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*Full Length Research Paper*

# Role of antioxidant scavenging enzymes and extracellular polysaccharide in pathogenicity of rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*

**Chithrashree and C. Srinivas***

Biocontrol Research Laboratory, Department of Studies in Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore-560 056, Karnataka, India.

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**In the present work, we studied the role of antioxidant scavenging enzymes of plant pathogenic bacteria: catalase, ascorbate peroxidase and a virulence factor; extracelluar polysaccharide production in determining the virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates and its differential reaction to rice cultivars. A varied level of antioxidant scavenging activity and exopolysaccharide production was observed among 34 isolates studied, and most of the *Xoo* isolates with higher catalase activity also exhibited higher ascorbate peroxidase activity. The maximum level of catalase (45 µM $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein), ascorbate peroxidase (29 µM ascorbate $min^{-1}$ $mg^{-1}$ proteins) activity and exopolysaccharide production (70 mg) was found in isolate *Xoo*32 which induced maximum lesion length on cultivar 'Jaya' upon clip inoculation in virulence assay. Among the 44 cultivars screened, cultivar 'Jeerigesanna' recorded least bacterial blight disease incidence, with 0.7 cm lesion length. The activity of catalase, ascorbate peroxidase and exopolysaccharide can be employed as bio-chemical markers in determining the virulence of *Xoo* under laboratory conditions.**

**Key words:** Paddy, plant pathogenic bacteria, antioxidant scavenging enzymes, exopolysaccharide, virulence, bio-chemical markers.

## INTRODUCTION

Rice (*Oryza sativa* L.) bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most devastating rice diseases worldwide (Nino-Liu et al., 2006). In rice, *Xoo* enters either through wounds or hydathodes, multiplies in the epitheme and moves to the xylem vessels where active multiplication results in blight disease symptoms on rice leaves. Plants, in response to invasion by microorganism, employ diverse defense mechanisms to contest pathogen, and production of active oxygen species (AOS) such as $O_2$, $OH^+$, $H_2O_2$ during oxidative burst is one of the earliest and most effective defense reactions. AOS have been suspected to play a role in many defense processes including direct antimicrobial action, lignin formation, phytoalexins production, hypersensitive response and trigger systemic acquired resistance (Mehdy, 1994; Baker and Orlandi, 1995; Peng and Kuc, 1992). There are strong suggestions that among the generated AOS, $H_2O_2$ play a central role in plant defense responses (Bestwick et al., 1998; Mehdy et al., 1996). The antioxidant defense system of the plant comprises a variety of antioxidant molecules and enzymes (Arora et al., 2002).

In response, pathogenic microorganisms have evolved a broad range of mechanisms to overcome this problem. Some virulent bacterial pathogens evolve to overcome

*Corresponding author. E-mail: srinivas_uom@rediffmail.com. Tel: +91-080-22961461. Fax: +91-080-23219295.

the toxic effects of reactive oxygen species (ROS) in plant tissues, by producing defensive antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase (Chamnogpal et al., 1995; Hammond-Kosack et al., 1996). Catalase and ascorbate peroxidase are two important antioxidant scavenging enzymes involved in the removal of $H_2O_2$. Catalase plays the role of a specific peroxidase (POX) protecting the cells from the toxic effects of $H_2O_2$ (Lebeda et al., 2001). The function of catalase in the cell is to remove the bulk $H_2O_2$, whereas ascorbate peroxidase would be involved mainly in scavenging the $H_2O_2$ that is not taken by catalase. The catalase/peroxidase system may thus act cooperatively to remove $H_2O_2$ at a minimum expense of reducing power. *Xoo* also produces a range of virulence factors, including extracellular polysaccharide (EPS), extracellular enzyme, iron-chelating siderophores, and the type III-secretion dependent effectors, which are collectively essential for virulence (Ray et al., 2000; Hu et al., 2007; Rajeshwari et al., 2005; Jha et al., 2007).

Xanthan is a major exopolysaccharide secreted by *Xanthomonas* spp. which induces plant susceptibility by suppressing callose deposition. Thus, Xanthan suppression effect on callose deposition seems to be important for *Xoo* infectivity. The hydrophilic polysaccharides create an environment favouring the pathogen growth in planta and could act as a protective barrier against toxic compounds from host origin (Leigh and Coplin, 1992). Some of the genes required for *X*. *oryzae* pv. *oryzae* virulence that have been previously described include *gumG* and *gumM,* involved in EPS biosynthesis (Dharmapuri and Sonti, 1999; Rajeshwari and Sonti, 2000); *rpfC,* involved in regulation of EPS biosynthesis (Tang et al., 1996).

