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ORIGINAL ARTICLE

Isolation of *Serratia marcescens* SR1 as a Source of Chitinase Having Potentiality of Using as a Biocontrol Agent

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Abstract *Serratia marcescens,* strain SR_1 was isolated from the local soil of a cultivated farm and it was screened as potent strain for chitinase production. Maximum chitinase production (77.3 u Mh^{-1} 100^{-1}) was observed after 96 h of incubation period with pH 5.5 at 30°C under shake conditions (120 rpm). Compare to still flasks, shake culture with prawn fish colloidal chitin of 0.5% (w/v) concentration, showed a better enzyme yield. Crude enzyme showed antifungal activity against plant pathogens.

Keywords Chitinase enzyme · Colloidal chitin · Plant pathogens · *Serratia marcescens*

Introduction

Chemical pesticides are widely used to control the plant diseases. However, most pesticides are hazardous to the ecological systems. Biological control promises to be a useful alternative approach in the control of plant pathogens in the sustainable agriculture system [1]. Biological pesticides are mainly formulated with the microbial metabolites. Among them, chitinase is a secondary metabolite novel enzyme, which effective against a number of plant pathogens. Chitinase catalyses the hydrolysis of chitin between the C_1 and C_4 of two consecutive *N*-Acetylglucosamines. The enzyme is regarded as important for the extensive carbon and nitrogen recycle in nature. Chitinase occurs widely in soil microorganisms and in some plants, fulfilling a possible defence role in them [2].

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Chitinase is found widely in the soil microorganisms, including *Trichoderma* sp. *Bacillus* sp., and *Serratia* sp have a chitinase producing ability [3–5]. It showed pesticidal effects against a number of plant pathogens including *Rhizoctonia solani* and *Fusarium oxysporium* causing wilt disease [6]. *Serratia marcescens*, a gram negative bacterium, soil inhabitant, is very efficient in degradation of chitin because of its ability to produce different chitinolytic enzymes [7]. Chitinase production and its activity depends on a number of limiting factors *viz*., culture state, temperature and pH of media etc.

In the present study, an attempt was made to optimize the production of chitinase enzyme from the isolated strain *S. marcescens* (SR_1) and screening of its biological activity against a number of fungal pathogens.

Materials and Methods

Isolation of Chitinolytic Microbes from Soil

A number of local strains of *Bacillus* sp., *Serratia* sp and *Trichoderma* sp. were isolated from the soil of Northern parts of Bangalore, India. The isolated strains were purified according to the standard microbiological techniques. Strains were identified as described [8]. The purified isolates were maintained in the nutrient agar and MEA slants at ±4°C in refrigerator.

Screening for Potent Strains

Purified strains were plated on the chitin plates for the determination of their chitinase activity. Chitin plates were made with use of chitin agar medium containing 2% 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 1 N NaOH/HCl. Colloidal chitin was processed using prawn shell [9]. The medium was autoclaved at 121°C for 15–20 min. The purified isolates were inoculated onto chitin plates and incubated for 3–5 days at 30 ± 2°C. The potent strain was selected on basis of the formation clear zones on the plates.

To standardize the enzyme production, a log phase culture of the potent strain (10^9 cells/ml) was inoculated into 100 ml (working volume) of sterile synthetic broth with colloidal chitin as substrate. The flasks were incubated at different time intervals, pH, temperature in both still and shake conditions. All experiments were carried out in triplicates.

Optimization studies for enzyme production and activity was continued through the fermentation of SR_1 using a 5 l glass fermentor with a provision of air at 0.8 vvm. The fermentor along with 3 l media with pH 5.0 was sterilized at 121°C for 15–20 min. The fermentor was inoculated with 300 ml log phase seed culture of SR_1 and fermentation was carried for a period of 8 days of incubation.

