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Studies on Interaction of *Serratia marcescens* Strain (SR $_1$) with Fungal Pathogens

K. Parani and B.K. Saha

Research and Development, Multiplex BiotechPvt. Ltd, Peenya 1st stage, Bangalore -560058, India

Abstract: Growth of the bacterium *i.e., Serratia marcescens strain* (SR_1) and its production clear zone was more in Chitin Agar (CA) medium compared with the Potato Dextrose Agar (PDA) medium. This bacterium was found to be antagonistic to fungal pathogens *viz., Alternaria alternate, Aspergillus niger, Fusarium oxysporum, Helminthosporium* sp, *Curvularia* sp, on CA and PDA media. S*erratia marcescens* strain (SR_1) was able to reduce the mycelial growth of *Aspergillus niger* recorded as 66.5 %, *Fusarium oxysporum* (64.4 %) when grown on PDA plates. *Aspergillus niger* and *Helminthosporium* sp showed the maximum radial growth (35.0 mm and 45.0 mm) with abundant mycelial proliferation on 7 days grown CA and PDA plates.

Key words: *Serratia marcescens* · Growth Inhibition · Colloidal Chitin · Antagonism

INTRODUCTION

Pathogenic micro - organisms affecting plant health are a major and chronic treat to food production and ecosystem stability worldwide. As agricultural production intensified over the past few decades, producers became more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However increasing use of chemical inputs causes several negative effects, *i.e.,* development of pathogen resistance to the applied agents their non - target environmental impacts [1]. Furthermore, the growing cost of pesticides, particularly in less - affluent regions of the world and consumer demand for pesticide - free food has led to a search for substitutes for these products. There are also a number of fastidious diseases for which chemical solutions are few, ineffective / non - existent [2]. Biological control is thus being considered as an alternative / a supplemental way of reducing the use of chemicals in agriculture [3].

Serratia marcescens, a gram negative bacterium, classified in the large family of Enterbacteriaceae, soil inhabitant, is very efficient in degradation of chitin because of its ability to produce different chitinolytic enzymes [3]. *Serratia* can be distinguished from other genera by its production of three special enzymes DNAase, lipase and gelatinase [4]. Major objective of this study was to isolate a potentially useful bacterial antagonist for biocontrol of fungal pathogens.

MATERIALS AND METHODS

Organisms: The fungal pathogens *viz., Aspergillus niger, Alternaria alternata, Fusarium oxysporum, Helminthosporium sp., Curvularia sp.* andbacteria *i.e., Serratia marcescens* were isolated from soil by serial dilution and pour - plate technique and identified as per the methodology described by Aneja [5]. These Fungal organisms were sub - cultured in PDA slants and *Serratia marcescens* was streaked in Nutrient agar slants by maintaining at ± 4°C.

Media Composition: Chitin plates were made with use of chitin agar medium containing 1% colloidal chitin (prawn shell), 0.5% yeast extract, 0.05% $MgSO_4$, 0.2% sodium nitrate, 0.05% KCl, $FeSO_4$ pinch, 0.1% K_2HPO_4 and 2% agar (w/v), adjusted to pH 6.0 using 1N NaOH/ HCl. Colloidal chitin was processed using prawn shell [6]. The medium was autoclaved at 121°C for 15-20 mins.

Antagonism Between Bacteria and Fungi: Antagonism between the bacteria and fungi was determined by placing 5mm discs of fungal pathogens were placed on one side of 2% PDA plates and Chitin agar (CA) plates containing colloidal chitin at 1.0% and incubated at 25±2°C for 2 days. After two days of incubation / growth, a loopful of overnight culture of *Serratia marcescens* strain (SR_1) was streaked on the opposite side of the fungus grown PDA and CA plates. The diameter (mm) of the inhibition