In the present study, 34 *X. oryzae* pv. *oryzae* isolates isolated from rice seeds of different cultivars grown in diverse agro climatic regions of Karnataka state, India were analyzed for their virulence by considering the level of two antioxidant scavenging enzymes; catalase and ascorbate peroxidase and EPS production as biochemical marker in the pathogenesis.

## MATERIALS AND METHODS

### *Xanthomonas oryzae pv. oryzae* isolates

Rice seed samples were collected from various agro climatic regions, public, private seed agencies, and from farmers of Karnataka, India. The collected samples were surface sterilized with 1% sodium hypochlorite for 3 min. Four hundred seeds of each sample were subjected to liquid assay onto growth factor (GF) medium (Mortensen, 2005). The plates were incubated for 3 to 5 days at 28 ± 2ºC. The number of colonies in each dilution was counted using a digital colony counter, and expressed in cfu/ml. For growing on test, 400 seeds of each rice cultivar were sown in earthen pots (25 cm diameter) at 25 seeds/pot, comprising of soil, sand and manure at 2:1:1 ratio. The pots were maintained under greenhouse conditions and were watered regularly. Seedlings showing typical bacterial leaf blight (BLB) symptoms; yellow to white water-soaked stripes along the lamina and leaf tip, were counted regularly up to 60 days after sowing. The identity of *Xoo* was confirmed by polymerase chain reaction (PCR) employing specific primers *Xoo* 80F and *Xoo* 80R designed to amplify the hypothetical protein of *Xoo* (Lang et al., 2010).

### Preparation of bacterial cell extract, catalase and ascorbate peroxidase assay

A total of 34 *Xoo* isolates were recovered from collected rice seed samples by liquid assay and were analyzed for catalase and ascorbate peroxidase assay. From each of the 34 *Xoo* isolates, 6 ml of 30 h suspension were inoculated to 100 ml nutrient broth in 250 ml flask individually, and incubated at 28 ± 2ºC on a rotary shaker at 150 rpm. The cells were harvested by centrifugation at 8000 rpm for 10 min and suspended 50 mM potassium phosphate buffer containing 0.1 mM EDTA. The cell suspension was disrupted by sonification with 0.5 s pulse for 2 min. Cell debris was pelleted by centrifugation at 13000 rpm for 10 min at 4°C, and the supernatant was used as an enzyme source (Subramoni and Sonti, 2005). Catalase was assayed using the method of Beers and Sizer (1951) with minor modifications. The 2.3 ml reaction mixture contains 50 mM potassium phosphate buffer (pH 6.0) and 100 mM $H_2O_2$, and the reaction was initiated by adding 200 µl of bacterial cell lysate. The decrease in absorbance at 240 nm was monitored spectrophotometrically for 3 min. The specific activity of catalase was expressed as µM $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein ($\varepsilon$ = 43.5 mM $cm^{-1}$).

Ascorbate peroxidase activity was determined spectrophotometrically by a decrease in absorbance at 265 nm ($\varepsilon$ = 13.7 mM $cm^{-1}$) (Nakano and Asada, 1981). The reaction mixture contained 50 mM potassium phosphate buffer pH 7.0, 5 mM ascorbate, 0.5 mM $H_2O_2$ and bacterial cell extract. Addition of $H_2O_2$ started the reaction. The rates were corrected for the non-enzymatic oxidation of ascorbate by the inclusion of reaction mixture without enzyme extract (cell lysate). Enzyme activity was expressed as µM ascorbate $min^{-1}mg^{-1}$ protein. Protein content of the bacterial cell lysate was determined by the method of Lowry et al. (1951) with BSA as the standard.

### Extracellular polysaccharide (EPS) production

A single colony forming unit of each *Xoo* isolate was inoculated in 40 ml of nutrient broth (NB) medium and incubated for 72 h at 28 ± 2°C with agitation. The optical density of the bacterial cultures was adjusted to 1.0 at 600 nm with NB. The culture supernatant was transferred to a new 50 ml flask and supplemented with 1.0% potassium chloride (w/v; final concentration). Two volume of absolute ethanol was added to each solution, and the tubes were placed at -20°C overnight. The precipitated crude EPS was collected by centrifugation for 30 min at 83000 × g. The EPS pellets were dried at 55°C for 12 h and the dry weight of each was measured (Jeong et al., 2008). Means and standard errors were calculated from three independent experiments.

