RESEARCH ARTICLE

Effect of *Streptomyces xantholiticus* on Rice Blast Disease Reduction and Enzyme Activity

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ABSTRACT

The rice blast infection triggered by *Pyricularia oryzae* Cav. is a grave issue restraining rice yields globally. Biological regulation has been seen as an encouraging option to such chemical fungicides. The biological control proficiency of 24 isolates of *Streptomyces* against *P. oryzae* (KT693184.1) has been examined. Analysis of defense enzymes such as polyphenol, peroxidase (PO), and total protein and phenol content was performed. The *Streptomyces* isolate that was determined to have maximum effectiveness was determined to be *Streptomyces xantholiticus*. The sequence was sent to the NCBI vide accession number MW029942. The *S. xantholiticus* isolate inhibited *P. oryzae* 92% using dual culture tests. The greenhouse experiments showed that the disease gravity in rice plants with *S. xantholiticus* isolate was considerably lower compared to plants sans the treatment. The *S. xantholiticus* isolate was analyzed for its effects concerning the activity of polyphenol and Po enzymes as well as the total protein content present in rice leaves under greenhouse conditions in both presence and absence of *P. oryzae*. It was found that rice species treated with the *S. xantholiticus* isolate had enhanced resistance to the rice blast pathogen along with increasing activity levels of the polyphenol and PO enzymes. There was a direct impact on pathogen growth along with the defense response triggered in the plants as a result of the treatment.

Keywords: *Pyricularia oryzae*; Peroxidase enzyme; Polyphenol oxidase; Plant growth; Biological control

INTRODUCTION

Rice is the principal food source for over three billion people across the globe. However, rice is susceptible to infections, the most damaging being rice blast, which is triggered by the fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*). This fungus attacks rice plants at all phases of development, leading to yearly losses of around 10–30% in different rice producing areas (Law et al., 2017 and Couch et al., 2005).

The efforts to reduce the usage of fungicides make it essential to formulate cost-effective and effectual approaches to attain products from endogenous microorganisms under biocontrol application (Adnan et al., 2019). The biocontrol factors lead to the development of induced systemic resistance (ISR), enhancement of the mechanical and physical strength of the cell wall. Furthermore, the host undergoes biochemical and physiological changes, thereby causing the production of compounds that act as a defense against the pathogens.

Streptomyces species have been observed to be biocontrol agents useful against many plant pathogens (Prabavathy

et al., 2006; Karthikeyan and Gnanamanickam, 2008) and have displayed the potential to create bioactive compounds, as well as decrease or deter mycelia growth of numerous fungi (Bressan and Figueiredo, 2008). The *Streptomyces* strain UPMRS4 was stated as a possible biocontrol agent for rice blast infection (Awla et al., 2016; Awla et al., 2017; Awla and Rashid, 2020). Furthermore, a higher activity of PO and polyphenol oxidase (PPO) in reaction to the infection by the pathogen is said to play a dynamic part in disease resistance in specific plant hosts. This is the pathogen interaction after the infection (Vidhyasekaran, 2004). According to Pradeep and Jambhale (2002), oxidative enzymes and phenolic compounds are said to be a vital biochemical factor for the aptitude of the plant to battle diseases. These bioactive compounds exhibit crucial uses in different domains. For instance, around 75% of commercially beneficial antibiotics were obtained from the genus *Streptomyces* and they are therefore the main antibiotic producing organisms manipulated by the pharmaceutical sector (Kashif et al., 2016). *Streptomyces* strains too have significant uses in the agricultural domain by means of their biological control ability against phytopathogens, mainly phytopathogenic fungi (González-Franco and Robles-Hernandez, 2009). Chung et al., (2005) approved the

ability of *Streptomyces xantholiticus* against *Rhizoctonia solani* to control of damping-off of Chinese cabbage (*Brassica chinensis*). Thus, the current work assesses the efficacy of *S. xantholiticus* to prompt systemic resistance against *M. oryzae* when contaminating rice plants.

MATERIALS AND METHODS

Sample Collection and Isolation

Samples of soil were gathered from rice irrigated fields of the Harir/Erbil rice growing region. Samples were picked from different depths from the surface and close to the root (Shahidi et al., 2005). The gathered samples were carried to the laboratory polymerase chain reaction for processing. Five spatulas from every soil sample were subject to air-drying at room temperature for 7–10 days (Shahidi et al., 2005). The *Streptomyces* were isolated using several dilutions using *Streptomyces* Isolation-agar (Difco™) medium along with cycloheximide (100 µg/mL) using 9 cm long Petri dishes. The incubation temperature was $28 \pm 2^\circ\text{C}$, and the samples were monitored for 5 days. Then, the untreated culture was retained in Potato Dextrose Agar slants at 4°C for future use (Rahman et al., 2011).

Antagonistic Activity

The evaluation of the antagonistic activity against *P. oryzae* (Accession no. KT693184.1) was performed *in vitro* using 24 *Streptomyces* isolates (Dean et al., 2005). The streak technique was used in triplicate (Ramesh et al., 2009), and the incubation temperature of the plates was set to $28 \pm 2^\circ\text{C}$. The antagonistic impact of the tested fungus was assessed through the formula given below:

$$I = \left[\frac{C - T}{C} \right] 100$$

where:

I = % of inhibition in the mycelia growth.

