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Bacterial chitin binding proteins show differential substrate binding and synergy with chitinases

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ABSTRACT

Glycosyl hydrolase (GH) family 18 chitinases (Chi) and family 33 chitin binding proteins (CBPs) from *Bacillus thuringiensis* serovar *kurstaki* (*Bt*Chi and *Bt*CBP), *B. licheniformis* DSM13 (*Bli*Chi and *Bli*CBP) and *Serratia proteamaculans* 568 (*Sp*ChiB and *Sp*CBP21) were used to study the efficiency and synergistic action of *Bt*Chi, *Bli*Chi and *Sp*ChiB individually with *Bt*CBP, *Bli*CBP or *Sp*CBP21. Chitinase assay revealed that only *Bt*Chi and *Sp*ChiB showed synergism in hydrolysis of chitin, while there was no increase in products generated by *Bli*Chi, in the presence of the three above mentioned CBPs. This suggests that some (specific) CBPs are able to exert a synergistic effect on (specific) chitinases. A mutant of *Bli*Chi, designated as *Bli*GH, was constructed by deleting the C-terminal fibronectin III (FnIII) and carbohydrate binding module 5 (CBM5) to assess the contribution of FnIII and CBM5 domains in the synergistic interactions of GH18 chitinases with CBPs. Chitinase assay with *Bli*GH revealed that the accessory domains play a major role in making *Bli*Chi an efficient enzyme. We studied binding of *Bt*CBP and *Bli*CBP to α- and β-chitin. The *Bt*CBP, *Bli*CBP or *Sp*CBP21 did not act synergistically with chitinases in hydrolysis of the chitin, interspersed with other polymers, present in fungal cell walls.

1. Introduction

Chitin, the second most abundant polysaccharide on earth, is composed of β-1,4 linked *N*-acetyl glucosamine residues. On the basis of arrangement of reducing and non-reducing ends of the polymer sheets, lying one above the other, two different forms of chitin (α and β) are known. Alpha-chitin present in arthropods and fungal cell walls is the most abundant form of chitin. The sheets in α-chitin are arranged antiparallel to each other. In β-chitin these sheets are parallel. Chitooligosaccharides (CHOS) generated by the hydrolysis of chitin find important medical, industrial and agricultural applications (Neeraja et al. 2010a). Structurally, chitinases possess two domains *viz*., a catalytic domain (bacterial chitinases belong to glycosyl hydrolase (GH18)) and a chitin binding module (CBM). Often, these two domains are linked together by accessory domains like fibronectin III (FnIII) domain, whose number may vary. Bacterial chitinases from different species of *Aeromonas* (Mehmood et al. 2010), *Arthrobacter* (Lonhienne et al. 2001), *Bacillus* (Hashimoto et al. 2000), *Chromobacterium* (Park et al. 2007), *Clostridium* (Morimoto et al. 1997), *Pseudomonas* (Folders et al. 2001), *Serratia* (Gal et al. 1997; Purushotham and Podile 2012), *Streptomyces* (Watanabe et al. 1999) and *Vibrio* (Ohishi et al. 2000) were reported. The involvement of different domains in the activity of chitinases (Chuang et al. 2008; Neeraja et al. 2010c) and the synergistic interaction of the chitinases and chitin binding proteins (CBPs) with the bacterial chitinases (Table 1) have been less studied.

Chitinolytic bacteria produce exo-chitinases, endo-chitinases and *N*-acetyl glucosaminidases. The presence of endo-chitinases increases the substrate availability for exo-chitinases (Brurberg et al. 1996) and glucosaminidase for a sequential cooperative degradation. In addition, majority of chitinases possess CBMs that help binding to insoluble chitin and increase substrate accessibility (Tjoelker et al. 2000; Hashimoto et al. 2000; Kojima et al. 2005). CBMs have been classified into 64 families in CAZy database (http://www.cazy.org) on the basis of amino acid similarity. The CBMs play a major role in ligand recognition and binding, and efficient substrate hydrolysis. The CBMs are also implicated in root colonization, pathogen defense, plant development and polysaccharide biosynthesis (Guillen et al. 2010). CBMs may also occur as individual chitin binding proteins (CBPs) as listed in Table 1. CBPs are found in families 14, 18 and 33 of CAZy database. Families 14 and 18 have small antifungal proteins which share a structurally similar CBM (Suetake et al. 2000). CBPs belonging to family 33 are mainly found in bacteria and viruses. The CBPs exhibit differential binding preferences (Table 1). CBPs also show binding to cellulose substrates (like avicel), with preference for chitin substrates (Purushotham et al. 2012a). The difference in substrate preference was mainly attributed to the difference in the sequence

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Table 1
Binding preferences of chitin binding proteins (CBPs) from bacterial sources.