### Virulence assay

The *Xoo* isolates were inoculated into peptone sucrose broth (PSB) and incubated at 28 ± 2°C on a rotary shaker at 150 rpm for 36 h and centrifuged at 8000 × g for 10 min. Bacterial cell pellets were washed thrice with sterile distilled water and the population was adjusted to 3 $\times 10^8$ colony forming units (CFU) as measured spectrophotometrically (Thompson, 1996). Greenhouse-grown 30-day-old susceptible rice cultivar 'Jaya' plants were clip inoculated with sterile scissors dipped in the bacterial suspension (3 $\times 10^8$ cfu/ml) (Kauffman et al., 1973). For each bacterial isolate, eight

replicates of 25 plants were clip inoculated. The plants were incubated under greenhouse conditions, with high relative humidity maintained by covering the pots with transparent polythene bags. The plants were carefully observed for the development of water-soaked streaks near tip and margins systemic infection. The lesion length was measured 14 days post-inoculation. Leaves clipped with scissors dipped in sterile water served as the control. The experiments were repeated thrice and the results were represented as mean lesion length (MLL).

#### Screening of rice cultivars for bacterial leaf blight (BLB)

Seeds of different rice cultivars (Table 2) collected were sown in earthen pots (18 cm diameter). After 25 days, seedlings were transplanted at 30 seedlings/pot. Thirty-day-old seedlings were clip inoculated with the virulent isolate (*Xoo*32) as described above (25 seedlings/pot, four replicates). In each pot, the number of plants showing symptoms was counted, and lesion length was measured after 14 days post-inoculation. Experiments were repeated thrice and the values are represented as percent disease incidence (DI) and MLL.

DI (%) = (Number of plants showing symptoms / Total number of plants inoculated) × 100

MLL = $n_1 + n_2 + n_3 + \ldots . n_{15} / 15$, where $n$ is the lesion length.

#### Statistical analysis

Data from laboratory and greenhouse were analyzed separately for each experiment and subjected to analysis of variance (ANOVA) and Duncan multiple range test (SPSS software version, 16).

## RESULTS

### *Xanthomonas oryzae pv. oryzae* isolates

A total of 236 rice seed samples were collected from different rice growing regions of Karnataka state, India. Among the samples subjected to liquid assay, 44 samples recorded small, yellow, dome-shaped and shining colonies in incubated GF medium plates. The colony forming units (4 × 1:10 dilution) ranged from 167 in cultivar Basmati followed by cultivar Jaya, which recorded 160 cfu/ml. The lowest cfu/ml was recorded with cultivar Jeerige sanna (40 cfu/ml) (Table 1). Among samples subjected to growing on test, highest disease incidence of 28% was recorded from cultivar CTH-1, followed by cultivar IR-64 (25%). The least incidence (6%) was observed in cultivar Naga rice (Table 1). The PCR conducted with specific primers *Xoo* 80F and *Xoo* 80R amplified an amplicon of 162 bp in all 44 samples, confirming the pathogen as *X. oryzae* pv. *oryzae*. The *Xoo* isolates confirmed by PCR were sub-cultured on yeast dextrose calcium carbonate agar (YDC) medium.

### Catalase and ascorbate peroxidase activity

The cell lysate of 34 *Xoo* isolates analyzed for catalase and ascorbate peroxidase activity revealed that the isolates with highest catalase activity also exhibited highest ascorbate peroxidase activity. Among 34 isolates, isolate *Xoo*32 recorded highest catalase and ascorbate peroxidase activity of 45.01 and 29.4 µM $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein, respectively followed by isolates *Xoo*17 and *Xoo*19 which recorded 41.7 and 41.5 µM $H_2O_2$ $min^{-1}$ $mg^{-1}$ catalase activity, respectively. The least activity of catalase and ascorbate peroxidase was recorded by isolates *Xoo*3 and *Xoo*1, respectively (Figure 1).

### Extracellular polysaccharide (EPS) production

All the 34 isolates of *Xoo* grown on NB medium for analysis of EPS production were positive for EPS production with varied range from 20 to 70 mg. The isolate *Xoo*32 recorded highest activity of catalase and ascorbate peroxidase activity (Figure 1) and also produced highest EPS of 76 mg. This was followed by isolate *Xoo*15 recording 50 mg of EPS production. The least EPS production was observed with isolate *Xoo*3 (Figure 2).

### Virulence assay

The 34 *Xoo* isolates clip inoculated onto 30-day-old seedlings of susceptible cultivar Jaya produced BLB symptoms with varied lesion length varying with isolate type. The isolate *Xoo*32 with higher antioxidant and EPS production caused a mean lesion of 5.07 cm length, followed by isolate *Xoo*33 which caused a mean lesion of 4.4 cm length. The lowest lesion length was recorded by isolate *Xoo*18 which caused a lesion of 0.87 cm (Figure 2). The *Xoo* isolates with least EPS production failed to develop blight symptoms on tested rice cultivar.

