test tubes were incubated for seven days and examined. Yellow colour indicated positive result for fermentation. Oxidative types of bacteria grow only in open tube (Huge and Leifson, 1953).

Gelatin liquefaction

The test medium containing beef extract 3 g; peptone, 5 g; gelatin, 120 g and distilled water, one l was dispensed in test tube, autoclaved at 121^{o}C for 12-15 minutes and cooled. After inoculation, the tubes were incubated at 20-22^{o}C for three days and kept at 4^{o}C for 30 minutes and observations were recorded (Schaad, 1992).

Arginine dihydrolase reaction

The bacterial cultures were stabbed into the semi-solid medium (Peptone- 1.0 g, Sodium chloride-5.0 g , Dipotassium hydrogen phosphate-0.3 g, Phenol red-0.01 g, Arginine HCl-10.0 ml, Agar agar-20 g, Distilled water-1000 ml and pH-7.2) of Thornley (1960) and the tubes were sealed with three ml of one per cent molten agar at 45°C. The tubes were incubated at 28°C for seven days and any colour change, indicative of change in pH under the agar seal was observed and recorded.

Sensitivity to erythromycin

Nutrient agar medium plus 1% dextrose was prepared and dispensed into tubes and flasks. The tubes and flasks were sterilized at 121^{o}C for 15 minutes. The medium was poured in Petri dish as

basal layer and one drop of nutrient broth was added on the basal layer as seeded medium. Filter paper disc dipped in antibiotic viz., erythromycin was placed on the surface of the seeded layer and incubated at 27°C for 24-48 h. Observation was made for the appearance of inhibition zone which was measured and recorded and tabulated (Schaad, 1992).

Results and Discussion

Physiological characteristics of P. chrysanthemi isolates:

Effect of temperature on growth: All the 15 isolates survived up to 37 °C. These isolates were not able to survive at 38 °C. So the thermal death point temperature for all isolates was 38 °C. The isolate I_9 (Melur) was the best recording the maximum growth (OD 1.356) followed by isolate I_{10} (0.917) while I_{11} isolate recorded the minimum of (0.548) growth at 37 °C. (Tables 1 and 2). Smadja *et al*. (2004) demonstrated that the multiplication of eight strains of *E. carotovora* subsp. *atroseptica* was high up to 37°C.

Table 1. Effect of temperature on the growth of *P.chrysanthemi* isolates *in vitro*

Isolates	Growth at temperature									
I	5 °C	10 °C	15 °C	20°C	25 °C		30 °C	35 °C	36 °C	37 °C	38 °C
I_{12}	-	-	-	-	+	+	+	+	+	-
I_3	-	-	-	-	+	+	+	+	+	-
I_4	-	-	-	-	+	+	+	+	+	-
I_5	-	-	-	-	+	+	+	+	+	-
I_6	-	-	-	-	+	+	+	+	+	-
I_7	-	-	-	-	+	+	+	+	+	-
I_8	-	-	-	-	+	+	+	+	+	-
I_9	-	-	-	-	+	+	+	+	+	-
I_{10}	-	-	-	-	+	+	+	+	+	-
I_{11}	-	-	-	-	+	+	+	+	+	-
I_{12}	-	-	-	-	+	+	+	+	+	-
I_{13}	-	-	-	-	+	+	+	+	+	-
I_{14}	-	-	-	-	+	+	+	+	+	-
I_{15}	-	-	-	-	+	+	+	+	+	-
Uninoculatedcontrol	-	-	-	-	-	-	-	-	-	-

+ Presence of growth, - No growth

Table 2. Effect of temperature on the growth of *P.chrysanthemi* isolates *in vitro*

Isolate	Growth at 37°C(OD value at 600 nm*)
I_1	0.861[e]
I_2	0.778[g]
I_3	0.901[c]
I_4	0.887[d]
I_5	0.664[k]
I_6	0.715[i]
I_7	0.801[f]
I_8	0.653[m]
I_9	1.356[a]
I_{10}	0.917[b]
I_{11}	0.548[n]
I_{12}	0.762[h]
I_{13}	0.661[l]
I_{14}	0.801[f]
I_{15}	0.685[j]
uninoculated control	0

* Mean of three replications,In a column, means followed by common letters are not significantly different at 5% level by DMRT

Effect of pH on growth

Among the different pH levels tested, pH 7.0 to 7.5 recorded the maximum growth of all the isolates and in pH 5.0, 5.5, 6.0, 6.5 and 8.0. Minimum growth was observed while all the isolates failed to grow at pH 8.5 (Table 3).

