DISCUSSION
4.1 *Stenotrophomonas maltophilia* is a potential chitinolytic bacterium

Chitin is a homo polymer of *N*-acetyl glucosamine linked by β- (1-4) glycosidic bonds. Chitin is the most abundant polysaccharide next to only cellulose, it is complex, crystalline, high molecular weight, extracellular amino-sugar polymer, which is non-toxic and biodegradable. Utilization of the chitin is difficult because of its insolubility in water (intermolecular hydrogen bonds), compact crystalline nature. To recycle such an abundant biomass, nature evolved several mechanisms involving microorganisms. Bacteria are the largest group explored for chitinolytic potential. Bacteria play a major role in turnover of chitin biomass and metabolize crystalline chitin as source of C and N. From ecological point of view, there is a great interest in the enumeration of chitinoclastic microorganisms from natural sources.

In the present study, we have isolated the chitinolytic genes and characterized the chitin degrading enzymes from *S. maltophilia* k279a which belongs to family Xanthomonadaceae. *Stm* represents a rhizosphere bacterial species of potential agronomic importance. The organism is aerobic, non-fermentative, Gram-negative, rod-shaped and its full genome sequence is available since 2008. Traits of *Stm* associated with biocontrol mechanisms include antibiotic production (Jakobi *et al*., 1996; O’Brien and Davis, 1982), extracellular enzyme activities such as protease and chitinase (Dunne *et al*., 1997; Kobayashi *et al*., 1995; O’Brien and Davis, 1982; Zhang and Yuen, 2000), and rhizosphere colonization (Dunne *et al*., 1997; Elliott *et al*., 1987; Kobayashi *et al*., 1995; Lambert *et al*., 1987; Lambert *et al*., 1990).
Complete genome sequence search of Stm k279a revealed 2 chitinases, 1 hexosaminidase, 2 chitin binding proteins (CBPs), two polysaccharide deacetylases (Pdas), and one unannotated GH19 sequence, which are capable of converting the chitin biomass to GlcNAc. Due to the presence of a rich diversity of genes for chitin degradation and/or modification, Stm was selected for the study. The domain architecture of these chitinases deduced from their amino acid sequences is depicted in Table 4.1.

The complete hydrolysis of chitin requires cooperative and synergistic action of chitinases and hexosaminidases. Zechmeister and Toth (1939) reported the key observation, the insoluble chitin polymer was converted to its monomer, GlcNAc, by two enzymes, a “polysaccharidase” (chitinase), and a “disaccharidase,” (β-N-acetyl glucosaminidase or chitobiase). Suzuki et al. (2002) reported the synergistic action of chitinases ChiA, ChiB, and ChiC1 of Serratia marcescens 2170 on chitin degradation. The hydrolytic activity of chitinases is either by single or multiple forms of the enzyme. The multiple forms may be due to expression from different genes or due to proteolytic processing, as in case of Bacillus circulans and Serratia marcescens (Alam et al., 1996; Gal et al., 1998).

Several methods were reported for the detection of chitinase activity using different chitinous substrates and chemicals. A simple and inexpensive method to identify chitinolytic microorganisms on agar containing chitinous polymer was developed (Howard et al., 2003). Since chitinases diffuse through agar, identification of chitinolytic bacteria or genomic clones encoding chitin-degrading enzymes was performed by monitoring the degradation of polymeric chitin incorporated into an agar medium. The larger and clearer zone of chitin hydrolysis around the colony was attributed to the chitinolytic ability of the organism. Stm was tested on chitinase detection agar medium, and a clear zone around the colony was
formed indicating the strong chitinolytic activity. A simple, rapid and less expensive method to detect chitinase activity on agarose plates using a fluorescent brightener Ranipal (Anil et al., 2007). Chitinase activity was detected after protein separation by SDS-PAGE and glycol chitin containing agar (Trudel and Asselin, 1989). Determination of chitinolytic activity of chitinase, glucosaminidase and chitobiosidase in solution can be achieved by rapid method as described by Tronsmo and Harman (1993). In the present study, we have estimated the chitinase activity calorimetrically by estimating the number of reducing amino sugars produced by using modified Schale’s procedure (Imoto and Yagashita, 1971). This method has the advantage of estimating the number reducing amino sugars in solution released due to chitinase activity compared to other known procedures.