C = the growth of pathogens in the control plates.

T = the growth of pathogens in the dual culture plates.

Streptomyces Identification

The optimal *Streptomyces* isolate was selected as per the antagonistic activity. The cetyltrimethylammonium bromide technique (Awla et al., 2017) was used for genomic DNA removal from the *Streptomyces* isolate. For the identified *Streptomyces* isolate, the 16S rDNA sequences were aligned as per the sequences of the indicative bacteria from the *Streptomyces* submitted to the GenBank (NCBI).

In vivo Activity

The soil was taken from Erbil Governorate (pH 5.5–6). Its constitution was sand, clay, and organic matter in 1:1:1 v/v

concentration. The soil sample was air-dried and sent through a sieve (0.5 mm); the sample was subsequently sterilized for 60 min at 121°C using an autoclave after which, it was left off to cool for 24 h before any subsequent test. The surface sterilization of the rice seeds was conducted by soaking them in 95% ethanol solution for 10 s; subsequently, the seeds were treated with 3% sodium hypochlorite solution for 60 s. Finally, the seeds were washed using sterile distilled water for a total of 6 times.

After surface sterilization, the rice seeds were planted directly into clean plastic seedling growth pots; the planting density was five seeds per pot. Tap water was used to water the plants every day. A completely randomized design comprising four replications was used to complete the experiment.

A conidial suspension comprising *S. xantholiticus* having a concentration of 10^9 cfu/ml was diluted using 5 L of water, thereby resulting in a solution have a concentration of 2×10^5 cfu/ml. 100 ml of the diluted solution was used of *S. xantholiticus* for every pot (Mahato et al., 2018). After 1 day *P. oryzae* conidia suspension containing 3×10^5 conidia/ml (15 ml/pot) (Chen et al., 2001) was sprayed on the rice seedlings and after 5 days, lesion lengths developed on inoculated leaves.

Total Protein Content Measurement

The protein concentration was ascertained through the Bradford assay technique, explained by Arora and Wisniewsk (1994).

Determination of Total Phenolic Content

The overall phenolic content of the inoculated and un-inoculated rice leaves was ascertained through Folin-Ciocalteu assay (Noreen et al., 2017).

Quantification of PPO Activity

The mixture used for the reaction comprised 0.5 ml of the enzyme extract was mixed with 2.3 ml phosphate buffer of 0.1 M concentration (pH–6.1). These solutions were mixed using a cuvette and adjusted so that the spectrophotometer reflected zero absorbance (Mahadevan and Sridhar, 1982). Subsequently, 0.2 ml of catechol solution of 0.1 M concentration was added to the mixture and mixed swiftly. Changes to absorbance, measured per minute, (ÅA/min) were used to determine enzyme activity; changes were observed at 400 nm just after 0.2 ml of the 0.1 M catechol solution was added. The catechol solution started the reaction.

Quantification of PO Activity

The activity of the PO enzyme was ascertained using the leaves of the un-inoculated rice plants, and those from

inoculated plants (Sreedevi et al., 2011). 0.5 g of the fresh harvest was ground using a pre-cooled mortar filled with 20 ml of the 0.1M concentration phosphate buffer (pH 7.1) at ice-cold temperature; subsequently, the solution was subjected to 2000 RPM centrifugation for 10 min. The supernatant used for the assay comprised 25 ml Pyrogallol reagent having 0.2 M concentration was freshly prepared, and 0.1 ml of this reagent was mixed with 1.4 ml phosphate buffer (0.1 M, pH-7.1) and 1.0 ml of enzyme extract using a cuvette tube. Immediately after mixing, the solution was adjusted so that it reflected zero absorbance on the spectrophotometer. Cuvette inversion was used to mix the 0.5 ml, 0.01 M hydrogen peroxide solution that was added to the initial solution. The change in absorbance per minute ($\Delta A/\text{min}$) at 430 nm was used to monitor enzyme activity.

RESULTS AND DISCUSSION

Antagonistic Activity

The results indicate that one isolate from a total of 24 produces the highest antagonism against *P. oryzae*, where the radial growth inhibition was 92%. The isolate has been determined to be *S. xantholiticus* and has been sent to the NCBI (accession number: MW029942). Several studies have indicated that *Streptomyces* spp. is able to control *P. oryzae* for example, *Streptomyces* spp. (Rhee, 2003; Law et al., 2017; Awla et al., 2017; Bibb, 2005); *Streptomyces sindenensis* (Zarandi et al., 2009), and *Streptomyces globisporus* (Li et al., 2011).

Disease Suppression

Plant growth and disease development witnessed significant impact as a result of the glasshouse experiment given the non-homogenous nature of the glasshouse. It took 3 days for the symptoms to appear as purple spots then changed to spindle-shaped lesions with gray center and reddish-brown border eventually surrounded by a yellow zone.

