

## SOME BIOCHEMICAL CHARACTERIZATION AGAINST *XANTHOMONAS ORYZAE* PV. *ORYZAE* CAUSE BACTERIAL LEAF BLIGHT OF RICE

Sadam Hussain Bhutto<sup>1</sup>, Javed Asghar Tarique<sup>2</sup>, Jamal-U-Ddin Hajano<sup>1</sup>, Rehana Naz Syed<sup>1</sup>, Ghulam Hussain Jatoi<sup>3+1</sup>, M. Ismail<sup>2</sup>, Qurban Ali Rattar<sup>1</sup> and Zeeshan Ghulam Nabi Gishkori<sup>1</sup>,

<sup>1</sup>Department of Plant Pathology, Sindh Agriculture University Tandojam, <sup>2</sup>Division of Plant Protection, Nuclear Institute of Agriculture Tandojam, Pakistan. <sup>3+1</sup>The State Key Laboratory for Bio-Pesticides Engineering of Plant Disease Biocontrol and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Science, No. 12 Zhong-Guan-Cun South Street, Beijing 100081, China  
E.mail: jatariq\_1411@yahoo.com

Article received 11.3.2018, Revised 30.5.2018, Accepted 7.6.2018

### ABSTRACT

In current studies enzymes production and antibiotics sensitivity of some strains of *Xanthomonas oryzae* pv. *oryzae* tested in *in-vitro* conditions. Results of biochemical tests like catalase, amylase (starch hydrolysis) and growth on Yeast Dextrose Calcium Carbonate Agar (YDCA) varied among the isolates. The results regarding catalytic and amylase activity showed that out of 19 isolates, 18 isolates (95%) were Catalase positive and 1 isolate (5%) Catalase negative. 16 strains (84%) were amylase positive and 4 strains (21%) were amylase negative. SHB-5, SHB-12, SHB-116 showed maximum inhibition zone against streptomycin where as SHB-18, SHB-19 showed minimum inhibition.

**Keywords:** *Xanthomonas oryzae* pv. *oryzae*, biochemical tests, rice (*Oryza sativa*), Bacterial leaf blight (BLB) disease, Sindh

### INTRODUCTION

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*), belongs to c-subdivision of the Proteobacteria. *Xoo*, a major bacterial pathogen that cause bacterial leaf blight disease (BLBD) of rice crop (*Oryza sativa* L.) (Ishiyama, 1922; Swings *et al.*, 1990). The BLBD disease is affecting crop throughout world in climatic conditions of irrigated, deep water, temperate, rain fed and tropical rice areas (Mew, 1987). In past, severe epidemics of the disease have been reported such as during 1884-5 the disease caused 25-30% in Japan, 50-60 in India, Indonesia, and 10-81 in Philippine (Ahmad and Singh, 1975). However, In Pakistan, the disease was first time observed in 1977 at Rice research Institute, Kala Shah Kaku on rice varieties Palman, Basmati 198 and IRRI-6 (Mew and Majid, 1977; Ahmad and Majid, 1980). Later the disease has spread to other major rice growing provinces like Khayber Phukhtoon Khaw (KPK), Punjab and Sindh (Akhtar and Sarwar, 1986; Akhtar and Akram, 1987). The epidemics of this disease was reported in 1997, 2002, 2006, 2007 and 2008 due the wide spread of susceptible variety for cultivation (Khan *et al.*, 2009). Since last few years, the incidence and severity of BLB in Sindh province has been increasing (Akhtar *et al.*, 2003, Bhutto *et al.*, 2018). If untreated seeds grown in the field then seed-borne pathogen reduce the crop

yield up to 15-90% (Zafar *et al.*, 2014; Bhutto *et al.*, 2018; Jatoi. *et al.*, 2016b). The bacterium enters through hydathodes or by wounds on the roots or leaves and its infection and symptoms are observed at tillering stage which depend on two phases leaf blight and kresek phase (Ou, 1985; Akhtar *et al.*, 2008). The disease is recognized with appearance of yellow lesions and leaf blade shows wavy margins which later on extend to leaf sheath; then the lesions turn whitish straw in color followed by the ooze of the bacterial from infected leaves (Mew, 1987; Jatoi *et al.*, 2016a). All commercial varieties have shown highly susceptibility to the disease (Cheema *et al.*, 1998; Khan *et al.*, 2000, Akhtar *et al.*, 2008; Ali *et al.*, 2009). Therefore, in this study efforts are taken for biochemical characterization of *Xoo* and their response against antibiotic.