We have amplified and sequenced the 16S rDNA gene of Stm to confirm the taxonomic affiliation of the isolate. The BLAST search of the 16S rDNA sequence showed 100% homology to S. maltophilia k279a as well as S. maltophilia IAM 12423 (GI: 343200890). Phylogenetic tree was constructed along with closely related 16S rDNA sequences of different bacterial species (Fig. 3.1). The strain used in the study was, therefore, considered as S. maltophilia k279a. Similarly, Tyler et al., (1995) distinguished between Stm and seven species of Pseudomonas by identifying differences in the sequences of 16S to 23S rDNA internal transcribed spacers.

4.2 Cloning and characterization of chitin degrading/modifying enzymes from Stm k279a

4.2.1 Search for homologs of Stm k279a chitin modifying enzymes at NCBI using BLAST
4.2.1.1 *Stm* ChiA

The amino acid sequence of *Stm* ChiA was BLASTed at NCBI database to search for protein homologues. The results displayed 75% sequence similarity to the sequence of ChiA from *Lysobacter enzymogenes* (AAT 77164.1), 65% to GH18 domain of *Xanthomonas albilineans* (YP003376658.1), 55% to chitinase of *Burkholderia ambifaria* (ZP02909207.1), and 47% to GH18 of *Streptomyces coelicolor* A3(2) (NP 629515.1).

The SignalP 3.0 ([http://www.cbs.dtu.dk/services/SignalP/](http://www.cbs.dtu.dk/services/SignalP/)) program predicted the presence of a putative signal peptide, typical of Gram-negative and Gram-positive bacteria, with a cleavage site located between amino acid residues Ala-28 and Ala-29. The sequences were tested for functional domains or motifs using SMART (a Simple Modular Architecture Research Tool) database. *Stm* ChiA contained an N-terminal chitin binding domain 3 (ChtBD3), polycystic kidney disease domain (PKD), fibronectin type 3 domain (FN3) and a family 18 catalytic domain (GH18).

4.2.1.2 *Stm* ChiB

The BLAST search for *Stm* ChiB displayed 96% identity to chitinase A1 of *Stenotrophomonas* sp. SKA14 (ZP 05136728.1), 41% to putative chitinase A1 from *Paenibacillus* sp. HGF 7 (ZP08512913.1), 40% to catalytic domain of ChiA1 of *Bacillus circulans* WL 12 (1ITX_A) and 38% to family 18 chitinase of *Chitiniphilus shinanonensis* (BAF 02588.1). *Stm* ChiB contained a single catalytic domain GH18 similar to that of *Serratia proteamaculans* ChiD (Purushotham, 2011).
4.2.1.3 Stm Nag

Stm Nag exhibited 62% identity to NAGase of *Pseudoxanthomonas suwonensis* (YP 004145298), 48% to NAGase from *Xanthomonas perforans* 91-118 (ZP06729723.1), 41% to Nag C from *Streptomyces thermoviolaceus* (BAC 76622), 36% to Nag 2 from *Vibrio harveyi* 650, 34% to GlcNAcase A from *Pseudoalteromonas piscicida* (BAB17855), and 26% to chitobiase from *Serratia marcescens*. Stm Nag contained family 20 catalytic domain, similar domain architecture was observed in *S. marcescens* chitobiase (Chb) (Tews et al., 1996).

4.2.1.4 Stm CbpD1

Stm CbpD1 had 53% homology with chitin binding protein D from *Pseudomonas aeruginosa* PA7 (YP 001350011.1), 48% to chitin binding protein from *Myxococcus xanthus* (YP 631148.1) and 35% to chitin binding domain 3 protein B from *Streptomyces* sp.4 (ZP 09182986.1) having a ChtBD3 as a conserved domain.

4.2.1.5 Stm Pda1

Stm Pda1 had 64% homology with polysaccharide deacetylase from *Xylella fastidiosa* Ann1(ZP 00680562.1), 61% to putative chitin deacetylase from *Xanthomonas vesicatoria* (ZP 08178658.1) and 43% to chitooligosaccharide deacetylase from *Ralstonia eutropha* H16 (YP 84181.1).

4.2.1.6 Stm Pda2

Stm Pda2 had 49% homology with polysaccharide deacetylase from *Rhodanobacter* spp. 2APBS1 (ZP 08953748.1), 28% to putative xylan/chitin deacetylase from *Rheinheimera* spp.
A13L (ZP 08570187.1) and 26% to chitooligosacchride deacetylase (Nodulation protein B) from *Paenibacillus polymyxa* E681 (YP 003870877.1).