### Reaction of different rice cultivars to virulent isolate Xoo32

When virulent isolate *Xoo*32 was clip inoculated to 30-day-old seedlings of different rice cultivars, the incidence of BLB ranged from 10 to 57%. The cultivar Jeerige sanna showed disease incidence of 10% with 0.3 cm mean lesion length, followed by cultivar Bangara sanna with disease incidence of 12% and mean lesion length of 0.6 cm. The highest BLB incidence (57%) was observed with the cultivar Jaya, which caused a mean lesion length of 5.8 cm. The isolate *Xoo32* produced highest mean lesion length of 6.2 cm in cultivar IR-64, with 56% of BLB incidence up on clip inoculation (Table 2).

## DISCUSSION

Bacterial blight is the most serious bacterial disease of rice, and in some areas, the most important of any

**Table 1.** Screening of rice seed samples for seed-borne *Xoo* infection.

| S/N | Cultivar | Place of collection | Liquid assay ($10^4$cfu/ml) (4×1:10) | Percent disease incidence in growing on test |
|---|---|---|---|---|
| 1 | IR-20 | H.N.Pura | 86.6±24$^{abcde}$ | 12±0.73$^{cdefghi}$ |
| 2 | IR-20 | Mandya | 66.6±6.6$^{abcd}$ | 10±0.6$^{abcde}$ |
| 3 | IR-64 | Hassan | 120±11.4$^{defgh}$ | 15±0.42$^{hijkl}$ |
| 4 | IR-64 | Hassan | 146.6±13.2$^{fgh}$ | 25±2.24$^{qr}$ |
| 5 | IR-30864 | Nanjangudu | 106.6±6.6$^{bcdef}$ | 10±0.58$^{abcde}$ |
| 6 | KRH-2 | Mandya | 113.4±17.6$^{cdefgh}$ | 15±1.26$^{hijkl}$ |
| 7 | THANU | Mandya | 113.4±17.6$^{cdefg}$ | 8±0.44$^{abcde}$ |
| 8 | Prakash | Mandya | 146.6±11.4$^{gh}$ | 24±0.76$^{pq}$ |
| 9 | Mangala | Mandya | 93.4±11.4$^{abcde}$ | 12±0.87$^{cdefghij}$ |
| 10 | KP-1 | K.R. Nagar | 53.4±6.6$^{ab}$ | 13±1.3$^{cdefghij}$ |
| 11 | Jaya | C.R. Patna | 146.6±6.6$^{fgh}$ | 14±0.88$^{fghij}$ |
| 12 | Jaya | Arasikere | 160±11.6$^{fgh}$ | 20±2.2$^{nop}$ |
| 13 | CTH-3 | Mandya | 80±6.6$^{abcde}$ | 7±1.15$^{ab}$ |
| 14 | CTH-1 (Mukthi) | Mandya | 120±11.4$^{defgh}$ | 28±1.76$^{r}$ |
| 15 | INTAN | Mandya | 73.2±17.6$^{abcde}$ | 8±1.2$^{abc}$ |
