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Enhancement of biocontrol potential of biocompatible bovine serum albumin (BSA) based protein nanoparticles loaded bacterial chitinase against major plant pathogenic fungi *Alternaria alternata*

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Abstract

Recently, principles of nanoscience and nanotechnology has being embraced in the field of agriculture rather than medicine and health care which has the potential to revolutionize modern day agriculture by effectively controlling insect pests and disease causing phytopathogenic microorganisms. In the present study, biocontrol potential of bovine serum albumin nanoparticles (BSA Nps) loaded extra cellular chitinase (BSA Np-CHS) produced by Serratia marcescens SU05 was studied against phytopathogenic fungi Alternaria alternata. Chitinase was extracted from the chitinase

production medium that produced by optimizing various nutrient and process conditions adopting Taguchi method (37 °C temperature, 7 pH, 50 rpm and 1.5 inoculum) shows a extended production of enzyme. Extracted enzyme was purified by ammonium sulphate precipitation and column chromatography separation. Purified enzyme thus obtained was loaded with BSA Nps by cocoeravation method. The method for the preparation of BSA Np-CHS conjugate was optimized by various parameters. Nano enzyme conjugate thus prepared using optimal condition reveals spherical nanosphere with the size range of 110–120 nm and changes in the functional group that was studied by Fourier transform infrared spectroscopy (FTIR) analysis. Biocontrol efficacy of the prepared BSA Np-CHS nano enzyme conjugate against tested fungal strain was studied by determination of fungal biomass and fungal hyphal fragments damage under *in vitro* condition. BSA Np-CHS nano enzyme conjugate brought about effective reduction of fungal biomass and high rate of fungal hyphae fragments damage in all the tested concentration. Further study will helpful to formulate, apply the nanoformulation under field trails for effective control of phytopathogenic fungi.

Introduction

Agriculture has a vital role in the economy of many developing as well as developed countries. In India, over twenty two percent of the rural households depend on agriculture as the principal means of livelihood (Singh et al., 2011). Among the various biotic and abiotic constraints in agriculture production, the insect pests, disease causing pathogens, weeds and nematodes play a vital role as biotic stress (Agrawal and Rathore, 2014). Recent studies reveals that the biotic stress were estimated to cause an annual loss of about 33–35% of the potential food production worldwide (Bhuiyan et al., 2012). About 70% of annual crop loss due to fungal mediated diseases was reported (Agrios, 2005). Furthermore, in India, the fungal related damages resulted in an estimated Rs. 50,000 crore annually in agricultural production in the field as well as storage in India (Singh, 2012).

Alternaria alternata is an ascomycetes fungi known to cause leafspot, rots, blight and other plant diseases in economic important crops. To overcome the losses due to the fungal diseases, the current control technology relies heavily on synthetic chemical fungicides. But the massive and / or over use of the synthetic fungicides and also their frequent usage led to various undesirable effects like development of resistance; elimination of beneficial organisms, toxicity hazards to man, plants, domestic animals and wild life; contamination of soil, water and food (Qiang et al., 2010). Because of the serious awareness of various environmental and health effects caused by synthetic

fungicides, there is a great need of development fungal control agents with effective, biodegradable and ecofriendly manner (Mishra et al., 2017).

Effective management of plant pathogenic fungi using microorganism especially antagonistic bacteria and fungi has received increasing attention in various parts of the world because of their specificity toward the target organism, safety to non-target organism like host plants and animals which make them effective, safe and alternative candidate in pathogen control. In agriculture, appropriate technological improvement that results in more effective use of natural resources is required; one of them is the use of microbial antagonists. Many microbial antagonists have been reported to possess activities against plant fungal pathogens, using various plant growth promoting rhizobacteria (PGRB) like *Pseudomonas fluorescens, Serratia* sp, *Agrobacterium radiobacter, Bacillus subtilis, B. cereus, B. amyloliquefaciens, Trichoderma virens, Burkholderia cepacia, Saccharomyces sp, Gliocadium sp* (Strange and Scott, 2005; Suprapta, 2012). Various types of mechanism of biocontrol agents like parasitism / predation, antibiosis, competition, lytic enzymes, and induced resistance (Pal and Gardener, 2006). The most effective biocontrol active microorganisms studied appear to antagonize plant pathogen employing several modes of actions. For example, *Pseudomonas* known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses. Additionally, DAPG-producers bacterial antagonists can aggressively colonize root, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of plant through competition for organic nutrients (Heydari and Pessarakli, 2010).

