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Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5

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Abstract

Two antifungal aliphatic compounds, SPM5C-1 and SPM5C-2 with a lactone and ketone carbonyl unit, respectively obtained from *Streptomyces* sp. PM5 were evaluated under *in vitro* and *in vivo* conditions against major rice pathogens, *Pyricularia oryzae* and *Rhizoctonia solani*. These compounds were dissolved in distilled water/medium to get the required concentrations. The well diffusion bioassay indicated that the of SPM5C-1 remarkably inhibited the mycelial growth of *P. oryzae* and *R. solani* in comparison to SPM5C-2. Though SPM5C-2 showed low antifungal activity against *P. oryzae,* it was not active against *R. solani.* Further, SPM5C-1 completely inhibited the growth of *P. oryzae* and *R. solani* at concentrations of 25, 50, 75 and 100 μg/ml. Greenhouse experiments revealed that spraying of SPM5C-1 at 500 μg/ml on rice significantly decreased blast and sheath blight development by 76.1% and 82.3%, respectively, as compared to the control with a corresponding increase in rice grain yield.

Keywords: *Streptomyces sp.*; Antifungal metabolites; Rice; Blast; Sheath blight; *Pyricularia oryzae*; *Rhizoctonia solani*

1. Introduction

Actinomycetes are potent producers of wide variety of secondary metabolites with diverse biological activities, which includes therapeutically and agriculturally important compounds (Tanaka and Omura, 1993; Lange and Sanchez Lopez, 1996). Actinomycetes effectively inhibit many soil fungi by degrading their chitinous cell walls (Keast and Tonkin, 1983; Xiao et al., 2002) and some of them are hyperparasites of fungal pathogens (Zuberu et al., 1988; Sutherland and Papavizas, 1991). They have been evaluated as biocontrol agents against various plant pathogens (Zheng et al., 2000; Ouhdouch et al., 2001; Sabaratnam and Traquair, 2002; Chung et al., 2005).

Actinomycetes play a pivotal role in maintaining a satisfactory biological balance in soil, largely because of their ability to produce antibiotics and other secondary metabolites (Strohl, 2004). Among actinomycetes, the members of the genus *Streptomyces* are considered economically important because they alone constituted 50% of the total soil actinomycetes population (Xu et al., 1996) and 75% of total bioactive molecules are produced by this genus (Demain, 2000). The streptomycetes produce an array of secondary metabolites such as enzyme inhibitors, herbicides and large number of antibiotics (Omura, 1992; Lange and Sanchez Lopez, 1996) and hence, they are widely recognized as important organisms from the academic and industrial point of view (Demain, 1999).

The antibiotic metabolites of microbial origin have been included in the biological control as supplement or an alternative to chemical control for the management of plant diseases (Fravel, 1988; Shimizu et al., 2000). The first success of microbial metabolites in plant protection was the use of streptomycin for the control of fire blight of apple and pear caused by *Erwinia amylovora* (Beer et al., 1984; Vanneste et al., 1992) and it was also used for the control of many other bacterial diseases of crop plants (Colins and Mc Carter, 1983; Colins and Chafik, 1986). Microbial metabolites attract increasing attention as potential plant protection agents because they are expected to overcome

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the pollution problems caused by the use of synthetic chemical pesticides. Several novel metabolites of actinomycetes have been discovered and proved as useful molecules for the control of plant diseases, insect pests and weeds (Copping, 1996; Yamaguchi, 1996; Li et al., 2003).

Indian soils are rich in microbial diversity especially actinomycetes (Malarvizhi and Mathivanan, 2004) and the wealth of indigenous micro-flora of India has not been fully explored. In this scenario, it is worthwhile to isolate microorganisms especially the streptomycetes from Indian soils to investigate the production of novel antimicrobial metabolites, which would eventually lead to the development of novel bioactive compounds. Hence, research has been initiated to identify novel metabolites from streptomycetes as agroactive molecules for controlling plant diseases. About 35 actinomycetes were isolated from soil samples collected from different parts of South India, of which, an isolate with a wide spectrum antimicrobial activity was selected and identified as *Streptomyces* sp. Two antimicrobial metabolites, SPM5C-1 and SPM5C-2 with lactone and ketone carbonyl functional group, respectively were isolated from the culture filtrate of *Streptomyces* sp. PM5 and characterized (Prabavathy, 2005). The purpose of this study was to evaluate these compounds in suppressing blast and sheath blight development in rice under greenhouse conditions.