| 16 | Mandya Vijaya | K.R. Nagar | 113.4±29$^{cdefg}$ | 15±0.63$^{ijkl}$ |
| 17 | BR-2655 | Mandya | 60±11.4$^{abc}$ | 14±1.09$^{ghijk}$ |
| 18 | Vikas | C.R. Patna | 86.6±17.6$^{abcde}$ | 16±0.88$^{jklmn}$ |
| 19 | Pushpa | Pandavapura | 126.6±6.6$^{efgh}$ | 20±1.2$^{mnop}$ |
| 20 | Sonam | Davanagere | 86.6±17.6$^{abcde}$ | 9±1.76$^{abcd}$ |
| 21 | Sonam | Mandya | 113.4±6.6$^{cdefg}$ | 12±1.48$^{bcdefghi}$ |
| 22 | Rasi | Tumkur | 53.4±24$^{ab}$ | 9±1.2$^{abcdef}$ |
| 23 | Rasi | Hassan | 106.6±24$^{bcdef}$ | 18±0.88$^{klmno}$ |
| 24 | BPT-5204 | Mandya | 73.4±13.2$^{abcde}$ | 20±2.02$^{nop}$ |
| 25 | MTU-1010 | Nanjangudu | 60±11.4$^{abc}$ | 11±1.15$^{bcdefghi}$ |
| 26 | MTU-1001 | Mandya | 113.4±17.6$^{cdefg}$ | 13±0.5$^{defghij}$ |
| 27 | KMP-105 | Mysore | 80±11.4$^{abcde}$ | 18±1.73$^{jklmno}$ |
| 28 | Salem | Mandya | 93.4±17.6$^{abcde}$ | 21±0.38$^{op}$ |
| 29 | Karibhatta | Belgaum | 80±20$^{abcde}$ | 16±1.04$^{jklm}$ |
| 30 | Gandhasali | Uttara Kannada | 93.4±13.4$^{abcde}$ | 16±1.73$^{jklm}$ |
| 31 | Mysore mallige | Uttara Kannada | 60±11.4$^{abc}$ | 12±1.2$^{cdefghij}$ |
| 32 | Naga rice | Uttara Kannada | 66.6±17.6$^{abcd}$ | 6±0.76$^{a}$ |
| 33 | Malgudi sanna | Dharwad | 86.6±17.6$^{abcde}$ | 9±1.10$^{abcde}$ |
| 34 | Red rice | Mandya(Shivalli) | 66.6±17.6$^{abcd}$ | 10±0.92$^{bcdefgh}$ |
| 35 | Basmati | Mysore | 166.6±6.6$^{h}$ | 19±1.23$^{lmno}$ |
| 36 | Raja Khaima | Haveri | 73.2±17.6$^{abcde}$ | 15±0.76$^{hijkl}$ |
| 37 | Mukanna sanna | Haveri | 80±23$^{abcde}$ | 14±1.88$^{efghij}$ |
| 38 | Bangara sanna | Haveri | 46.6±17.6$^{a}$ | 8±0.14$^{abc}$ |
| 39 | Jeerigesanna | Belgaum | 40±11.4$^{a}$ | 8±0.87$^{abc}$ |
| 40 | Maradda | Uttara Kannada | 60±11.4$^{abc}$ | 18±1.9$^{klmno}$ |
| 41 | D-Basmati | Uttara Kannada | 153.4±17.6$^{fgh}$ | 20±0.96$^{mnop}$ |
| 42 | Hamsa | Srinivaspura | 73.4±17.4$^{abcde}$ | 15±3.54$^{ijklm}$ |
| 43 | Sona Masuri | Davanagere | 86.6±4$^{abcde}$ | 8±0.63$^{abc}$ |
| 44 | Sona Masuri | Davanagere | 86.6±6.6$^{abcde}$ | 16±1.45$^{jklm}$ |