### MATERIALS AND METHODS

**Collection of diseased samples:** Before collection of the samples, a survey was conducted to collect information about the diseased crops in farmer fields at Hyderabad, Tando Muhammad Khan, Thatta and Badin District of Sindh Province. The diseased leaves samples were collected from different localities after every 10-20 kilometers. Bacterial blight was identified on the basis of visual observation of 10 leaves of infected plants selected randomly from rice field.

**Isolation and Purification of BLB pathogen:**

The infected rice leaf (28×7 mm) was cut into pieces with a sterile scalpel. Ethanol at concentration of 75% was used to sterilize the leaf pieces for three minutes and then washed with sterilized distilled water. Six to seven infected leaf pieces were transferred to nutrient agar medium and incubated at 25±2 °C for 72 hours. The cultures were purified by streaking method and were preserved in Sterilized Distilled Water (SDW) and silica gel as reported by (Wilson *et al.*, 1993).

**Biochemical characterization:** The biochemical characterization of all the isolates was carried out as per the procedure outlined in Maheshwari and Dubey (2006). The tests performed are detailed below.

**Gram staining:** Bacterial smear was prepared in a drop of water on clean glass slide and heat fixed by gently over the flame on the spirit Lamp. One to two-drop of crystal violet was applied over the smear and left for 1 min and then washed with sterile distilled water. Then few drops of Gram's iodine solution were applied for 1 min and then washed with 95% alcohol. Finally, safranin was applied for 30 seconds and smear was washed with sterile distilled water. After the slide air dried and examined under light microscope under oil immersion lens by using one drop of immersion oil. The Gram positive bacterial cells appeared violet while gram negative bacteria turned pink to red (Vincent, 1970).

**Potassium hydroxide (KOH) test:** Potassium hydroxide test was conducted to confirm the results obtained during gram staining test with procedure described by Suslow *et al.*, 1982. For this purpose, the fresh bacterial growth was harvested from an agar plate with help of tooth pick and placed on glass slide in a drop of 3% KOH solution. Then the growth was stirred into KOH for 10 seconds maximum of 1 minute using a quick circular motion of hand (Ryu, 1940). Slowly lifting the loop frequently raise the loop 1 cm off surface to test if the mixture is becoming viscous and has the Gram negative: Mixture becomes viscous and "strings out". Gram positive: After 1 minute the mixture is not viscous and does not string out.

**Catalase test:** Bacterial smear was placed on a microscopic slide containing a drop of hydrogen peroxide to perform this test. Growths were categorized as Catalase-positive or negative either bubbles or froth were produced or not.

**Amylase test (starch hydrolysis):** Starch hydrolysis was tested for isolated strains to determine the capability of using carbon source like starch as described by De Oliverira (2007). The medium was inoculated with bacterial strains in signal Petri plate four bacteria were streak with help of loop and incubated at 25±2°C for 172 hours. After full growth of the deferent bacterial strains the drop of the Iodine was applied on the surface and again incubated 25±2°C for 24 hours. A color change i.e blue color formation indicates that the bacteria negative of starch and other not formation in blue color were recorded as positive.

**Growth on (Y D C A) media:** The yeast extract granulates 5g, Dextrose 10g, Calcium carbonate 10g and Ager technical 7g, these ingredients mix with each other and autoclave at 120 °C for 15 minutes. Isolated strains were streak in Petri plate, in signal plate four bacteria were streak different bacterial lines and incubated at 25±2 °C for 172 hours. The strains which were grown on media it means positive and which not grow on (Y D C A) media it means negative.

