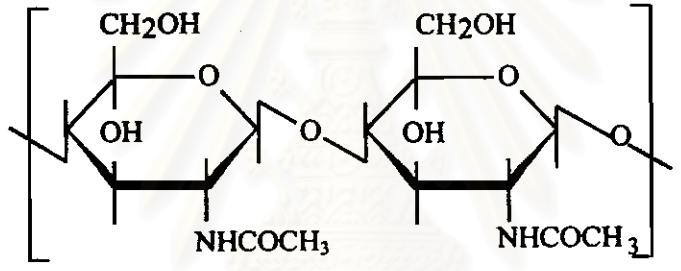
CHAPTER I

INTRODUCTION

Chitin

Chitin, a (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan, is one of the most abundant polysaccharides in nature (Figure 1). The distribution of chitin and its quantitative importance in living beings is now well known (Jeuniaux, 1971). Chitin is synthesized by some unicellular organisms such as diatoms and protozoa. Chitin and/or chitosan are constituents of cell wall in most fungi, molds and yeasts. Chitin is present in cuticular or exoskeletal structures of most Invertebrates. The amount of chitin with respect to total dry weight is the highest in Crustaceans. This observation, together with the availability of waste material from canning food industries, may explain the use of Crustacean shells as main source of chitin by most chemical industries.

Electron microscopy shows that chitin has a fibrous morphology: the polymer is semicrystalline, and the amorphous regions probably correspond to the less ordered edges of the fibrous crystallites (Blackwell, 1988). Comparison of the X-ray data from different sources has revealed the existence in nature of more than one polymorphic form. Polymorphism is a common phenomenon in crystalline polymers, because there is frequently relatively little difference in potential energy between several methods of packing the chains. By the molecular conformation, chitin is classified into 3 groups, α-chitin, β-chitin and γ-chitin. The α-chitin is tightly arranged in antiparallel fashion, whereas β-chitin is in a parallel form. The γ-chitin is in mixed strand form. Most chitins, including those from crustaceans, insects, and fungi, are in the so-called α-form. However, a rare second form know as β-chitin has been identified in four sources: the spines of the polychaete *Aphrodite*, the pen of the squid *Loligo*, the tubes of *Pogonophora*, and the spines of certain marine diatoms. A third form, γ-chitin, has been reported from the stomach lining of *Loligo*.



N-acetylglucosamine N-acetylglucosamine

Chitin

Figure 1 Chitin is a β(1→4)-linked homopolymer of N-acetyl-D-glucosamine

Chitin and its deacetylated form, chitosan, have been used in industrial applications to produce high value products such as cosmetics, drug carrier, food additives, semi-permeable membranes and pharmaceutics (Table 1) (Goosen, 1997; Shahidi, Arachchi, and Jeon, 1999). Chitin is now produced commercially from crab and shirmp shells by treatment with dilute NaOH solution for deproteinization, followed by treatment with dilute HCl solution for demineralization (Figure 2). Chitosan is produced by treatment of chitin with concentrated NaOH at elevated temperature.

Chitin and chitosan oligomers has continuously attracted much attention in the food and pharmaceutical application. These oligomers may be more advantageous than chitin and chitosan as polymers in the field of food additives and nutraceuticals in human health, because chitin and chitosan could not be degraded in the human digestive system due to the absence of enzymes such as chitinase and chitosanase. Most physiological activities and functional properties of chitin and chitosan oligomers has been clearly shown to be depend upon their molecular weight. A chain length of at least five residues is required for plant immune elicitor and animal lysozyme production.

There are two hydrolytic methods, to prepare chitin and chitosan oligomers: acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis with inorganic acids leads to the formation of oligomers with a low degree of polymerization (DP), varying from monomer to trimer. The yield of oligomers with relatively higher DP, such tetramer to heptamer, which are desirable as biologically active oligomers, are low. In contrast to acid hydrolysis, enzymatic hydrolysis of chitin and chitosan by chitinase, chitosanase and lysozyme readily allows production of high DP oligomers for different applications (Aiba, 1994).

