Biocontrol efficacy of two *Bacillus* species against chilli pepper anthracnose causal agent *Colletotrichum truncatum*

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ABSTRACT

Applicability of two *Bacillus* strains isolated from standard compost was assessed as a reliable alternative in the management of the anthracnose in chilli pepper. Out of twenty promising antagonistic bacterial isolates, two were genotypically characterized as *Bacillus* spp. based on 16 S rRNA analysis. Selected two antagonistic *Bacillus* strains significantly suppressed the mycelial growth and the spore germination of chilli pepper anthracnose causal agent *Colletotrichum truncatum* ($p<0.05$). Malformation of the fungal hyphae was dominant in the area subjected to antagonism. One of the major antagonistic mechanisms extended towards the anthracnose pathogen *C. truncatum* by these two *Bacillus* strains was detected as antibiosis in the cellophane overlay technique. They produced antifungal peptides, lipopeptides and also minute amount of organic solvent soluble molecules as antifungal compounds against *C. truncatum*. These antifungal compounds showed minimum inhibitory concentrations of 3200 ppm and 2460 ppm on *C. truncatum*. Also these metabolites are thermally stable up to 120 ºC while they could retain the antifungal properties more than 10 weeks under cold conditions and up to eight weeks at room temperature. One of the *Bacillus* strains produces a bacteriocin like small peptide inhibitory against *C. truncatum*. Induction of latent host defense mechanisms is another antagonistic mechanism employed by this *Bacillus* sp. upon *C. truncatum* infection in chilli pepper. The tested *Bacillus* sp. could enhance the expression of defense related PAL and PO enzymes in chilli pepper plants upon *C. truncatum* infection. Also these antagonistic *Bacillus* strains promote chilli pepper plants' growth through the production of significant amounts of Indole Acetic Acid (IAA) which is a growth hormone as an indirect mechanism against *C. truncatum*. Also they showed a prominent swimming and swarming ability indicating their high potentiality in colonizing plant tissues paving the way for successful green house and field applications. These two strains effectively managed the seed borne infection of *C. truncatum* resulting healthy chilli pepper seedlings with good vigour. Also they effectively managed the fruit decay caused by *C. truncatum* at colour breaking stage and green stage suggesting the suitability of *Bacillus* spp. as a potential candidate to be used in the development of a biocontrol agent against chilli pepper anthracnose causal agent *C. truncatum*.

Keywords: Anthracnose, Antibiosis, *Bacillus* spp., Chilli pepper, *C. truncatum*, Defense induction, Growth promotion

INTRODUCTION

- Anthracnose is a destructive postharvest disease
- Extensive application of synthetic fungicides is the only control measure for this
- Synthetic fungicides have many harmful effects on living beings and the environment
- Therefore, attention has been paid on eco-friendly biological control methods
- Most focused biological control agents of phytopathogens
  - Beneficial microorganisms (PGPR)
  - Antimicrobial herbal preparations
- PGPR applied through
  seeds, soil, foliage, rhizomes or as a combination of several methods of delivery (Nakkeeran *et al.,* 2005)
- Antagonistic mechanisms can be antibiosis, competition, host defense induction or plant growth promotion

OBJECTIVE

To evaluate the biocontrol efficacy of two *Bacillus* species against *C. truncatum* in managing the chilli anthracnose disease

METHODOLOGY

Isolation of potential candidates

Isolation from standard compost → Screening 1. Co-cultivation 2. Dual culture → Identification → Effect on spore germination and hyphal morphology

Determination of antifungal metabolites production

a) Cellophane overlay technique (Nourozian *et al*., 2006)
b) Agar strip removal technique (Choi *et al*., 2006)

Properties of extracellular cell free culture supernatants

TSS; MIC; Heat stability; Time stability

Detection of plant growth promoting characteristics

1.) IAA production 2.) Phosphate solubilization 3.) Siderophore production 4.) Swimming & swarming