**Antibiotic sensitivity assay:** Antibiotic sensitivity testing was conducted using the cup assay method. Petri plates containing sterilized nutrient agar media were inoculated with bacterial suspension through spread plate method. Six holes were prepared in plates containing Nutrient Agar medium with the help of cup borer. 10 ul of Streptomycin (100 ppm) was poured on each cups of pre-inoculated plates. All the plates were incubated at 28±2 °C for up to 8 days. The inhibition zone around each cup was recorded in mm with the help of scale. Plates without any amendment of antibiotic were kept as control

**RESULTS**

**Identification of bacterial isolates:** Identification was done using manual and books. Bacterial strains isolated were tentatively identified as belong to genus *Xanthomonas oryzae* pv. *oryzae* (Table. 1)

**Biochemical characterization: Gram staining:** On the basis of Gram reaction out of 19 strains, most of strains such as SHB-1, SHB-3, SHB-4, SHB-5, SHB-6, SHB-7, SHB-8, SHB-9, SHB-11, SHB-12, SHB-13, SHB-14, SHB-15, SHB-17, were found gram negative. Whereas other 5 remaining strains SHB-2, SHB-10, SHB-16, SHB-18, and SHB-19, were Gram negative. This shows that there is 73% contribution of Gram negative to total collected strains and

remaining contribution of 26% was of Gram positive strains.

**Catalytic and amylase activity:** The results regarding catalytic and amylase activity showed that out of 19 isolates, 18 isolates (94%) were Catalase positive and 1 isolate (6%) Catalase negative, whereas 16 (84%) and 4 strains (21%) were amylase positive and negative, respectively.

**Growth on (Y D C A):** Among 19 strains, 14 strains i. e SHB-1, SHB-2, SHB-3, SHB-6, SHB-7, SHB-9, SHB-10, SHB-11, SHB-12, SHB-13, SHB-14, SHB-15, SHB-18 and SHB-19, were positive on Y D C A, while remaining 5 strain such as SHB-4, SHB-5, SHB-8, SHB-

16, SHB-17, were negative on Y D C A. This result showed YDCA positive were *Xoo* strains (Table. 2).

**Antibiotic sensitivity assay:** All the tested strains of *Xoo* showed different level of reaction against Streptomycin. None of them was found to show complete resistance at 100 ppm up to 8 days interval. Moreover, Streptomycin response against tested strains resulted formation of maximum inhibition zone i.e 8,9 and10 mm after eight days of incubation against strains SHB5, SHB-12, SHB-16 and minimum inhibition zone i.e 3 mm against SHB-18 and SHB-19 (Table. 3).



**Zone Produced by Streptomycin**



**Catalase Tested Slide**

**Table. 1 Outline of bacterial isolates *Xanthomonas* spp.**

Strain Name	Host	Origin	Tentative identification
S.H.B-1	Rice plant	Hyderabad	<i>Xanthomonas</i>
S.H.B-2	Rice	Hyderabad	N.D
S.H.B-3	Rice	Hyderabad	<i>Xanthomonas</i>
S.H.B-4	Rice	Tando-M.Khan	<i>Xanthomonas</i>
S.H.B-5	Rice	Thatta	<i>Xanthomonas</i>
S.H.B-6	Rice	Thatta	<i>Xanthomonas</i>
S.H.B-7	Rice	Thatta	<i>Xanthomonas</i>
S.H.B-8	Rice	Thatta	<i>Xanthomonas</i>
S.H.B-9	Rice	Badin	<i>Xanthomonas</i>
S.H.B-10	Rice	Badin	N.D
S.H.B-11	Rice	Badin	<i>Xanthomonas</i>
S.H.B-12	Rice	Tando-M.Khan	<i>Xanthomonas</i>
S.H.B-13	Rice	Tando-M.Khan	<i>Xanthomonas</i>
S.H.B-14	Rice	Tando-M.Khan	<i>Xanthomonas</i>
S.H.B-15	Rice	Badin	<i>Xanthomonas</i>
S.H.B-16	Rice	Badin	N.D
S.H.B-17	Rice	Badin	<i>Xanthomonas</i>
S.H.B-18	Rice	Badin	N.D
S.H.B-19	Rice	Hyderabad	N.D

**Table. 2 Biochemical characterization of bacterial blight strains**

Strains	Gram reaction	Catalase (Test)	Amylase Test (Starch Hydrolysis)	Y D CA (Test)
S.H.B-1	-	+	+	+
S.H.B-2	+	+	+	+
S.H.B-3	-	+	+	+
S.H.B-4	-	+	-	-
S.H.B-5	-	+	+	-
S.H.B-6	+	+	+	+
S.H.B-7	+	+	-	+
S.H.B-8	+	+	+	-
S.H.B-9	-	+	+	+
S.H.B-10	+	+	-	+
S.H.B-11	-	+	+	+
S.H.B-12	-	+	+	+
S.H.B-13	-	+	+	+
S.H.B-14	-	-	-	+
S.H.B-15	-	+	+	+
S.H.B-16	+	+	+	-
S.H.B-17	-	+	+	-
S.H.B-18	-	+	+	+
S.H.B-19	+	+	+	+