Assay of Cultures

At different incubation time intervals, cultures were clarified by filtration through Whatmann No.1 filter paper and centrifuged (1,800×*g* for 15 min at 1°C). Enzyme activities were determined after dialysis at 4°C for 24 h against about 200 vol. of distilled water, pH 6.0. The assay was based on the estimation of reducing sugars released during the hydrolysis of colloidal chitin. The reaction mixture, containing 1.0 ml of 0.5% colloidal chitin (suspended in 50 mM sodium acetate buffer, pH 5.2) and 1.0 ml of enzyme solution was incubated for 1 h at 40°C with intermittent shaking and then centrifuged at 4,000 rpm for 5 min. The amount of reducing sugar released in the supernatant was determined by using *N*-acetylglucosamine as standard [10].

Biological Activity Test

The cell free culture filtrate of the potent strain was tested against *Fusarium oxysporum, Rhizoctonia solani, Helminthosporium, Xanthomonas putida* by cup-plate method.

Results and Discussion

Identification of the Potent Strain

Comparative studies for chitinase activity showed that the strain SR_1, *Serratia marcescens* was the potent among the other isolates. Chitinase production in *S. marcescens* are induced by the inducer, are soluble oligomers derived from chitin, but not the monomer (*N*-acetylglucosamine) [11].

Effect on Age of the Culture

The chitinase activity of SR_1 was maximum at 96 h of incubation (77.3 μM h^{-1} 100^{-1}) when it was grown in liquid synthetic media, supplemented with prawn shell

Table 1 Effect of incubation time (h) on chitinase activity (μM/h/100 ml) by *Serratia marsecens* strain (SR_1) grown in still and shake cultures

S. no.	Incubation time (h)	Chitinase activity (μM/h/100 ml)	
		Still culture	Shake culture
1.	0	0	0
2.	24	17.3 ± 0.8	25.8 ± 1.3
3.	48	43.7 ± 2.1	50.1 ± 2.1
4.	72	59.4 ± 2.5	61.2 ± 2.5
5.	96	65.3 ± 3.2	77.3 ± 4.2
6.	120	48.6 ± 2.2	54.3 ± 2.5
7.	144	11.3 ± 0.9	21.2 ± 1.3

Table 2 Effect of temperature (°C) on chitinase activity (μM/h/100 ml) by *Serratia marsecens* strain (SR_1) grown in still and shake cultures

S. no.	Temperature (°C)	Chitinase activity (μM/h/100 ml)	
		Still culture	Shake culture
1.	0	0	0
2.	20	8.2 ± 0.42	18.7 ± 0.8
3.	25	23.4 ± 1.3	51.6 ± 2.3
4.	30	56.7 ± 2.5	76.7 ± 4.3
5.	35	52.1 ± 2.3	65.5 ± 3.7
6.	40	42.5 ± 2.1	55.3 ± 3.1
7.	45	35.3 ± 3.3	44.7 ± 2.1
8.	50	23.8 ± 1.3	35.2 ± 1.8
9.	55	14.5 ± 1.3	23.6 ± 1.3

Table 3 Effect of pH on chitinase activity (μM/h/100 ml) by *Serratia marsecens* strain (SR_1) grown in still and shake cultures

S. no.	pH	Chitinase activity (μM/h/100 ml)	
		Still culture	Shake culture
1.	0	0	0
2.	3.0	17.4 ± 0.8	26.4 ± 1.3
3.	5.0	57.3 ± 2.5	76.6 ± 4.1
4.	7.0	40.5 ± 2.1	60.3 ± 2.5
5.	9.0	29.3 ± 1.3	48.4 ± 2.1

Table 4 Effect of cell free culture filtrate of *Serratia marsecens* strain (SR_1) on growth of fungal pathogens