CHITINASE

Chitinase (EC 3.2.1.14) is an enzyme that catalyzes the degradation of chitin. Chitinase is present in a wide range of organisms, including viruses, bacteria, fungi, insects , plants and animals. For those organisms that utilize the structural properties of chitin such as insects, yeast and fungi, chitinases are critical for normal life cycle functions of

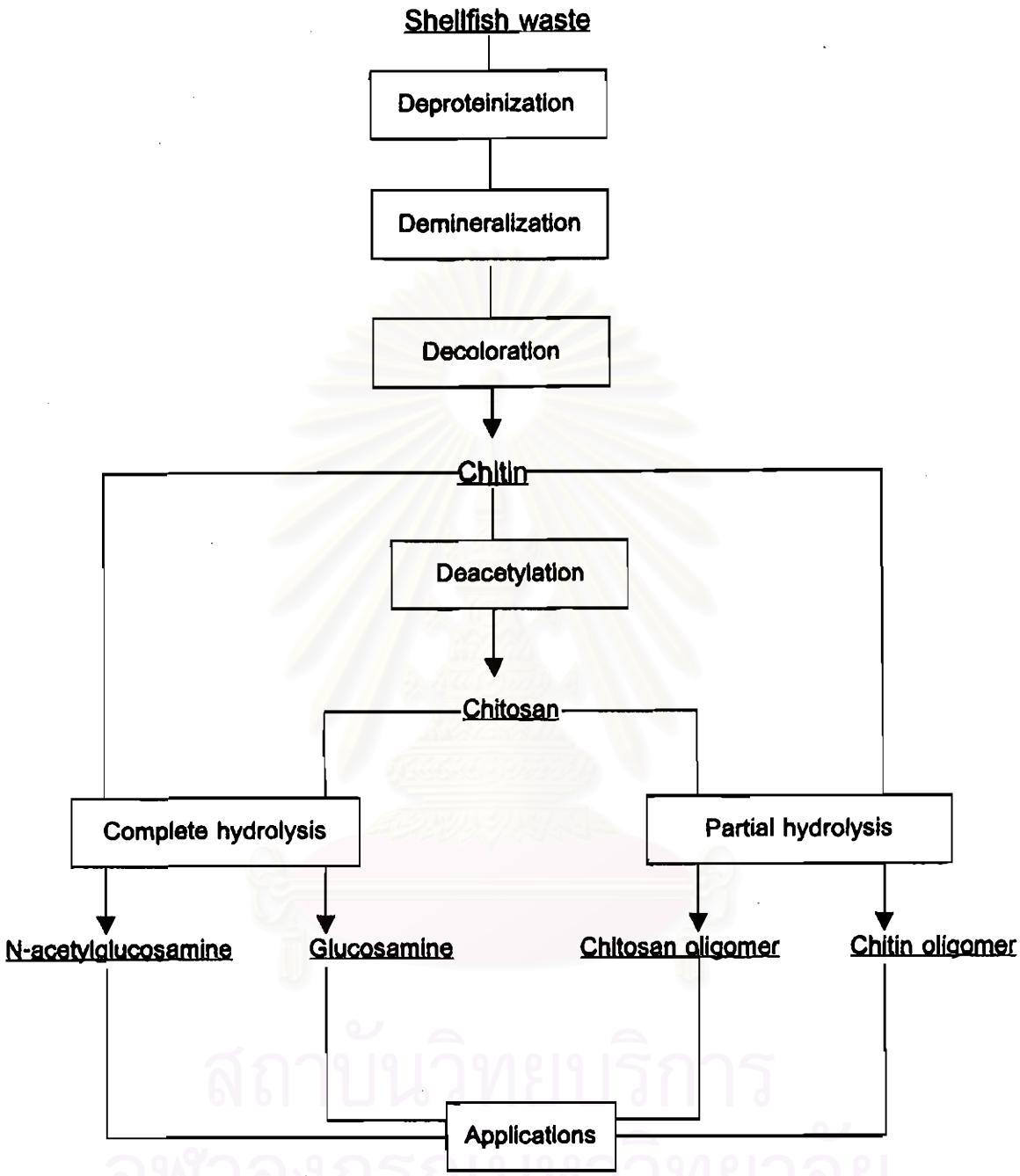


Figure 2 Simplified flowchart of preparation of chitin, chitosan and their oligomers from shellfish waste (Shahidi *et al.*, 1999)