**Table. 3 Response of Bacterial isolates against streptomycin**

Strains	Zone Diameter Produced by Streptomycin at Different Days					
	3 DAYS		5 DAYS		8 DAYS	
S.H.B-1	2 mm	0.0±	3 mm	0.0±	6 mm	2.0±
S.H.B-2	0 mm	0.0±	2 mm	1.0±	4 mm	0.0±
S.H.B-3	2 mm	0.0±	4 mm	1.0±	5 mm	0.0±
S.H.B-4	3 mm	1.0±	4 mm	0.0±	4 mm	0.0±
S.H.B-5	4 mm	0.0±	7 mm	3.0±	10 mm	0.0±
S.H.B-6	2 mm	0.0±	2 mm	0.0±	3 mm	0.0±
S.H.B-7	4 mm	1.0±	5 mm	0.0±	7 mm	0.6±
S.H.B-8	2 mm	0.0±	3 mm	0.0±	5 mm	2.0±
S.H.B-9	3 mm	1.0±	4 mm	0.0±	5 mm	1.0±
S.H.B-10	2 mm	0.0±	4 mm	1.0±	5 mm	0.0±
S.H.B-11	1 mm	0.0±	3mm	2.0±	5 mm	0.0±
S.H.B-12	3 mm	1.0±	6 mm	2.0±	9 mm	1.0±
S.H.B-13	2 mm	0.0±	3 mm	1.73±	4 mm	0.00±
S.H.B-14	3 mm	1.0±	5 mm	1.15±	6 mm	0.00±
S.H.B-15	4 mm	0.0±	4 mm	1.15±	6 mm	1.00±
S.H.B-16	2 mm	0.0±	5 mm	1.15±	8 mm	2.00±
S.H.B-17	3 mm	1.0±	4 mm	1.15±	5 mm	0.00±
S.H.B-18	0 mm	0.0±	2 mm	2.31±	3 mm	0.00±
S.H.B-19	0 mm	0.0±	2 mm	2.31±	3 mm	0.00±

## DISCUSSION

Bacterial leaf blight has been found very destructive disease in rice growing regions of world including Pakistan. Its frequency is increasing in Punjab as well as in Sindh province from last 3 year. To tackle the disease, current studies resulted with following findings:

All bacterial isolates in present studies were identified as *Xanthomonas oryzae* pv *oryzae*. Our procedure of identification is reliable and

coincides with the results of (Muneer *et al.*, 2007). Rafi *et al.*, (2013) and Joint (2016) reported formation of light yellow, mucoid, circular dome shaped and smooth colonies on nutrient Agar. The yellow color and mucoid colonies is cultural characteristics of *Xanthomonads* that was due to the production of extracellular polysaccharides (EPS in media containing sugar). More over they stated that *Xanthomonads* are reported to produce colonies that are yellow,

mucoid, convex, and texture in shiny. Such colonies were consistently observed and tentatively identified of as *Xanthomonas oryzae* pv. *oryzae*. These bacterial strains also need identification on molecular level for authentication.

14 strains were identified as gram positive and 5 strains as gram negative. All strains except one were found catalase negative. This is also clue for identification of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Joint (2016) also considered all his catalase positive strains as *Xoo* and discarded catalase negative from his studies. In our study different results were recorded for other biochemical tests like amylase and YDCA. Starch hydrolysis positive results are found at incubation period of a week, but it is not common feature of *Xoo* (Swings *et al.*, 1990). Similarly, we found that some strains of *Xoo* are starch hydrolysis positive and other negative.