Corresponding Author: K. Parani, Research and Development, Multiplex BiotechPvt. Ltd, Peenya 1st stage, Bangalore -560058, India

zone between the bacteria and the fungus was used as an indication of the extent of antagonism. Three plates were used as replicates for each particular treatment. The plates were again incubated at 25 ± 2°C to study the colonization of fungus mycelium by bacteria *Serratia marcescens* strain (SR_1). Percentage Inhibition was calculated as:

$$\frac{\text{Colony growth diameter in checked plates - Colony growth diameter in each treatment}}{\text{Colony growth diameter in checked plates}} \times 100$$

Growth Studies: The effect of different media on fungal growth was studied under *in vitro* conditions. Fungal growth was determined by inoculation of PDA and CA plates with an agar disc (5 mm) diameter of actively growing young mycelium placed in centre of the medium. All fungal cultures were incubated in a dark cultivation chamber at 25±2°C with 70 % relative humidity for one week. Three plates were used as replicates for each particular treatment.

RESULTS AND DISCUSSION

Data in Table 1, show the Morphological characters, Colony characters of isolated fungi from the soil. The isolated identified fungi were as *Aspergillus niger, Alternaria alternate, Fusarium oxysporum, Helminthosporium* sp, *Curvularia* sp.

The fungi tested were found to grow well on the Chitin agar and Potato dextrose agar medium. Fungi generally did not exhibit good / normal growth on the media that are commonly used as bacterial media. It was sometimes difficult to determine if the antagonistic reaction was the result of inadequate nutrients / of pH requirements for fungal growth.

The maximum radial growth was showed by *Aspergillus niger* and *Helminthosporium* sp, (45.0 mm) on PDA medium, while they showed 35.0 and 36.0 mm as radial growth when grown on CA plates (Table 2) other organisms showed similar trend in a lower extent.

The bacterium *Serratia marcescens* strain (SR_1) was found to cause a clear zone around its growth on Chitin Agar medium containing colloidal chitin as substrate. As the bacterium starts to digests the colloidal chitin supplemented in Chitin Agar medium, ultimately the production of red pigment was more. But in PDA inoculated plates, production of red pigment was not observed. Reduction in fungal growth as a result of the antagonist *S.marcescens* was more obviously when they grew on CA medium compared with PDA medium. The highest inhibition percentage was observed in The growth of *Aspergillus niger* as (66.5 %), *Fusarium oxysporum* (64.4 %) and *Curvularia* sp (57.52%) (Table, 3).

In Chitin supplemented agar medium, the growth and production of red pigment by *Serratia marcescens* (SR_1) was highly antagonist to the fungal pathogens *viz., Alternaria alternata, Aspergillus niger, Fusarium oxysporum, Helminthosporium* sp, *Curvularia* sp, which suggests that degradation of hyphal cell wall / cell death due to the production of chitinase ezymes. These results were positively correlated with Minerdi *et al.* [7]. They observed that the presence of a consortium of ectosymbiotic bacteria belonging to *Serratia, Achromobacter, Bacillus* genera associated to the mycelium of the pathogen *Fusarium oxysporum* MSA 35 showed that a depletion in production of micro - conidia, aerial hyphae and a change in shape and dimension of the conidia. Nobutaka *et al.* [8] reported that a synergistic inhibitory activity of prodigiosin by

Table 1: Identification of Fungal Pathogens from isolated soil

Isolate	Morphological Characters	Colony Characters	Identification
1.	Colony Growth: Fast Colour of Spores: Black Colony Texture: Velvetty	Conidiophore arising from a Foot Cell, Conidia on Phialides	*Aspergillus niger*
2.	Colony Growth: Slow Colony Colour: Grayish green	Transversely and longitudinally septate in acropetal manner, Beaked conidia	*Alternaria alternata*
3.	Colony Growth: Woolly Colony Colour: White to Pink	Sicke shaped transversely septate macro - conidia produced in sporodochia	*Fusarium oxysporum*
4.	Colony Growth: Fast Colony Colour: Gray to Black	Dark large, transversely Septate Conidia	*Helminthosporium* sp
5.	Colony Growth: Slow Colony Colour: Gray to Black	Conidia dark, more/less end cells lighter, fusiform typically bent / curved with one / two of the central cells enlarged	*Curvularia* sp

