Editorial

Bio-control Potential of Designed Bacterial Chitinase

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Editorial

Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects from ancient time leading to drastic decrease in yields. It has been estimated that half of the total loss is due to plant diseases and one-third of them are due to fungal infections [1]. Hence, the fungal infections are one of the major concerns of good agriculture production in developing countries. With the advent of chemical pesticides, this calamity was resolved to a great degree. However, the over dependence on chemical pesticides and eventual uncontrolled use of them has necessitated for alternatives mainly for environmental concerns.

Bio-pesticides as an alternative to chemical pesticides based on pathogenic and non-pathogenic microorganisms offer an ecologically sound and effective solution to pest problems. Chitinases are known to protect plants from entering fungal pathogens by degrading the chitin of fungal cell walls [2]. Chitinolytic bacteria as bio-control agents have also showed potential antagonistic activity against pathogenic fungi by degrading the cell wall [3,4]. Among hundreds of different microorganisms tested for chitinolytic properties, *Serratia marcescens* was identified as the most efficient one. It has been reported producing multiple chitinases like ChiA, ChiB, ChiC, CBP21 and chitobiase [5]. ChiA and ChiB are processive chitinases, ChiC is an endochitinase, CBP21 shows sequence similarity with monooxygenase and chitobiase [5]. There protein structures, mechanism of action and synergistic behavior of them have been reviewed by Vaaje-Kolstad *et. al* [5]. Bacterial chitinases generally comprise of a catalytic domain fused with chitin binding domain & fibronectin type III like domain. These enzymes are capable of degrading the chitin in the cell walls of fungi and the exoskeletons of insects. Structural and functional analyses show that the ChiA and ChiB are processive enzymes and ChiC is non-processive. ChiA is described to be acting from reducing end and ChiB is from non-reducing end of the polysaccharide. Remarkably, both the exo-chitinases have long hydrophobic tunnel made up of aromatic amino acid side chains [6,7]. This tunnel supports the substrate to remain bound to the ChiA and ChiB [6-8]. These chitinases have ability to slide on the polysaccharide chain while hydrolyzing them. ChiC lacks the hydrophobic tunnel formed by aromatic amino acid residues and has a much shallower substrate-binding cleft, suggesting a non-processive and endo-chitinase behavior. This editorial provides insights on the potentials of engineered bacterial chitinases as biopesticides. Chitinases also have significance in various industrial applications including bioconversion of chitin waste from crustacean shells into chito-oligosaccharide-based value-added products.

Crystalline chitin is an insoluble molecule, which is not easily accessible by the enzymes and need to be processed. As it is mentioned above that for complete digestion of chitin, synergistic activity of endo- and exo-chitinases are required. Sequence analysis of exo- and endo-chitinases reveals little difference in their catalytic domains, which unwraps a possibility of designing a single chitinase for complete degradation. In this regard, not much work has been done towards the improvement of overall chitin degradation by using single chitinase. From structural data analyses and the experimental data available for the most studied endo-chitinase 'ChiB', it is revealed that the mechanism of catalysis involves a large number of residues beyond the key residues Asp142, and Glu144. In fact a greater part of the TIM-barrel core may be involved in catalysis. Highly conserved residues like DXXDXDXE and SXCG motifs of ChiB have been recognized to be involved in catalysis [5].

Many investigators have designed chimeric chitinases by applying domain swapping. Chitinase Chit42 from *Trichoderma atroviride PTCC5220* lacks a Chitin-Binding Domain (ChBD). Fusing to Chit42 a ChBD from ChiB of *S. marcescens* created a chimeric chitinase with stronger chitin-binding capacity. The fusion of ChBD improved the affinity to crystalline and colloidal chitin and also slightly increased the enzyme activity of the chimeric chitinase [9,10]. When the chitin-binding domain of ChiA1 of *Bacillus circulans* was replaced with ChBD of *Bacillus cereus* ChiCW, the chimeric chitinase named ChiAAAW exhibited both high enzyme activity and antifungal activity [11]. The results indicate that ChBD may play an important role in the antifungal activity of ChiCW. In spite of several efforts of the designing of chimeric chitinases, there is no significant improvement in the chitinase activity to achieve the required catalytic activity and hence its use as bio-pesticides.

Since last one decade, many laboratory techniques for the redesigning of proteins have been described which broadly includes directed evolution and rational protein designing based on random and site directed in vitro mutagenesis respectively [12,13]. Directed laboratory evolution has been proven to be an efficient technique particularly when the structure of protein is not known. This has been applied for the significant improvement of enzyme activities for agricultural, industrial and therapeutic applications [14]. Since the detailed protein structures of the chitinases of *S. marcescens* have already been reported [5], a semi-rational approach may be applied for the redesigning. This approach of protein engineering requires *in silico* identification of the probable amino acid residues that can be targeted for site directed mutagenesis. Although, designing of a broadly specific chitinase, which can accommodate both exo- and endo-chitinase activities for processing as well as degradation of crystalline chitin, appears to be a nightmare. A broadly specific chitinase could be designed by applying directed laboratory evolution

technique. Based on the identifications of similar catalytic machinery of many chitinases, it may be accomplished that these are evolved from a common ancestral chitinase. A single engineered chitinase gene might be able to provide the simplest and cost effective chitinolytic machinery, which can be easily expressed in a soil bacterial strain for field applications and utilized to generate recombinant fungal resistant plants for direct defense.

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