The BLAST search of protein homologues for different *Stm* chitinases revealed the evolutionary divergence of *S. maltophilia* from different genera. However, it has close affinity with *Stenotrophomonas* spp. SKA14 and *Lysobacter enzymogenes*.

### 4.2.2 Catalytic activity

The presence or absence of the accessory domains in the protein sequence of the chitinases and chitin modifying enzymes influence the catalytic activity. *Stm* chitinases differ amongst each other with different domain organization. The role of accessory domains in the specific activity of enzymes was assessed by performing kinetic studies for *Stm* chitinases.

Colloidal chitin or chitin derivatives are mostly used for activity measurements in enzyme assays. However, these substrates are not suitable for kinetic analysis as they are of heterogeneous nature (Bokma *et al.*, 2000). Hence, we have used a soluble substrate, chitin hexamer. Michaelis-Menten kinetic parameters were determined using different concentrations of chitin hexamer as a substrate.

Kinetic analysis of *Stm* chitinases revealed that *Stm* ChiB had highest catalytic efficiency ($k_{cat}/K_m$) and substrate affinity ($K_m$) than *Stm* ChiA and *Stm* Nag. *Stm* ChiB displayed two-fold high substrate affinity and overall catalytic efficiency compared to *Stm* ChiA and *Stm* Nag (Fig 3.6). From this analysis it can be assumed that GH18 domain has preference towards hexameric substrate than GH20 domain. The kinetic analysis for the *Stm* Nag showed higher substrate affinity and overall catalytic efficiency over Nag 2 from *Vibrio*
**Discussion**

*harveyii* 650 [ $k_{cat}/K_m$ 166 s$^{-1}$ M$^{-1}$, $K_m$ 421 µM ] (Suginta *et al.*, 2010). NagC from *Streptomyces thermoviolaceus* (Kubota *et al.*, 2004) and chitobiose from *Vibrio parahemolyticus* (Zhu *et al.*, 1992) showed similar kinetic parameters like *Stm* Nag.

Among *Bacillus* chitinases, *B. thuringiensis* chitinase displayed five-fold greater affinity and overall catalytic efficiency when compared to *B. licheniformis* chitinase. This was observed with both polymer and oligosaccharide substrates. In *S. proteamaculans* chitinases, *Sp* ChiD exhibited 3-fold greater affinity towards hexamer substrate when compared to ChiA and ChiB, and 10-fold greater affinity than ChiC. Comparative kinetic analysis of a few bacterial chitinases revealed that among all, *Sp* ChiD is catalytically active towards hexamer, and *Stm* ChiB exhibited high activity in terms of $k_{cat}$ and $k_{cat}/K_m$ among *Stm* chitinases. Unique feature among these two enzymes was to have a single GH18 domain. We can infer that single catalytic domain favoured efficient hydrolysis of soluble CHOS. Our results in the present study ascertained this fact by the behavior of these two enzymes on polymer substrates. The enzymes *Sp* ChiD and *Stm* ChiB did not show significant activity towards chitinous polymers as both these enzymes do not contain accessory domains. Enzymes without accessory domains are not active on polymer substrates as these domains are helping in binding to the polymeric substrates making them accessible for enzymatic hydrolysis (Kawase *et al.*, 2006).

The PKD domain was found in N-terminal region of both the chitinases that were effective in hydrolysis of crystalline α-chitin (Orikoshi *et al.*, 2005). So, we can assume that the enzymes containing accessory domain cleave the polymer to shorter chain length oligomers making them better substrates for *Sp* ChiD and *Stm* ChiB, to release products like GlcNAc, the energy source for the bacterium. Kinetic analyses for *Sp* chitinases towards colloidal chitin
are in contrast to hexamer substrate. Sp ChiA exhibited highest substrate affinity and overall catalytic efficiency on colloidal chitin and Sp ChiD showed very poor $K_m$ & $k_{cat}$/ $K_m$ values (Purushotham et al., 2012). Our results are in conformity with the earlier findings on Sp chitinases. The results revealed that, among different bacterial chitinases used in this study, Sp ChiD exhibited highest $K_m$ & $k_{cat}$/ $K_m$ and Bli Chi showed low $K_m$ & $k_{cat}$/ $K_m$ values (Table 3.3).