Values are the means ± SE of data from three different independent experiments. Mean values within the column followed by the same letters are not significantly different according to Duncan's multiple range test (DMRT) at P ≤ 0.05, DI (disease incidence).

disease of rice, carrying the potential to reduce yields by as much as 50% (Mew, 1987). From a broader perspective. *Xoo* belongs to a diverse and highly adapted genus *Xanthomonas* that includes more than 20 plant- associated

**Table 2.** Reaction of different rice cultivars to virulent isolate *Xoo*32.

| S/N | Cultivar | Lesion length | Disease incidence (%) |
|---|---|---|---|
| 1 | IR-20 | 1.6±0.12$^{cd}$ | 21±0.8$^{cdef}$ |
| 2 | IR-20 | 1.6±0.88$^{cd}$ | 25±1.3$^{cdefghij}$ |
| 3 | IR-64 | 6.2±0.19$^{o}$ | 56±1.7$^{op}$ |
| 4 | IR-64 | 4.8±0.18$^{n}$ | 48±0.7$^{no}$ |
| 5 | IR-30864 | 2.2±0.18$^{efgh}$ | 31±1.8$^{ghijkl}$ |
| 6 | KRH-2 | 5.7±0.23$^{o}$ | 51±2.8$^{nop}$ |
| 7 | THANU | 1.6±0.04$^{cd}$ | 27±1.5 $^{defghijk}$ |
| 8 | Prakash | 5.9±0.05$^{o}$ | 56±1.4$^{op}$ |
| 9 | Mangala | 2.3±0.01$^{fghi}$ | 35±2.7$^{kl}$ |
| 10 | KP-1 | 2.7±0.06$^{hij}$ | 23±1.3$^{cdefg}$ |
| 11 | Jaya | 5.8±0.22$^{o}$ | 57±1.5$^{p}$ |
| 12 | Jaya | 4.9±0.1$^{n}$ | 51±1.3$^{nop}$ |
| 13 | CTH-3 | 2±0.15$^{defg}$ | 28±1.6$^{efghijk}$ |
| 14 | CTH-1 (Mukthi) | 3.3±0.05$^{klm}$ | 57±1.4$^{p}$ |
| 15 | INTAN | 2.6±0.15$^{hij}$ | 26±1.9$^{defghi}$ |
| 16 | Mandya Vijaya | 2.4±0.1$^{fghi}$ | 45±0.6$^{mn}$ |
| 17 | BR-2655 | 1.6±0.04$^{cd}$ | 27±1.5$^{defghij}$ |
| 18 | Vikas | 1.8±0.12$^{def}$ | 22±1.0$^{cdefg}$ |
| 19 | Pushpa | 2.8±0.07$^{ijk}$ | 27±1.4$^{defghijk}$ |
| 20 | Sonam | 3.6±0.13$^{m}$ | 34±1.7$^{jkl}$ |
| 21 | Sonam | 2.5±0.15$^{hij}$ | 30±1.0$^{fghijk}$ |
| 22 | Rasi | 2.5±0.1$^{hij}$ | 44±4.0$^{mn}$ |
| 23 | Rasi | 1.8±0.18$^{def}$ | 25±1.3$^{cdefghij}$ |
| 24 | BPT-5204 | 1.6±0.02$^{cd}$ | 34±1.7$^{jkl}$ |
| 25 | MTU-1010 | 2.7±0.12$^{hij}$ | 23±2.1$^{cdefgh}$ |
| 26 | MTU-1001 | 3.6±0.02l$^{m}$ | 39±3.7$^{lm}$ |
| 17 | KMP-105 | 0.9±0.02$^{b}$ | 32±1.0$^{jklm}$ |
| 28 | Salem | 0.6±0.02$^{ab}$ | 31±3.7$^{ghijkl}$ |
| 29 | Karibhatta | 0.5±0.05$^{ab}$ | 16±1.0$^{abc}$ |
| 30 | Gandhasali | 0.7±0.05$^{ab}$ | 16±1.7$^{abc}$ |
| 31 | Mysore mallige | 0.7±0.02$^{ab}$ | 22±1.2$^{cdefg}$ |
| 32 | Naga rice | 1.6±0.05$^{cd}$ | 22±1.3$^{cdefg}$ |
| 33 | Malgudi sanna | 0.7±0.04$^{ab}$ | 20±0.8$^{bcde}$ |
| 34 | Red rice | 2±0.03$^{cdef}$ | 12±1.7$^{ab}$ |
| 35 | Basmati | 0.9±0.7$^{b}$ | 28±0.7$^{efghijk}$ |
| 36 | Raja Khaima | 0.8±0.02$^{ab}$ | 20±3.1$^{bcde}$ |
| 37 | Mukanna sanna | 0.7±0.04$^{ab}$ | 24±1.8$^{cdefghi}$ |
| 38 | Jeerigesanna | 0.3±0.03$^{a}$ | 10±1.2$^{a}$ |
| 39 | Bangara sanna | 0.6±0.03$^{ab}$ | 12±1.8$^{ab}$ |
| 40 | Maradda | 3±0.2$^{jkl}$ | 18±1.9$^{abcd}$ |
| 41 | D-Basmati | 1.7±0.03$^{de}$ | 27±2.4$^{defghij}$ |
| 42 | Hamsa | 1.1±0.1$^{bc}$ | 23±0.9$^{cdefg}$ |
| 43 | Sona Masuri | 2.5±0.22$^{ghi}$ | 27±1.0$^{defghijk}$ |
| 44 | Sona Masuri | 3.3±0.1$^{klm}$ | 32±0.6$^{ijklm}$ |

Values are the means ± SE of data from three independent experiments. Mean values within the column followed by the same letters are not significantly different according to Duncan's multiple range test (DMRT) at P ≤ 0.05, DI (disease incidence).

or plant pathogenic species. Each species may comprise one or more pathogenic varieties (pathovar; pv.), which demonstrate distinct host plant specificity or modes of infection. Collectively, different *Xanthomonas* species