Table 2: Radial growth (mm) and their mycelial proliferation of fungal pathogens on two different media after 7 days incubation at 25±2°C

	Potato Dextrose Agar Medium		Chitin Agar medium	
Organisms	Radial Growth (mm)	Mycelial Proliferation	Radial Growth (mm)	Mycelial Proliferation
Aspergillus niger	45.0±2.1	++++	35.0±1.5	++
Alternaria alternata	42.0±1.5	++++	30.0±1. 2	+++
Fusarium oxysporum	42.0±1.5	+++	27.0±1.1	+
Helminthosporium sp.	45.0±2.3	++++	36±1. 4	++
Curvularia sp.	40.0±2.2	++++	30.0±1.4	++

++++: Heavy / Abundant growth ; +++: Moderate growth ; ++: Sparse; +: poor growth

Table 3: Inhibition Percentage (%) of *Serratia marcescens* strain (SR_1) against fungal pathogens on different media after 7 days incubation at 25±2°C

	Inhibition Percentage (%)	
Organisms	Potato Dextrose Agar Medium (PDA)	Chitin Agar Medium (CA)
Aspergillus niger	20.0±1.0	66.5±2.7
Alternaria alternata	33.3±1.3	50.0±2.1
Fusarium oxysporum	36.3±1.3	64.4±2.3
Helminthosporium	20.0±1.0	45.83±1.6
Curvularia	24.2±1.1	57.52±2.2

Serratia marescens and chitinolytic enzymes was observed against spore germination of *Botrytis cinerea*. Thus *Serratia marcescens* strain has multiple modes of action against the fungal pathogen.

However, the growth inhibition of these fungi by *Serratia* (SR_1) was markedly reduced on chitin agar media, where neither hydrolyzation of colloidal chitin nor production of reddish pigment by *Serratia* (SR_1) were detected. These findings were also positively correlated with Someya *et al.* [9]. The reddish pigment was fungitoxic to fungal pathogens and these results are compatible with previous reports on the antifungal action of *Serratia marcescens* and its chitinase towards other fungi [10,11].

The growth inhibition of fungal pathogens could be attributed to the production of extracellular enzymes of *Serratia marcescens* Strain (SR_1). In this regard, Sindhu and Dadarw [12] observed that five *Pseudomonads* strains were found to produce appreciable amounts of cellulose and chitinase enzymes in culture - free supernatants and showed growth inhibition of the two fungi *Pythium aphanidermatum* and *Rhizoctonia solani* in plates on PDA medium. Vaidya *et al.* [13]. *Alcaligenes xylosoxydans* was isolated and showed potential use as a antifungal biocontrol agent for the control of two fungal plant pathogens.

Chitinase produced by *Serratia plymuthica* C_{48} inhibited spore germination and germ - tube elongation of *Botrytis cinerea* [14]. Ordentlich *et al.* [15] observed that the ability to produce extra - cellular chitinases is considered as crucial for *Serratia marcescens* to act as antagonist against *Sclerotium rolfsii* and for *Paenibacillus* sp strain 300 and *Streptomyces* sp strain 385 to suppress *Fusarium oxysporum*. Although biocontrol activity of micro - organisms involving synthesis of allelochemicals has been studied extensively with free - living rhizobacteria, since they can also synthesize metabolites with antagonistic activity toward plant pathogens [16].

On the other hand, various factors produced by bacterium *Serratia marcescens* and *Pseudomonas* sp. [17,18] have been reported as elicitors including systemic resistance to plant diseases. Oligosaccharide - elicitors, which are produced by the degradation of the fungal cell wall with chitinase / ß - 1, 3 - glucanase, have been reported to play an important role in signal transduction essential for various plant defense mechanisms (e.g., Production of phytoalexins, lignification and induction of hyper sensitive reactions) reported by Lotan and Fluha, [19]. The bacterium produces chitinolitic enzymes, which causes degradation of the fungal cell walls and induction of plant defense reaction, in addition to the antifungal low - molecular weight molecule. These results suggest that this bacterium may be an effective and persistent biocontrol agent for both air borne and soil borne plant pathogens.

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