The difference in the kinetic parameters among bacterial chitinases also could be due to difference in the amino acid sequences of catalytic domains in conjunction with the presence (Stm ChiA, Sp ChiA- Sp ChiC, Bt Chi and Bli Chi) or lack of (Stm ChiB, Stm Nag and Sp ChiD) of different accessory domains (Table 4.1). It is evident that the primary role of these accessory domains (CBMs, PKD and FN3) is to potentiate catalytic activity by disrupting the substrate, rather than simply to promote enzyme-substrate binding. Watanabe et al. (1994) showed that deletion of the two FN3 domains of ChiA1 from Bacillus circulans did not affect chitin-binding, but strongly reduced chitin hydrolyzing activity.

The difference in the kinetic parameters of Stm chitinases could be attributed to the difference in the amino acid sequence. More specifically, the variation in the affinity of the chitinases could be due to amino acid sequence variation that imparts a strong or weak binding affinity towards the polymeric substrate or a more or less efficient catalytic process.

4.2.3 Effect of temperature on chitinolytic activity

All the three Stm chitinases exhibited highest specific activity at an optimum temp of 40°C (Fig 4A), similar to S. proteamaculans chitinases (Purushotham et al., 2012) and Chi L of B. pumilis SG2 (Ghasemi et al., 2010) and CHIT100 from Serratia plymuthica HRO-C48 (Frankowski et al., 2001), while chitinases from B. thuringiensis and B. licheniformis
Discussion

exhibited optimum temperature at 60°C (Neeraja et al., 2010c) higher than Stm chitinases. Stm Nag was active at 40°C (Fig.3.8 A), similar to β-N-acetylglosaminidase from Alteromonas spp. strain 0-7 (Tsujibo et al., 1995), β-N-acetylglosaminidase from V. furnissii (Keyhani and Roseman, 1996). The three Stm chitinases retained their specific activity with a marginal loss at 40°C after pre-incubation of 4 h. Stm ChiA showed higher thermal tolerance than Stm ChiB and Stm Nag. Stm ChiA was thermally stable as it retained more than 90% of its initial activity between 30-50 °C up to 1 h. Stm ChiB and Stm Nag were thermally stable up to only 40°C, while Stm ChiB lost 80% of the initial activity at 50°C. On the other hand 50% of the initial activity was retained at 50°C for Stm Nag.

4.2.4 Effect of pH

The three Stm chitinases were optimally active in acidic to neutral pH (3.0-7.0) (Fig 3.9).

Stm ChiA displayed activity over a broad range of pH (7.0-12.0) similar (but not identical) to Pt ChiA from Paecilomyces thermophila (Kopparapu et al., 2011; Kudan and Pichyangkura, 2009). Stm ChiA exhibited highest specific activity at sodium citrate buffer of pH 3.0 and sodium acetate buffer pH 5.0. The Stm ChiB was optimally active at pH 7.0 similar to that of CHIT 100 of Serratia plymuthica HRO- C48 (Frankowski et al., 2001). The Stm Nag exhibited activity over a broad pH range similar to Bacillus cereus TKU 006 (Wang et al., 2009) and B. atrophaeus SC081(Cho et al., 2011) with an optimum activity at pH 5.0. The chitinases showing optimum activity in both alkaline and acidic pH is not uncommon (Kawase et al., 2006) as observed for Stm ChiA and Stm Nag in this study. Although not clear one possible explanation could be such chitinases have a deep catalytic cleft formed by the two insertion domains. These domains make the top of the catalytic cleft narrow and might interfere with the entrance of long-chain soluble substrate into the catalytic cleft in the
neutral pH range. At extremely high or low pH, conformational change occurs that makes the top of the catalytic cleft wider or the cleft structure more flexible. This results in long-chain soluble substrate easily entering into the catalytic cleft and the chance of hydrolysis increases.

4.2.5 Activity on chitosans of different Degree of De Acetylation (DDA)

Chitinases are reported to be chitosanolytic as well. Chitosans are very valuable substrates for in depth studies of processivity in family 18 chitinases (Sikorski et al., 2006). Stm ChiA was able to hydrolyze chitosans of different DDA, but preferably water soluble chitosan. Stm Nag has very feeble activity towards water soluble chitosan, and Stm ChiB did not hydrolyze any of the chitosan.