Streptomycin at concentration of 100 ppm was used to observe the sensitivity of *Xoo* strains and was found to form zones of inhibitions up to 8 days of incubation. Similarly, Zhang *et al.*, (2011) found growth of 11 *Xoo* isolates grew well in Streptomycin amended medium at 100 µg ml<sup>-1</sup>. Khan *et al.*, (2012) evaluated efficacy of different antibiotics for controlling *Xoo*. They concluded that, Chloramphenicol, Ampicillin and Dihydrostreptomycin were most effective for controlling *Xoo* (Fitt *et al.*, 1992). The present study showed that 100 ppm concentration was found to be the best among all treated concentration for the controlling of *Xanthomonas Oryzae* pv. *Oryzae*.

## REFERENCES

- Ahmad, W and Majid, Incidence of bacterial blight of rice in Punjab (Pakistan). (IRRN), **5**: 5 (1980).
- Ahmed, K.M. and R.A. Singh, Disease development and yield losses in rice in rice varieties by bacterial leaf blight. Indian Phytopathology **28**: 502-507 (1975).
- Akhtar, M.A., A. Rafi and A. Hameed, Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivars. Pak. J. Bot. **40**(5): 2171-2175 (2008).
- Akhtar, M.A. and M. Akram, Incidence of bacterial blight of rice in the Punjab (Pakistan). Int. Rice. Res. Newslett. **5**: 5 (1987).
- Akhtar, M.A. and M. Sarwar, Major Rice diseases and their control. In progressive farming. nat. Agric. Res. Center, Islamabad, Pakistan. **6**: 68-70 (1986).
- Ali, A., M.H. Khan, R. Bano, H. Rashid, N.I. Raja and Z. Chaudhry, Screening of Pakistan rice (*oryzae sativa*) cultivars against *Xanthomonas oryza* pv. *oryzae*. Pak. J. Bot. **41**(5): 2595-2604 (2009).
- Amna, N., Studies on production of transgenic rice plants resistant bacterial blight using agro bacterium mediated transformation. Ph.D. Dissertation, Deptt. of Biochem. Quaid-i-Azam Univ., Islamabad, Pakistan. (2008).
- Bhutto, S.H., J.A. Tariq, R.N. Syed and G.H. Jatoi, Isolation and characterization of bacteria isolated from the rice crop in lower Sindh. Pak. J. Biotechnol. **15**(1): 151-154 (2018).
- Cheema, A.A., M.A. Awan and Y. Ali, Screening of basmati rice mutants against prevalent diseases in the Punjab province. Pak. J. Phyt. **10**: 39-41 (1998).
- Fitt, W. K., G. A. Heslinge, T. C. Waston, Used of antibiotic in the mariculture of giant clams (*F. tridacnidae*). Aquaculture. 104: 1-10 (1992).
- Gnanamanickam, S.S., V.B. Priyadarisini, N.N. Narayanan, P. Vasudevan and S. Kavitha, An overview of bacterial blight disease of rice and strategies for its management. Current Science **77**(11): 1435-1443 (1999).
- Guvera, Y and A. Marsella, Rice bacterial blight in Venezuela. Agri. Trop. Maracay **49**: 505-516. (1999).
- Ishiyama, S. Studies on bacterial leaf blight of rice. In report of agricultural experiment station, Tokyo **45**: 233-261 (1922).
- Jatoi, G.H, M.A. Abro, J.A. Tariq, S. Memon, N. Mangi, A.D. Keerio, M.A. Gadhi, S. Hussain and D. Qiu, Screening of potential bacterial bio-control agents against the *Helminthosporium oryzae in-vitro* conditions. caused by brown spot of rice. Pak. J. Biotechnol. **13**(2):95- 100. (2016a)
- Jatoi, G.H., A. G.r Bhatti, Hidayatullah, S. Memon, M.U. Bhatti, M.A. Abro and S. Memon, Rhyzobacteria associated from rhyzospheric soil and their antifungal activity against Brown spot fungus (*Helminthosporium oryzae*). Sci. Int-(Lahore) **28**(4):4119-4122. (2016b)
- Jonit, N. Q., Y. C. Low and G. H. Tan, *Xanthomonas oryzae* pv. *oryzae*, Biochemical tests rice (*Oryza sativa*), bacterial leaf blight (BLB) disease, Sekinchan. J. App. Env. Mic. **4**(3): 63-69 (2016).