Detection of host defense induction ability

PAL Activity; PO Activity; PPO Activity

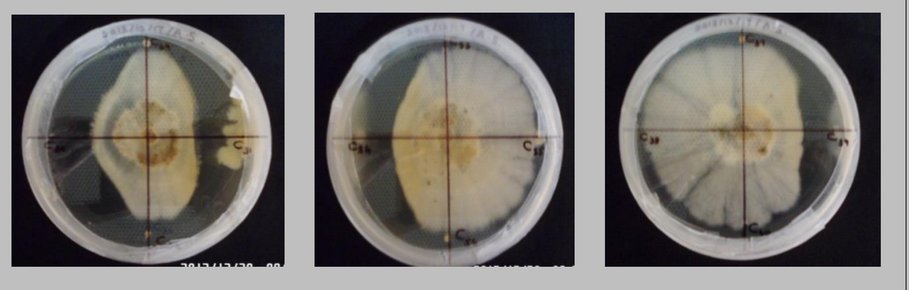
Biocontrol efficacy

a). At seed germination stage b). Different fruit growth stages

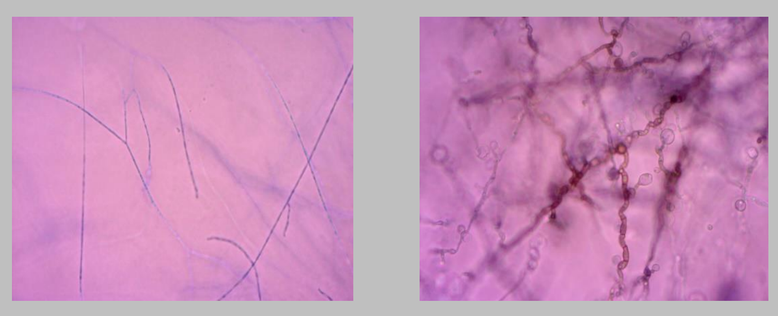
RESULTS AND DISCUSSION

Isolation and screening of antagonists

- Out of 84 isolates 69 were significant in co-cultivation
- Out of 69, 20 significantly inhibited *C. truncatum* in dual culture



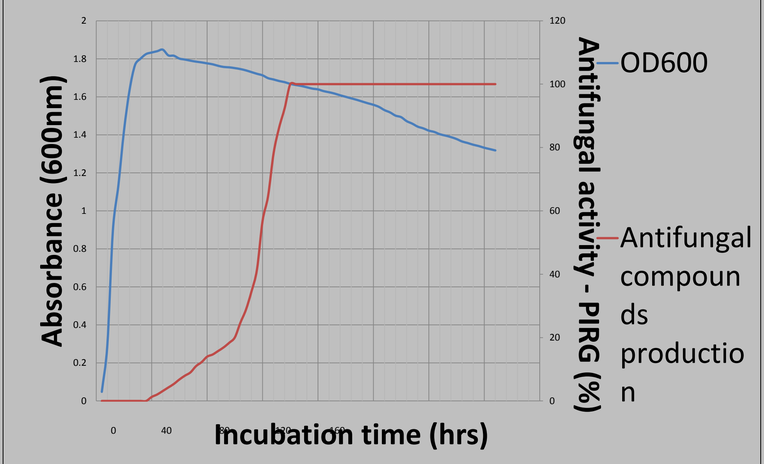
Hyphal deformations due to antagonism



Control Treated

***Bacillus* spp.**

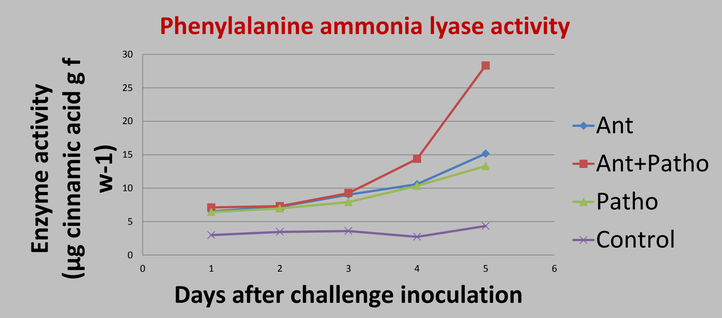
Antifungal metabolites Production

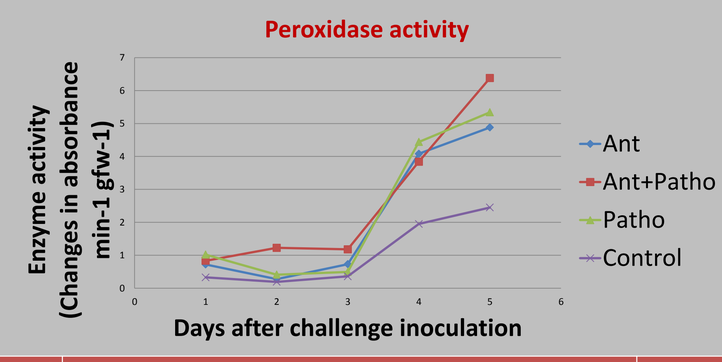


Properties of extra cellular metabolites

Culture filtrate	Initial bacterial cell concentration (cells/ml)	TSS (ppm)	MIC (ppm)	Heat stability (up to)	Durability (up to)	
					At room temperature	At 4 ºC
Bacillus sp. 1	10^8	9620	3200	100 ºC	8 weeks	10 weeks
Bacillus sp. 2	10^8	12340	2460	120 ºC	8 weeks	10 weeks

Host Defense Induction





Antagonist	PGP Test					
	IAA production (µg/ml)	Phosphate solubilization	Siderophore production	Exo-polysaccharides production	Swarming	Swimming
Bacillus sp. 1	21	n.d.	n.d.	n.d.	+	+++
Bacillus sp. 2	36	n.d.	n.d.	n.d.	++	+++

CONCLUSION

Two studied *Bacillus* spesies are effective in managing chilli anthracnose disease

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