4.2.6 Substrate binding properties of Stm chitinases

4.2.6.1 Insoluble substrates

To establish the relationship between specific activity and the substrate binding capability of Stm chitinases on different insoluble polymers was assessed and compared with different substrates (Fig. 3.11). Stm ChiA did not show binding towards Avicel. Stm ChiB showed binding preference to β-chitin, but did not show binding to α-chitin and chitosan. However, binding towards Avicel was negligible. The importance of the accessory domains, most importantly ChBD (chitin-binding domain) in binding to insoluble substrates was known. The C-terminal binding region was important for strong affinity of the enzyme to insoluble chitin (Watanabe et al., 1990). Morimoto et al. (1997) explained the role of highly conserved aromatic amino acids tryptophan (W) and tyrosine (Y) in chitin binding domain (ChBD). These amino acids may play a crucial role during binding to the pyranosyl rings of
N-acetyl glucosamine residues in chitin. Deletion of the CBD from the *B. circulans* chitinase A1 reduced the rate of hydrolysis of colloidal chitin by approximately 50% (Svitel and Kirchman, 1998). *Stm* Nag exhibited binding towards all the polymers.

### 4.2.6.2 Soluble substrates

Binding of three *Stm* chitinases to soluble polysaccharides was investigated by native PAGE with and without polysaccharides. Electrophoretic mobility of the *Stm* chitinases was only affected by the presence of glycol chitin (Fig. 3.12). Among *Stm* chitinases only *Stm* ChiA exhibited strong affinity towards the glycol chitin substrate indicating that accessory domains are crucial for the hydrolysis of chitin polymer which is in accordance with the previous results. The binding ability of *Stm* ChiA is similar to that of BliGH, BliGh-CeBD towards glycol chitin substrate (Neeraja *et al*., 2010a).

### 4.2.7 Hydrolytic activities on chitinous substrates

#### 4.2.7.1 TLC analysis of products

Only *Stm* ChiA was active on polymers probably due to the presence of accessory domains. To check the hydrolytic activity of *Stm* ChiA on colloidal chitin was assessed by TLC was performed and followed the products formation over a period of time (Fig 3.13). We could see the concentration of dimer enhanced in addition to very faint concentrations of higher chain oligosaccharides observed during extended incubation. Colloidal chitin hydrolysis by chitinase A of *Vibrio carchariae* and *B. licheniformis* (Suginta *et al*., 2005; Songsiriritthigul *et al*., 2010 also had similar pattern. Our results reveal the endo-mode of action of chitinase A on chitin polymers.
4.2.7.2 HPLC analysis of products

4.2.7.2.1 Polymeric substrates

Activity profile of *Stm* ChiA on α- chitin and β- chitin was observed for 12 h, to see the activity profile from 0 min to 12 h (Fig 3.15). The results indicated that *Stm* ChiA preferentially hydrolyzed β- chitin. Similarly, chitinase A from *S. marcescens* preferentially hydrolyzed β- chitin than α- chitin and predominantly released dimers (Horn et al., 2005).