- Khan, J.A., M.I. Arshad, F.F. Jamil and S. Hasnain, Evaluation of rice genotypes against bacterial leaf blight (BLB) disease. *Pak. J. Phytopathol.* **21**(1): 26-30 (2009).
- Khan, J.A., R. Siddiq, H.M.I. Arshad, H.S. Anwar, K. Saleem and F.F. Jamil, Chemical control of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*. *Pak. J. Phytopathol.* **24** (2): 97-100 (2012).
- Khan, J.A., F.F. Jamil and M. Gill, Screening of rice varieties lines against bakanae and bacterial leaf blight (BLB). *Pak. J. Phyt.* **12**(1): 6-11 (2000).
- Li, J., Yie, R. W. Foo, J.M.L. Ling, H. Xu and N.Y.S. Woo, Antibiotic resistance and plasmid profiles of *Vibrio* isolates from cultured silver sea bream, *Sparus sarba*. *Mar. Poll. Bull.* **39**: 245-249 (1999).
- Maheshwari, D.K., R.C. Dubey and S.C. Kang, Biotechnological applications of microorganisms- a techno-commercial approach. I. K. International Publ. House P Ltd, New Delhi. p. 400 (2006).
- Mew, T. W., M. A. Alvarez, E. J. Leach and J. Swings. Focus on bacterial blight of rice. *Plant Dis.* **77**: 5-12 (1993).
- Mew, T.W and A. Majid, Bacterial blight of rice in Pakistan. *IRRN.* **2**(1): 5 (1977).
- Mew, W.T., Study on rice bacterial blight syndrome. International research institute, P.O. Box 933, Manila, Philippines. (1987).
- Muneer, N., A. Rafi and M.A. Akhtar, Isolation and characterization of *xanthomonas oryzae* pv. *oryzae* isolates from north west frontier province (nwfp) Pakistan. *Sarha. J. Agric.* **23**(3): 743-751 (2007).
- Oliveria, A.N., L.A. Oliveria J.S. Andrade and A.F.C. Junior, Rhizobia amylase production using various starchy substances as C substrates, *Braz. J. Microbiol.* **38**: 208-218 (2007).
- Ou, S. H., Rice Diseases 2nd edition common wealth Mycological Instt. Kew, Surrey, England. pp: 61-96 (1985).
- Rafi, A., A. Hameed, M.A. Akhtar, Farhatullah, K. Sohail, M. Shahid and M. Fahim, Identification and characterization of *Xanthomonas oryzae* pv. *oryzae* in north-west of Pakistan Sarhad. *J. Agric.* **29**(3): 423-432 (2013).
- Ryu, E., A simple method of differentiation between gram positive and gram-negative organisms without staining. *Arch. Exp. Med.* **17**: 58-63 (1940).
- Son, R., G. Rusul, A.M. Sahilah, A. Zainuri, A.R. Raha and I. Salmah, Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish telapia (*Telapia mossambica*). *Let. Appl. Microbiol.* **24**: 479- 482 (1997).
- Suslow, T.W., N. Schrotl and M. Isaha, Application of a rapid method for gram differentiation of plant pathogenic bacteria without staining. *Phytopathol.* **72**: 917-918 (1982).
- Swings, J., Van den, M. Mooter, L. Vauterin, B. Hoste and M. Gillis, Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of Rice as Pathovars of *Xanthomonas oryzae* (ex- Ishiyama 1922) sp. nov., nom. rev. *Int. J. Syst. Micro.* **40**(3): 309-311 (1990).
- Vincent, J.M., A manual for practical study of root nodule bacteria. IBP Handbook No. 15, Blackwell Scientific Publishers, Oxford, Pp. 164 (1970).
- Waheed, M.A., I. Ahmad, H. Sirajuddin, H. Ali, A.Q. Khan and A. Khan, Evaluation of rice genotypes for resistance against bacterial leaf blight. *Pak. J. Bot.* **41**(1): 329-335 (2009).
- Wilson, M. and S.E. Lindow, Interaction between the biological control agent *Pseudomonas fluorescens* A 506 and *Erwinia amylovora* in pear blossoms. *Phytopathol.* **83**: 117-123 (1993).
- Zhang, Y., J. Li, X. W. Ma, X.F. Zhu, F. Zhang and M.G. Zhou, A study on the mechanism of resistance to streptomycin in *Xanthomonas oryzae* pv. *oryzae*. *Afr. J. Biotechnol.* **10**(79): 18167-18173 (2011).