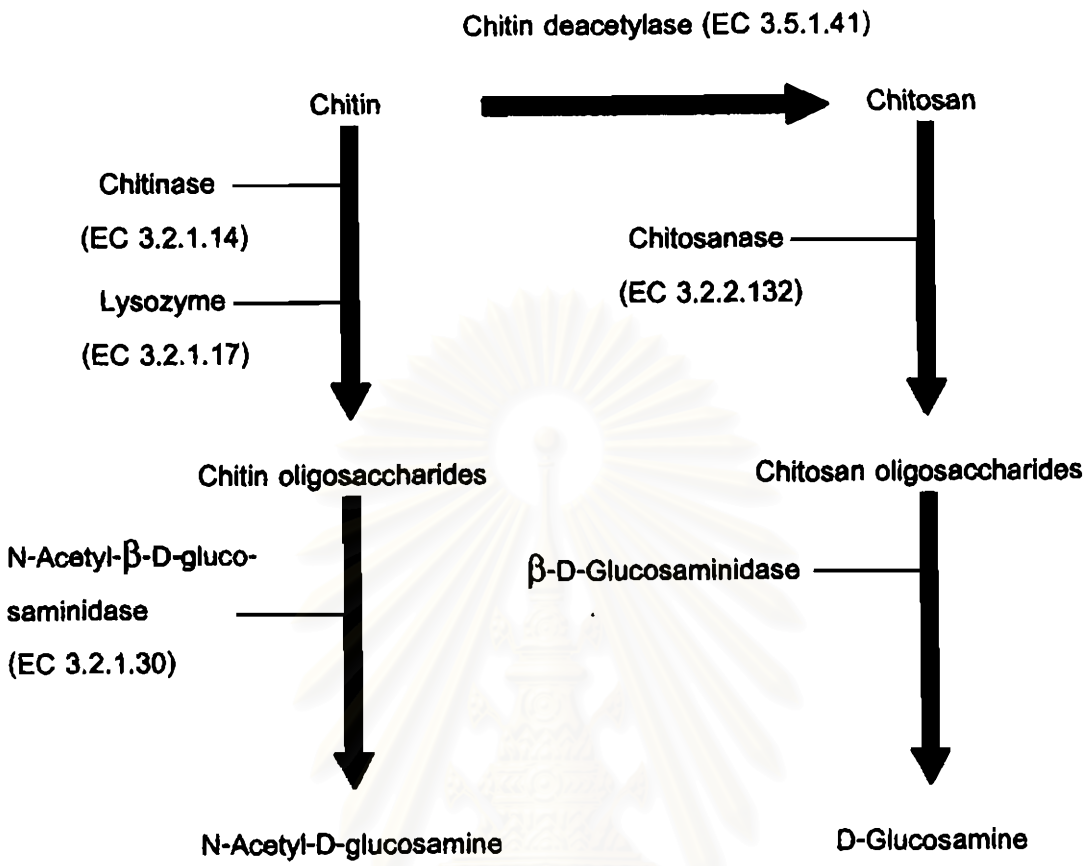


Figure 3 Enzymes for the hydrolysis of chitin and chitosan
(Goosen, 1997)

Table 1 Current practical uses of chitin, chitosan and their derivatives

Area of Application	Example
Agriculture	- Plant seed coating - Fertilizer
Food Industry	- Color stabilization - Emulsifying agent - Clarification and deacidification of fruits and beverages - Dietary fiber
Medical	- Dressing materials for the burns and skin lesions of humans and animals - Controlled release of drugs
Wastewater Treatment	- Recovery of metal ions, pesticides and phenols - Removal of dye
Cosmetics	- Moisturizer - Face, Hand and Body creams
Paper	- Surface Treatment - Photographic Paper
Biotechnology	- Enzyme immobilization - Chromatography