4.2.7.2.2 Oligomeric substrates (DP2-DP6)

Reaction time course of CHOS hydrolysis was analyzed by HPLC (Fig. 317-3.21). Hydrolysis of DP2 with *Stm* ChiA yielded very low amount of DP1 after 24 h indicating that DP2 is not preferred substrate for the *Stm* ChiA hydrolysis. Similarly, chitinase A from *V. carchariae* and *B. licheniformis* did not hydrolyze DP2 substrate after 57 h and 18 h, respectively (Suginta et al., 2005; Songsiriritthigul et al., 2010). Sakai et al. (1998) showed that three chitinases (Chi L, Chi M and Chi S) from *Bacillus* strain MH-1 did not hydrolyze DP2 substrate at all. On DP3 substrate, *Stm* ChiA produced low concentration of monomer and dimer after 1 h. In contrast *V. carchariae* chitinase required 57 h to completely hydrolyze trimer (Suginta et al., 2005). *Stm* ChiA hydrolyzed DP4 readily from the 0 min, and it cleaved tetramer mainly in the middle, releasing dimers. Trimer was also produced but in lower quantity (< 20% of the dimers at 60 min of reaction). *Stm* ChiA exhibited transglycosylation activity at the 60th min. Based on these results we can infer that *Stm* ChiA prefers higher chain length oligosaccharides for the hydrolysis. As soon as the substrate was a limiting factor, the enzyme transglycosylated the available oligomers (Fig 1.4). DP5 substrate got readily cleaved by *Stm* ChiA at the initial time points. On DP6 substrate also
Stm ChiA exhibited transglycosylation activity. The increase in the concentration of the hexamer at 15th min could be due to the transient transglycosylation activity of the enzyme where DP2, DP3, and very low concentrations of DP4 are acting as substrates for transglycosylation. Stm ChiA is exhibiting transglycosylation on even chain length oligosaccharides starting from DP4. Hydrolysis pattern of Stm ChiA was similar to chitinase A from B. licheniformis (Songsiriritthigul et al., 2010). Stm ChiB and Stm Nag hydrolyzed DP2-DP6 to give DP1 as the major end product. The formation of other intermediate oligomers indicated that these enzymes are endo–acting enzymes on oligomeric substrates. Stm Nag was truly a chitobiase with dimer. Unlike Stm Nag, Nag2 from Vibrio harveyii 650 required 25 h for complete hydrolysis of the DP6, β-N-acetylglucosaminidase from Pseudomonas fluorescens JK-0412 (Park et al., 2010a) and Aeromonas hydrophila strain SUWA-9 (Lan et al., 2004) attained complete DP5 hydrolysis by 24 and 7 h, respectively. NagC from Streptomyces thermoviolaceus exhibited activity only on DP2-DP5 substrates and no activity on DP6 substrate (Kubota et al., 2004). The activity of Stm Nag on DP2, in the present study, started at 0 min and completely hydrolyzed within two minutes indicating the rapid rate of hydrolysis unlike other known hexosaminidases. Our results establish the fact that Stm Nag is an exclusive chitobiase.

4.2.8 Antifungal activity

Chitinolytic bacteria have received much attention as potential biocontrol agents due to their ability to lyse hyphae of fungal pathogens. Hyphal tips and germinating spores appear to be susceptible to the lytic activities of chitinolytic bacteria. Purified chitinase preparations of potent biocontrol strains can cause deformation of fungal hyphae (Ordentlich et al., 1988; Zhang and Yuen, 2000; Manjula et al., 2004).
S. maltophilia k279a is well known for its biocontrol ability due to its ability to produce diverse group of enzymes. Kobayashi et al. (2002) reported that chitinase from the strain Stm 34S1 had the ability to suppress summer patch disease on Kentucky bluegrass, supporting a role for the enzyme in the biocontrol activity. But, very few biocontrol traits have been characterized in detail. The present study reports the involvement of Stm chitinase A in alterations in the structure of F. oxysporum fungal mycelium and spores. It was confirmed by scanning electron microscopy (Fig 3.23.2). Bacillus cereus chitinase showed antifungal activity towards F. oxysporum and R. solani (Pleban et al., 1997).

Genome sequence of Stm k279a consists of putative un annotated GH19 gene sequence. This gives us a clue that the role of Stm k279a in biocontrol ability could also be due to the presence of a variety of chitinase gene sequences in the genome. Most probably, family 19 chitinases were transferred from plants to bacteria by horizontal gene transfer. In plants, family 19 chitinases are thought to form part of a defense mechanism against chitin-containing fungal pathogens. The family 19 chitinases may attack the hyphal tips, which are believed to consist of newly synthesized chitin that is not firmly crystallized (Kawase et al., 2006). Only a few chitinolytic bacteria possess family 19 chitinases, and these also display antifungal activity (Watanabe et al., 1999; Kawase et al., 2006). Role of chitinases in antifungal activity and presence of different glycosyl hydrolase domains contribute to overall biocontrol ability of S. maltophilia k279a towards fungal pathogens. Presence of C-terminal ChBD in Streptomyces griseus HUT 6037 increased the antifungal ability of the chitinase (Itoh et al., 2002).

Our present work focused on cloning and characterization of chitin degrading/modifying enzymes from S. maltophilia k279a and the production of CHOS by the action of these
enzymes. A search for effective biological plant protection methods currently is of high interest in view of the growing area of ecological crops. Stm chitinases can be used in industries for conversion of chitinous waste into biologically active CHOS. Results presented in this study will be useful to design appropriate strategies for enzymatic synthesis of CHOS.