Table 3. Effect of pH on the growth of *P.chrysanthemi* isolates *in vitro*

Isolate	Growth at pH							
	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
I_1	+	+	+	+	++	++	+	-
I_2	+	+	+	+	++	++	+	-
I_3	+	+	+	+	++	++	+	-
I_4	+	+	+	+	++	++	+	-
I_5	+	+	+	+	++	++	+	-
I_6	+	+	+	+	++	++	+	-
I_7	+	+	+	+	++	++	+	-
I_8	+	+	+	+	++	++	+	-
I_9	+	+	+	+	++	++	+	-
I_{10}	+	+	+	+	++	++	+	-
I_{11}	+	+	+	+	++	++	+	-
I_{12}	+	+	+	+	++	++	+	-
I_{13}	+	+	+	+	++	++	+	-
I_{14}	+	+	+	+	++	++	+	-
I_{15}	+	+	+	+	++	++	+	-
Uninoculated control	-	-	-	-	-	-	-	-

+ Growth

++ More growth

- No growth

Acid production from carbon sources:

A comparison of acid production by all the 15 isolates of *P. chrysanthemi* from different carbon sources revealed that four isolates *viz*., $I_{9,}$ I_{10} and I_{14} produced moderate amount of acid from nine, eight and eight numbers of carbon sources respectively. All the isolates failed to produce acid from adonitol, dulcitol, inocitol, lactose and maltose. Nine isolates *viz*., $I_{1,}$ $I_{2,}$ $I_{3,}$ $I_{4,}$ $I_{5,}$ $I_{6,}$ $I_{7,}$ $I_{8,}$ I_{11} $I_{12,}$ I_{13} and I_{15} produced slight acid from the majority of the carbon sources tested. Arabinose supported slight production of acid by 11 isolates *viz*., $I_{1,}$ $I_{3,}$ $I_{7,}$ I_{12} and I_{15} and moderate acid production by $I_{2,}$ $I_{4,}$ $I_{5,}$ $I_{6,}$ $I_{8,}$ $I_{11,}$ I_{13} and $I_{15.}$ Cellobiose supported slight production of acid by five isolates

Table 4. Acid production from carbon sources by *P.chrysanthemi* isolates *in vitro*

Isolates	Adonitol	Arabinose	Cellobiose	Dulcitol	mannose	Ribose	Inocitol	Lactose	Maltose	Mannitol	Rhamnose	Salicin	Sorbitol	Xylose
I_1	N	S	M	N	M	S	N	N	N	M	M	S	M	S
I_2	N	M	M	N	S	M	N	N	N	S	M	M	S	M
I_3	N	S	S	N	M	M	N	N	N	M	S	M	M	M
I_4	N	M	M	N	S	M	N	N	N	S	M	S	M	M
I_5	N	M	M	N	M	S	N	N	N	M	M	M	S	M
I_6	N	M	S	N	M	M	N	N	N	S	M	M	S	M
I_7	N	S	S	N	M	M	N	N	N	M	M	S	M	S
I_8	N	M	M	N	S	S	N	N	N	M	M	M	M	M
I_9	N	M	M	N	M	M	N	N	N	M	M	M	M	M
I_{10}	N	M	M	N	M	M	N	N	N	M	M	M	M	S
I_{11}	N	M	S	N	S	S	N	N	N	M	M	M	S	M
I_{12}	N	S	M	N	M	M	N	N	N	M	M	M	M	S
I_{13}	N	M	S	N	S	M	N	N	N	S	M	M	S	M
I_{14}	N	M	M	N	M	S	N	N	N	M	M	M	M	M
I_{15}	N	S	M	N	M	S	N	N	N	M	S	M	M	S
Uninoculated control	-	-	-	-	-	-	-	-	-	-	-	-	-	-

M-Moderate growth, S-Slight growth, N- No acid

viz., $I_{3,}$ $I_{6,}$ $I_{7,}$ I_{11} and I_{13} and moderate acid production by $I_{1,}$ $I_{2,}$ $I_{4,}$ $I_{5,}$ $I_{8,}$ $I_{9,}$ I_{10} , $I_{12,}$ I_{14} and $I_{15.}$ Mannose supported slight production of acid by five isolates *viz*., $I_{2,}$ $I_{4,}I_{5,}$ $I_{8,}$ I_{11}, and I_{13} and moderate acid production by $I_{1,}$ $I_{3,}$ $I_{5,}I_{6,}$ $I_{7,}$ $I_{9,}$ $I_{10,}$ $I_{12,}$ I_{14} and $I_{15.}$Ribose supported slight production of acid by six isolates *viz*., $I_{1,}$ $I_{5,}$,$I_{8,}$ $I_{11,}I_{14}$and I_{15} and moderate acid production by $I_{2,}$ $I_{3,}$ $I_{4,}I_{6,}$ I_{7} , $I_{9,}$ $I_{10,}$ I_{12} and $I_{13.}$ Mannitol supported slight production of acid by 4 isolates *viz*., $I_{2,}$ $I_{4,}$ I_{6} and I_{13} and moderate acid production by $I_{1,}$ $I_{3,}I_{5,}$ I_{7} ,$I_{8,}$ $I_{9,}$ $I_{10,}$ $I_{11,}$ $I_{12,}$ $I_{14}$and $I_{15.}$ Rhamnose supported slight production of acid by two isolates *viz*., I_3 and I_{15} and moderate acid production by $I_{1,}$ $I_{2,}$ $I_{4,}$ $I_{5,}$ $I_{6,}$ $I_{7,}$ $I_{8,}$ $I_{9,}$ $I_{10,}$ $I_{11,}$ $I_{12,}$ I_{13} and $I_{14.}$ Salicin supported slight production of acid by three isolates *viz*., $I_{1,}$ I_4 and I_7 and moderate acid production by $I_{2,}$ $I_{3,}$ $I_{5,}$ $I_{6,}$ $I_{8,}$ $I_{9,}$ $I_{10,}$ I_{11}, $I_{12,}$ I_{13} I_{14} and $I_{15.}$ Sorbitol supported slight production of acid by four isolates *viz*., $I_{2,}$ $I_{5,}$ I_6 and I_{11} and moderate acid production by $I_{1,}$