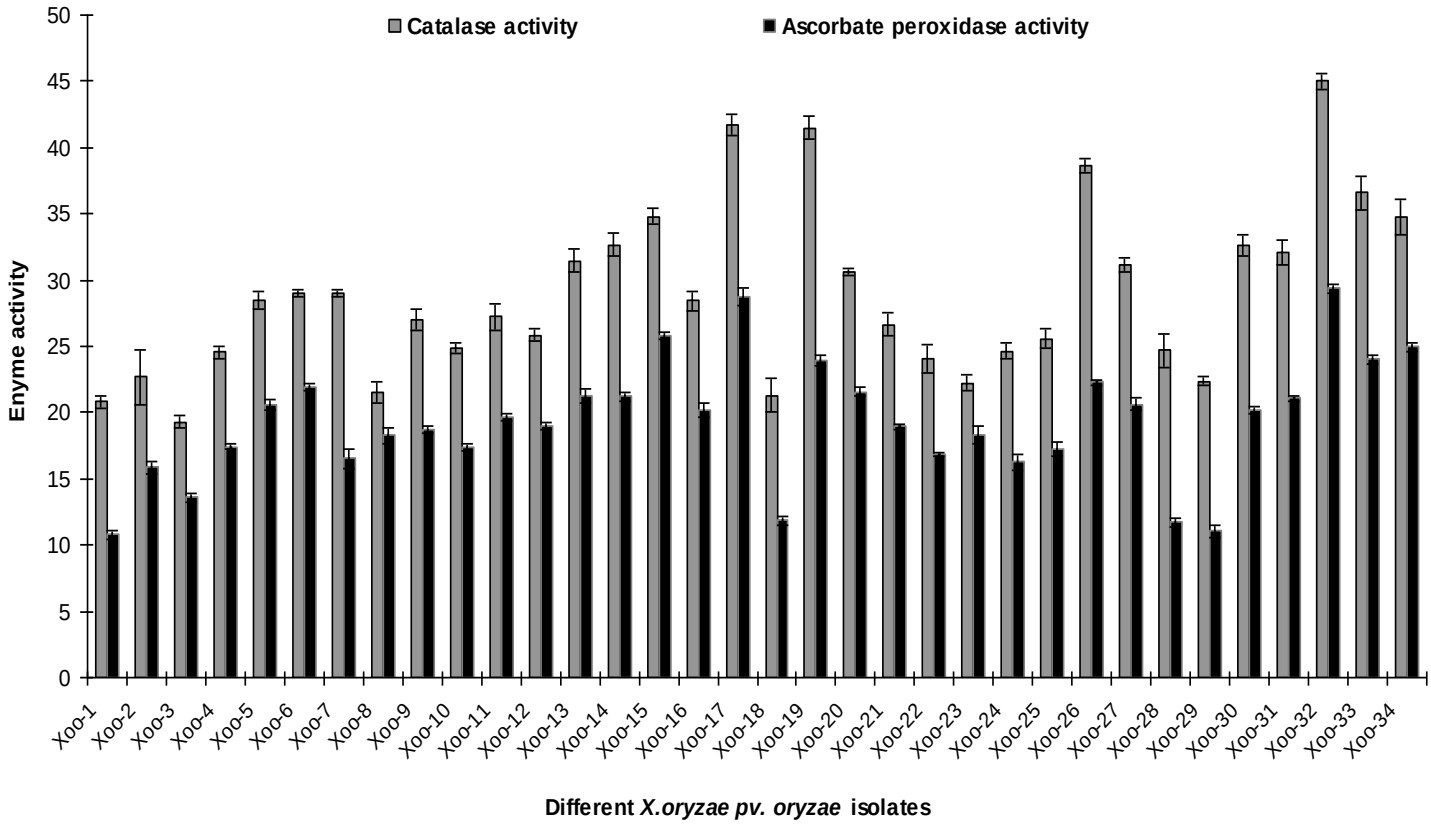


**Figure 1.** Catalase and ascorbate peroxidase activity of *X. oryzae* pv. *oryzae* isolates. Bars represents catalase and ascorbate peroxidase activity, respectively with SE.

and pathovars cause diseases in over 390 host plant species (Hayward, 1993).

Numerous attributes collectively contribute to the success of pathogenic bacteria in the host environment. The results in the present study strongly correlate the production of catalase, ascorbate peroxidase and EPS with virulence of *Xoo.* The 34 *Xoo* isolates studied differed in their ability to produce antioxidant scavenging enzymes and EPS. *Xoo* isolates clip inoculated to susceptible rice cultivar Jaya produced diverse lesion lengths which were strongly correlated with their catalase and ascorbate peroxidase producing ability. The isolate *Xoo*32 which showed highest of both catalase (45 µM $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein) and ascorbate peroxidase activity (29 µM ascorbate $min^{-1}$ $mg^{-1}$ proteins) produced maximum lesion length of 5.07 cm on susceptible rice cultivar Jaya. Previously, both the ability of bacterial isolate to produce catalase and the rate of lesion development were used as major criteria for classifying the pathogenic variability and differentiate among BLB-resistant cultivars, respectively (Koch and Mew, 1991; Chamnogpol et al., 1995). Similar observations were made by Choodamani et al. (2009), Cruz and Mew (1989), Kumar et al. (2000) and Kunstler et al. (2005). Choodamani et al. (2009) reported strong correlation among *Xoo* isolates with highest Catalase activity and higher lesion length in susceptible rice cultivar upon clip inoculation. The results from the catalase enzyme analysis related the *Xoo* isolates having variable level of catalase activity and differential expression of isoforms with their virulence. A virulence assay conducted on the susceptible rice cultivar Jaya revealed that the bacterial isolate with the highest level of catalase activity caused the maximum lesion length and vice versa. Subramoni and Sonti (2005) observed reduced catalase activity, and hypersensitivity to $H_2O_2$ was observed in virulence deficient *Xoo* mutant for ferric uptake regulator (*fur*) gene *Xoo*.