Designation of CBP	Size (kDa)	Organism	Binding preference	References
ChbB	19.8	*Bacillus amyloliquefaciens*	α-chitin and β-chitin	Chu et al. (2001)
*Ll*CBP33A[a]	20.9	*Lactococcus lactis* ssp. *lactis*	α-chitin and β-chitin	Vaaje-Kolstad et al. (2009)
Chb3	14.9	*Streptomyces coelicolor*	α-chitin, β-chitin, colloidal chitin (CC) and chitosan	Saito et al. (2001)
Cbp50	49.8	*B. thuringiensis* sv. *konkukian*	α-chitin, β-chitin and CC	Mehmood et al. (2011)
E7[a]	21.3	*Thermobifida fusca*	Prefers β-chitin followed by α-chitin and bacterial microcrystalline cellulose (BMCC)	Moser et al. (2008)
E8[a]	44.8	*T. fusca*	α-chitin, β-chitin and BMCC	Moser et al. (2008)
*Sm*CBP21[a]	21	*Serratia marcescens*	Strictly to β-chitin	Suzuki et al. (1998)
CHB1	18.7	*Str. olivaceoviridis*	Strictly to α-chitin	Schnellmann et al. (1994)
CHB2	18.6	*Str. reticuli*	Strictly to α-chitin	Kolbe et al. (1998)
*Sp*CBP50[a]	50	*S. proteamaculans*	α-chitin, β-chitin and CC	Purushotham et al. (2012a)
*Sp*CBP21[a]	18.6	*S. proteamaculans*	α-chitin, β-chitin and CC	Purushotham et al. (2012a)
*Sp*CBP28	28	*S. proteamaculans*	Does not show binding	Purushotham et al. (2012a)
*Ef*CBM33A[a]	18.3	*Enterococcus faecalis*	α-chitin and β-chitin	Vaaje-Kolstad et al. (2012)

[a] CBPs reported to show synergism with chitinases

of solvent exposed functionally important polar residues in the respective CBPs. Three-dimensional structure of family 33 CBP was made available for *Sm*CBP21 from *Serratia marcescens* (Vaaje-Kolstad et al. 2005b) and *Ef*CBM33A from *Enterococcus faecalis* V583 (Vaaje-Kolstad et al. 2012). *Sm*CBP21 showed preference to β-chitin (Suzuki et al. 1998), while *Ef*CBM33A binds to both α- and β-chitin (Table 1). More recently, Aachmann et al. (2012) proposed that the binding residues in *Sm*CBP21 line up in a narrow stretch along the substrate binding surface that could match the width of a single polysaccharide chain.

The CBPs in bacteria were assumed to increase the efficiency of chitinases during chitin degradation. Vaaje-Kolstad et al. (2010) demonstrated that the *Sm*CBP21 that generated oxidized CHOS was indeed a chitin oxidohydrolase. Since the fungal cell wall is chiefly made up of chitin, in addition to glucans and mannans, CBPs from plants and bacteria have been explored for their antifungal activity (Huang et al. 2000; Van Parijs et al. 1991). CBP from *Streptomyces tendae* Tu901 interferes with growth polarity in fungi (Bormann et al. 1999), while Cbp50 from *Bacillus thuringiensis* serovar *konkukian* was antifungal (Mehmood et al. 2011).