Among the different interaction of biocontrol agents with the pathogenic microorganism chitinase is an important lytic enzyme produced by biocontrol agent known to elicit kill the pathogens. Chitinases (EC 3.2.1.14) can catalyze the hydrolysis of chitin to its monomer *N*-acetyl-D-glucosamine. Chitin forms the exoskeleton of many invertebrates and is a major component in the cell wall of fungi (Kamensky et al., 2003). There are enormous chitin cassations causing remarkably environmental problem per annum in the world. Chitinases may find important industrial applications to the treatment of chitin, especially derived from sea-food-processing units (Liu et al., 2003). Chitinases may be used to convert chitin-containing biomass into useful (depolymerized) components. Chitinases can be exploited for their use in control of fungal and insect pathogens of plants. Fungal protoplasts have been exploited as a very efficient experimental means to study the synthesis of cell wall, enzyme synthesis and secretion and strain improvement for biotechnological applications (El-Katatny et al., 2003; Andrés et al., 2014). Chitinase activity also acts as an indicator showing the activity of fungi in soil. It has been reported that there is a strong association between chitinase activity and fungal population in the soil.

Improvement of enzyme activity using enzyme immobilization techniques has been used in the various sector. There are various methods are used for the immobilization using entrapment, covalent Binding, cross-linking and adsorption to improve the stability and kinetic properties (Ansari et al., 2012).

Advanced nanotechnology inspired researchers to immobilize the industrial important enzymes in chemically modified nanopreparation which have aroused innovative industrial interest for using supporting nanomaterials to provide high surface area/ volume ratio, low mass transfer limitation and excellent particle mobility in enzyme catalysed reactions (Rani et al., 2015). Developments in nanotechnology can be expected to become the main economic driving forces in the long run and benefit consumers, producers, farmers, ecosystems, and the general society at large.

In this case, nanoformulation of agro active agents adopting nanotechnology principles are highly effective than the conventional type such as decreased rate of removal of the fertilizers from the soil by rain or irrigation water, sustained supply or minerals for a prolonged time, increased efficiency of the fertilizer, lower frequency of application in accordance with normal crop requirement, minimized potential negative effects associated with over dosage and reduced toxicity (Petkova et al., 2012).

Nanotechnology is being visualized as a rapidly evolving field that has potential to revolutionize agriculture and food systems and improve the conditions of the poor (Xu et al., 2014). Proteins are a class of natural molecules that have unique functionalities and potential applications in both biological as well as material fields (Jahanshahi et al., 2008; Kreuter Ramge et al., 2003). Nanomaterials derived from proteins, especially protein nanoparticles are biodegradable, non-antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. Because of the defined primary structure of proteins, the protein-based nanoparticles may suggest various possibilities for surface alteration and covalent drug attachment (Sharma, 2013).

Role of BSA nanoparticles as drug carrier against cancer and human pathogenic diseases, recent studies reveals the effective use of BSA Nps in enzyme immobilization. Chemically modified albumin nanoparticles formulation of amylase, glucose oxidase, alkaline protease and their enhanced kinetic properties has been reported (Rani, 2014, Namasivayam et al., 2014). The present study, bovine serum albumin nanoparticles was selected for chitinase by coceravtion method which biochemically engineered enzyme without affecting enzyme stability, kinetic properties and opens a new strategy in agriculture sector to manage disease causing organism.

Section snippets

Bacterial strain

The bacterial strain were isolated from the prawn culture farms near Chennai (12°48'30.8"N 80°14'50.6"E), India, the organisms were purified, screened and characterized for the maximum estimation of the chitinase enzyme.

Taguchi method for optimization

The parameters such as pH, temperature, Inoculum size and Agitation with different ranges (Table 1) were taken as variables for the analysis, Further the Design of Experiment (DoE) was suggested by using Minitab-16 Statistical software, version -16.1.1. The enzyme activity was

Bacterial isolation and characterization

Serratia marcescens was isolated from the soil of shrimp cultivated pond and identified by 16rRNA sequencing. The nucleotide sequence was submitted in NCBI and the accession number KX002030 ↗ was procured. The isolated organism was screened for the production of enzyme using colloid chitin subjected plates. The zone of utilization show the productivity of the enzyme by the organism. The sequence was subject to BLAST in NCBI and its phylogenetic tree was created to understand the ancestral

Conclusion

Serratia marcescens was isolated and identified from the prawn cultivation farm soil and the 16s rRNA sequence was characterized and submitted in NCBI as the sequence was unique and named as *Serratia marcescens* strain SU05. The physical condition were optimized using Taguchi methods and the active ingredients among the parameters were identified and the interactive roles were analyzed. The physical and the nutritional parameters were optimized and the maximum productivity was

obtained. After

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> ...Three replicates and control were maintained. Fragmentation efficacy expressed in percentage was determined by measuring the differences in the number of fragmented fungal hyphae in control and treatment (Narendrakumar et al., 2018). 2.4.4....

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