Previous studies indicated that EPS of *Xoo* could play an important role in pathogenesis of *Xoo* (Goto, 1972; Huang and De Cleene, 1988; Watanabe et al., 1993). Our data in the present study are consistent with their results, in that virulence of *Xoo* seemed to be affected by the amount of EPS production. The isolate *Xoo*32 which produced highest EPS (70 mg) induced maximum lesion length on susceptible Jaya cultivar. Vidhyasekaran et al. (1989) reported EPS accumulated in tiny veinlets might block water transfer in xylem vessels of rice plant. EPS productivity of *X. campestris* pv. *oryzae* affects movement of bacteria in infected xylem vessels (Watabe et al., 1993). Alfen et al. (1987) reported that the physical pressure exerted by bacterial EPS in xylem vessels may be important in facilitating the movement of bacteria in

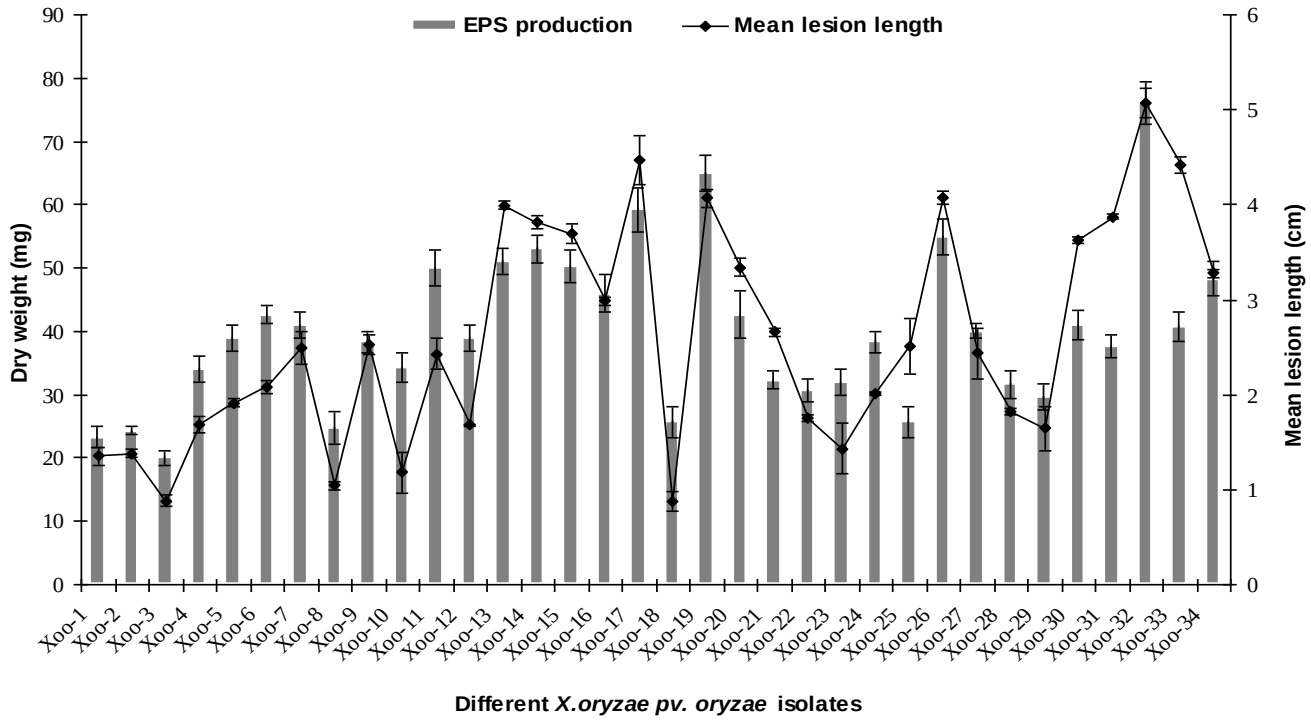


**Figure 2.** Extracellular polysaccharide (dry weight) production and virulence of *X. oryzae* pv. *oryzae* strains (Bar and line graph represents EPS production and mean lesion length), respectively with SE.

the vessels of infected plants.

The leaf clipping procedures of Kauffman et al. (1973) with the virulent *Xoo* is an effective method to determine the host resistance/susceptibility of rice to BLB disease. Resistance to BLB, as assessed by lesion length, increases considerably, with the plant reaching the maximum increase 30 to 50 days post-sowing (Koch and Mew, 1991). In our study, we used clip inoculation method to determine the virulence of bacterial isolates and reaction of rice cultivars to virulent isolate *Xoo*32. Among 44 cultivars screened, cultivar 'Jeerigesanna' recorded least bacterial blight disease incidence, with 0.7 cm lesion length. However, none of the cultivar tested were completely resistant to *Xoo* infection. This may be because of host-resistance, which may be due to the level of ROS more than the tolerance capacity of the pathogen (Mew et al., 1992). The analysis of antioxidant scavenging enzymes, catalase and ascorbic peroxidases, along with EPS production under laboratory conditions, is an alternative and accurate method to detect pathogenecity and virulence of *Xoo*.

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