Culture filtrate (ml) of *Serratia marsecens* (SR_1)	*Fusarium oxysporum*		*Sclerotium rolfsii*		*Rhizoctonia solanii*		*Alternaria alternata*	
	Radial growth (mm)	% Inhibition zone	Radial growth (mm)	% Inhibition zone	Radial growth (mm)	% Inhibition zone	Radial growth (mm)	% Inhibition zone
Control	45.0 ± 2.1	0	45.0 ± 2.1	0	45.0 ± 2.1	0	45.0 ± 2.1	0
1.0	15 ± 0.7	66.6 ± 3.1	12.0 ± 0.81	73.3 ± 3.4	17.0 ± 0.8	62.2 ± 2.6	18.0 ± 0. 73	60.0 ± 2.5
3.0	10 ± 0. 52	77.7 ± 3.64	8.0 ± 0.34	82.2 ± 4.1	13.0 ± 1.1	71.1 ± 5.5	14.0 ± 0.63	68.8 ± 3.2
5.0	5.0 ± 0.23	88.8 ± 4.1	3.0 ± 0.12	93.3 ± 4.62	6.0 ± 0.24	86.6 ± 3.2	5.0 ± 0.23	88.8 ± 4.2

colloidal chitin, as the sole carbon source, at 30°C with pH 5.0 in shake culture (120 rpm) (Table 1). Compare to the shake culture, the still culture showing less activity (65.3 μM h^{-1} 100^{-1}). Similar results were observed in *Trichoderma harzianum* (39.1 μM h^{-1} 100^{-1}) [12].

Effect of pH and Temperature

A wide range of effect of pH on chitinase activity was shown by SR_1 (Table 3). The rate of hydrolysis of chitin was increased almost linearly between pH 3.5–5.0 in both still and shake cultures. A fairly broad optimum pH occurred with maximal activity at pH 5.0. Beyond which the activity slowly declined. Initial pH value of the culture medium of pH 5.0 showed the highest chitinolytic activity in 7-day-old shaken culture using *Aspergillus carneus* strain [13]. Studies over a different periods of incubation of SR_1, determined the optimum temperature of chitinase activity was at 30°C (Table 2). The optimum temperature reflects a balance between the effect on the activity and the maximum yield of the enzyme protein. This was positively correlated on the chitinase production by *Trichoderma harzianum* in submerged fermentation [14, 15].

Fermentations

The chitinase activity was increased at the beginning of the cultivation and reached to a maximum level at the log phase. Subsequently, the chitinase activity kept as plateau/decreased. The highest chitinase activity appeared on the fourth day from the day of inoculation. Started with fifth day, the production of chitinase was going to decreased. The decrease in the chitinase yield may be due to shear inactivation of the enzyme [16]. Similarly, the highest chitinase activity (18.2 mμ/ml) was obtained under the optimal cultivation conditions of aeration rate at 0.6 vvm, pH 4.0, agitation rate at 150 rpm and 24°C with a 5 l stirred tank bioreactor (STR) [17]. The analysis on fermentation process indicated that chitinase belonged to the growth associated enzyme and also an inducible enzyme. Accordingly, the synthesis of this kind of enzyme can be induced [18]. However, when the inducer is removed/the cell is in stationary phase, the enzyme synthesis will be blocked, indicating that, the mRNA of this kind of enzyme is unstable. Within a certain pH range would enhance the stability of the mRNA and thus prolonged the duration of enzyme production [19].

Effect of *S. marcescens* strain (SR_1) cell free filtrate on fungal pathogens: in order to find out, whether the bacterial component was responsible for the antagonistic/antifungal activity, the effect of culture filtrate on growth of fungal pathogens *viz., Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia solanii, Alternaria alternate* were studied. The addition of 1 ml cell free culture filtrate of *Serratia marcescens* was sufficient to inhibit the fungal growth by 60.0–73.3% for fungal pathogens with 3–4 days of incubation (Table 4). Increasing the amount of culture filtrate to 3 and 5 ml reduced the fungal biomass by 85–93% for fungal pathogens respectively.

Major objective of this study was to isolate a potentially useful bacterial antagonist for biocontrol of fungal pathogens. Chitinase plays a major role in degrading fungal cell walls [20]. Micro organisms capable of producing and excreting chitinase have been shown to be efficient biocontrol agents [21–23]. This study has demonstrated the presence of cell wall degrading enzymes in *Serratia marcescens* which is mainly responsible for antifungal activity.

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