Total protein Content

A marked decrease was observed concerning the total soluble protein amount in the case of rice plants infected by *P. oryzae*, the measurements were taken after 5, 10, 15, and 20-day intervals, as showed in Figure 1. *S. xantholiticus* was observed to have higher effectiveness in increasing the total protein content for infected plants compared to the control samples. Observations indicated a significant rise in proteins for all the cases where the rice plants inoculated with the pathogen and *S. xantholiticus*. At the same time, the plants that received only the pathogen inoculation had the lowest amount of protein. The observed and significant decrease in rice tissue due to the infection could be attributed to specific activities triggered by a hypersensitive response (Chandra and Bhatt, 1998). Such defense reactions are triggered as a result of the build-up of PR proteins such as phenylalanine ammonia-lyase, phenolics, citinase, α -1,3-glucanase, phytoalexins, and PO (Christopher et al., 2010).

PO Enzyme Activity

Figure 2 showed the activity specific to the PO enzyme. A significant increase in PO was observed for all samples compared to the control sample after increasing the period. All rice plants except those inoculated with *S. xantholiticus* and the control samples, PO activity witnessed a rise till the 10th day; subsequently, the activity levels were either stable or reducing.

Several antioxidants such as PPO and PO may be responsible for oxygen metabolism happening in a diseased plant (Morkunas and Gmerek, 2007). The previous research indicates the higher activity of such enzymes at the host-tissue level as a consequence of the pathogenic infections (Abo-Elyousr and El-Hendawy, 2008, Christopher et al., 2010; Ojha and Chatterjee, 2012).

Figure 3 showed the total phenol level as a consequent of *S. xantholiticus* and the pathogen. The control specimens

Figure 1: Effect of *Streptomyces xantholiticus* on the total protein content (mg/g) of the inoculated rice plants with *P. oryzae*. (Co.=Control, Cof = Inoculated plant, Ts = Plant with *Streptomyces*, and Tsf = Inoculated plant with *Streptomyces*)

Figure 2: Effect of *Streptomyces xantholiticus* on the peroxidase activity of the inoculated rice plants with *Pyricularia oryzae*

Figure 3: Effect of *Streptomyces xantholiticus* on the total phenol of the inoculated rice plants with *Pyricularia oryzae*

were found to have the lowest phenol levels at the 5th and the 10th days after beginning the treatment; a small increase in the phenol level is observed on the 15th day. Samples inoculated with the pathogen, *S. xantholiticus*, and a combination of these two demonstrated an increase on the 5th and 10th days of the treatment. Concerning the plans inoculated with only the pathogen were found to have lesser phenol on the 15th day, as opposed to an increase on the 20th day. At the same time, the plants treated with *S. xantholiticus* are found to have the highest total phenol level, and they retain that level.

On the 5th and the 10th day of the experiment, the rice plants inoculated with *S. xantholiticus* were found to have higher polyphenol activity, which decreased slightly on the 15th day and witnessed an increase on the 20th day [Figure 4]. Control specimens and the plans inoculated only with the pathogen were found to have the lowest polyphenol level for the complete duration of the treatment. At the same time, specimens inoculated with the pathogen and then treated using *S. xantholiticus* were found to have a high

increase in polyphenol levels on the 5th day, which then decreased gradually during the remaining duration of the experiment.

Numerous studies suggest a rise in polyphenol and PO enzyme levels during the use of *Streptomyces*. One example is soil application and seed treatment using *Streptomyces* sp., which has a very pronounced effect on the polyphenol and PO activity levels in mung bean (*Vigna radiata*) (Adhilakshmi et al., 2014). Higher activity of the PO (POX) and PPO enzymes in the plants treated using *Streptomyces* indicates the development of biocontrol-ISR in the case of cucumber, unlike *Phytophthora drechsleri* (Sadeghi et al., 2017). The results indicate that treatments using *Streptomyces hygroscopicus* subsp. *angustmyceticus* NR8-2 led to an increase in the activity levels of PO, phenylalanine ammonia-lyase, and PPO enzymes present in oil palm (Sunpapao et al., 2018). Higher activity levels of PPO-1 and PPO-2 of the treated samples compared to the control samples were observed for groundnut species treated using the three strains of *Streptomyces* sp. using a seed-and-soil

Figure 4: Effect of *Streptomyces xantholiticus* on the polyphenol activity of the inoculated rice plants with *Pyricularia oryzae*

combination, only seed, or control rotting of the stem as a consequence of *Sclerotium rolfsii* (Adhilakshmi and Velazhahan, 2013).

One study concerning *Bacillus subtilis* for controlling *M. oryzae* indicated higher PO and PPO activities. At the same time, the activity of superoxide dismutase was significantly higher for rice species sprayed with *B. subtilis* (Sha et al., 2016).

CONCLUSION

Treated rice plant with *S. xantholiticus* is observed to have an ability for inducing specific localized and systemic resistance in response to *P. oryzae* infection. The plant responds by effecting a change in PO activity, PPO activity, and the total levels of phenol and protein. Such mechanisms facilitate resistance build-up in rice species. The present study suggests that triggering a plant's natural defense systems using biocontrol substance is a useful technique for disease management in rice.

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