The chitinolytic machinery of bacteria primarily consists of CBPs and chitinases. To further understand the synergy between chitinases and CBPs from bacterial sources, we have selected CBPs and chitinases from *B. thuringiensis* serovar *kurstaki, B. licheniformis* DSM13 and *Serratia proteamaculans* 568. The domain organization of the selected chitinases and CBPs that share GH18 or family 33 CBM, respectively is shown in Fig. 1. CBPs may consist of only CBMs or additional FnIII domains connecting the CBMs as seen in E8 from *Thermobifida fusca* (Moser et al. 2008) and Cbp50 from *B. thuringiensis* serovar *konkukian* (Mehmood et al. 2011). The CBP from *B. thuringiensis* serovar *kurstaki* also possesses two FnIII domains and a C-terminal CBM5. Chitinase (*Sp*ChiB) and CBP (*Sp*CBP21) from *S. proteamaculans* 568 and chitinases from *B. thuringiensis* serovar *kurstaki* (*Bt*chi) and *B. licheniformis* DSM13 (*Bli*chi, and its deletion mutant, *Bli*GH) were characterized earlier (Purushotham et al. 2012a,b; Neeraja et al. 2010b,c). Molecular mass of *Bt*CBP, *Bli*CBP and *Sp*CBP21 was 50, 21 and 18.6 kDa, respectively.

We have performed a chitinase assay for the selected chitinases (in combination with the selected CBPs) to study the importance of CBPs in hydrolysis of chitin by chitinases and showed that CBP was always not synergistic to the hydrolytic activity of all bacterial chitinases. To assess the relative contribution of the accessory domains to the activity of the selected chitinases, we have made use of a deletion mutant *Bli*GH generated by Neeraja et al. (2010b). The activity of *Bli*GH on chitin substrates was also studied in presence or absence of *Bt*CBP, *Bli*CBP or *Sp*CBP21. We further report that CBPs work synergistically with the bacterial chitinases, other than their own, in hydrolysis of pure substrates, but may not assist in the antifungal activity where the chitin is present along with other polymers in the fungal cell wall.

2. Materials and methods

2.1. Chemicals and enzymes

Restriction enzymes, T4 DNA ligase and P*fu* DNA polymerase were from MBI Fermentas (Ontario, Canada). Oligonucleotide primers were purchased from MWG Biotech (Ebersberg, Germany). All chemicals used in the present investigation were of analytical grade, procured from Sigma–Aldrich (MO, USA), GE Health Care (Uppsala, Sweden), Promega Life Science (Madison, USA), Fermentas (Ontario, Canada), Himedia labs (Mumbai, India), Thermo Scientific (USA) and Qualigens fine chemicals (Mumbai, India). The polymeric substrates α-chitin (extracted from shrimp shells, 60 mesh powder) and β-chitin (extracted from squid pen, 200 μm) were provided by Dr. Dominique Gillete, Mahtani Chitosan (Veraval, India).

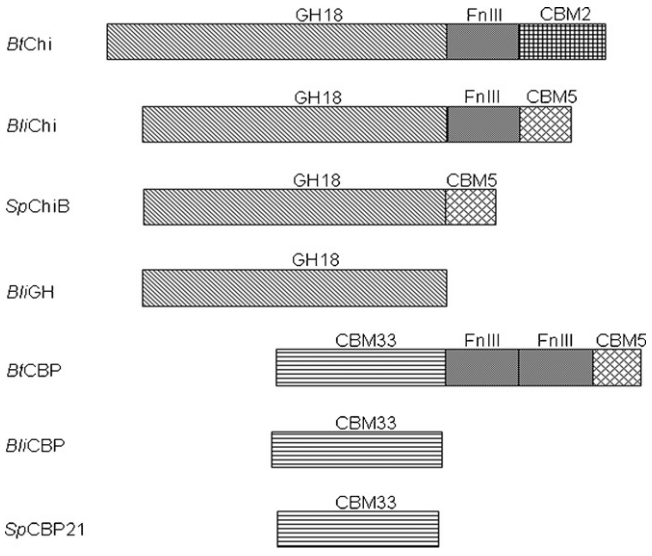

Fig. 1. Schematic representation of domains architecture in chitinases and CBPs of *B. thuringiensis*, *B. licheniformis* and *S. proteamaculans* 568. GH18 represents glycosyl hydrolase domain belonging to family 18, FnIII is type III fibronectin domain. CBM represents carbohydrate binding module and 2, 5 and 33 represent their family as per CAZy database.

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