Table 5. Biochemical diagnostic tests for *P. chrysanthemi*

Bio chemical test	Isolate														
	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13}	I_{14}	I_{15}
Indole production test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red and Voges Proskauer tests	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Caesin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reductase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidation – Fermentation test (0 – F test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sensitivity to erythromycin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

$I_{3,}$ $I_{4,}$ I_7 ,$I_{8,}$ $I_{9,}$ $I_{10,}$ $I_{12,}$ I_{13} $I_{14}$and $I_{15.}$ Xylose supported slight production of acid by eleven isolates *viz*., $I_{1,}$ I_7 , $I_{10,}$ I_{12} and $I_{15}$and moderate acid production by $I_{2,}$ $I_{3,}$ $I_{4,}I_{5,}I_{6,}$ $I_{8,}$ $I_{9,}$, I_{11}, I_{13} and I_{14} (Table 4).

Biochemical characteristics of P. chrysanthemi isolates

In the present study, the results of the biochemical tests were pertinent to P. *chrysanthemi as* documented by Dye (1969) and Mandal (2005). All the 15 isolates gave positive result to utilization of citrate, nitrate reductase test, catalase test, Oxidation – Fermentation test (0 – F test), gelatin liquefaction, casein hydrolysis, anaerobic growth and sensitivity to erythromycin, while recording negative results to indole production, starch

hydrolysis, H_2S production, oxidase test, methyl red and Voges Proskauer tests and arginine dihydrolase reaction (Table 5).

References

Aneja, K.R. 1993. Experiments in Microbiology, Plant Pathology and tissue culture. Wishwa Prakashan, New Delhi. 117-195pp.

Anselm, A. 2004. Nature power. 3rd Edn. Fr. Anselm Adodo, SB Ewu-Esan, Nigeria, 288p.

Blumenthal, M. 2003. *Clinical Guide to Herbs.* Austin,TX: American Botanical Council; 214–225pp.

Davis, R.H., Leithner, M.G., Russo, J.M. and Bryne, M.E. 2000. Advance methods in plant breeding and biotechnology. *J. Am. Ped.,* **79**: 559-562.

Dowson, W.J. 1957. Plant Disease due to Bacteria. 2nd Ed. Cambridge Univ. Press, London. 232p.

Dye, D.W. 1969. A taxonomic study of the genus Erwinia. II. *The "Carotovora" group.* New zealand. *J. Sci. 12 : 81-97pp.*

Hecht, A. The overselling of aloe vera. FDA Consumer 1981, 15, 26-29.

Huge, R. and Leifson, E. 1953. The taxonomic significance of fermentation versus oxidative metabolism of carbohydrates by gram negative bacteria. *J. Bacteriol*, **66**: 24-26.

Mandal, K. and Maiti, S. 2005. Bacterial soft rot of aloe caused by *Pectobacterium chrysanthemi.* National Research Centre for Medicinal and Aromatic Plants, Gujarat, India.

Robert, B. and Hentry, T. 2004. Medicinal Plants. Asiatic Publishing House, New Delhi, 308p.

Schaad, M.W. 1992. Laboratory guide for identification of Plant Pathogenic Bacteria Second edition. International Book Distributing Co., Lucknow, 44-58pp.

Smadja, B., Lantour, X., Trigui, S., Burini, J.F., Chevalier, S. and Orange, N. 2004. Thermodependence of growth and enzymatic activities implicated in pathogenlcity of two *Erwinia carotovora* subspecies (*Pecto bacterium* spp.) *Can.J.Microbiol*., **50**: 19-27.

Sofowora, A. 1984. Medicinal Plants and Traditional Medicines in Africa, John Wiley and Sons Ltd., New York, 256p.

Swaminathan, M.S. and Kochhar, S.L. 1992. Comparative petiole anatomy as an aid to the classification of Africa Genius. CAB International Wallingford, 409p.

Thornley, M.J. 1960. The differentiation of *Pseudomonas* from other gram negative bacteria on the basis of arginine metabolism. *J. Appl. Bacteriol*., **1**: 37-52

Tyler, V. 1994. Herbs of Choice: The therapeutic Use of phytomedicinals. New York: Pharmaceutical Products Press, 76–77pp.

Waksman, S.A. 1952. *Soil Microbiology***,** Chapman and Hall Ltd., London, 356p.

Received: September 14, 2011; Accepted: May 13, 2012