2. Materials and methods

2.1. Production, purification and characterization of antimicrobial compounds from Streptomyces sp. PM5

Different experiments have been previously reported to standardize fermentation parameters for *Streptomyces* sp. PM5 and optimized the production of antimicrobial metabolites, including SPM5C-1 and SPM5C-2 (Prabavathy, 2005). *Streptomyces* sp. PM5 was cultured in a 14 L total volume capacity fermentor (Biostat-B, B-Braun, Germany) using 8 L of dextrose soybean meal yeast autolysate broth (DSYB) containing (g/L) dextrose, 40.0; soybean meal, 12.5; yeast autolysate, 7.5; pH 7.2. The fermentation process was initiated by the inoculation of three days old pre-cultured *Streptomyces* sp. PM5 at 6% (v/v) in to the fermentor vessel. The culture medium was agitated at 250 rpm and the aeration flow was maintained at 2 volume of air/volume of liquid/minute (VVM). The fermentation was carried out for five days at 28 °C.

The metabolites were extracted from cell free fermented broth using ethyl acetate and concentrated to a viscous material. The concentrated crude metabolites were partially purified by passing through 60–120 mesh followed by 100–200 mesh silica gel column chromatography. From the partially purified metabolites, two major compounds were successfully purified by vacuum column chromatography using thin layer chromatography (TLC) grade silica gel (ACME, Mumbai, India) and coded as SPM5C-1 and SPM5C-2.

After purification, various spectral analyses were carried out to characterize the compounds. Both of them were UV-inactive and the probable molecular mass was determined as 310.6 and 434.1, respectively, for SPMC-1 and SPMC-2. IR spectra of both SPM5C-1 and SPM5C-2 showed the presence of hydrogen bonded-OH group and they were aliphatic in nature. The SPM5C-1 had a lactone carbonyl unit whereas, SPM5C-2 had a ketone carbonyl unit. The ^{1}H NMR spectral analyses confirmed the absence of aromatic C–H protons and the presence of different types of aliphatic protons in both SPM5C-1 and SPM5C-2. These purified compounds, in aqueous solutions were used for bioassays and greenhouse experiments against rice pathogens.

2.2. In vitro bioassay of purified compounds on rice pathogens

The antifungal activity of the purified compounds of *Streptomyces* sp. PM5 was studied by agar diffusion test using potato dextrose agar (PDA). Mycelial discs of *P. oryzae* (Couch and Kohn, 2002) and *R. solani* were cut from 7 and 5 days old cultures, respectively and placed in the center of the respective PDA plates. Three wells were made in each plate using a sterile cork borer and 50 µl aqueous solutions of each compound at 50 µg/ml concentration was placed in respective wells. Sterile water was placed in the control wells. Triplicate plates were maintained for these experiments. The plates of *P. oryzae* and *R. solani* were incubated at 23 ± 1 °C and room temperature (28 ± 2 °C), respectively. The zones of inhibition were measured after 9 and 5 days for *P. oryzae* and *R. solani*, respectively.

In another experiment, increasing concentrations of the purified SPM5C-1 were tested against the rice pathogens by poison plate method using PDA. Calculated amounts of SPM5C-1 were added to the molten PDA to obtain concentrations of 25, 50, 75 and 100 µg/ml. PDA alone served as the control. Mycelial discs of *P. oryzae* and *R. solani* were placed in the centre of the SPM5C-1 amended plates and incubated. Triplicate plates were maintained for each treatment. The mycelial growth of *P. oryzae* and *R. solani* was recorded after 9 and 5 days, respectively.

2.3. Evaluation of purified compounds on blast and sheath blight diseases of rice in greenhouse

2.3.1. Experimental details

The purified compounds, SPM5C-1 and SPM5C-2, were evaluated against blast and sheath blight diseases of rice caused by *P. oryzae* and *R. solani*, respectively, in two different potted plant experiments using a complete randomized block design with four replications. Wet nursery was prepared in earthenware pots and the rice seeds of IR50 (susceptible to blast and sheath blight) were sown. Rice