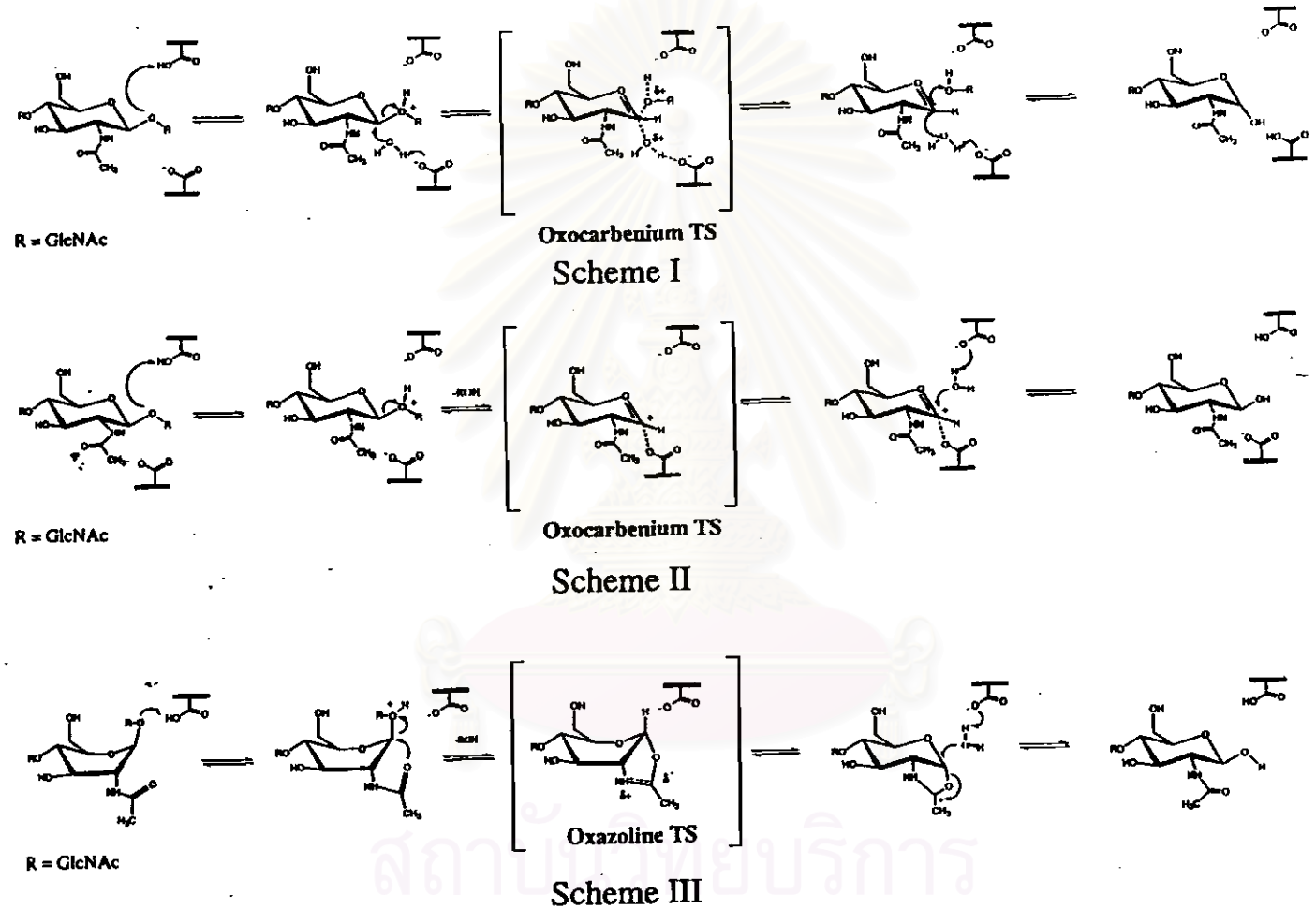


Figure 4 Comparison of family 19 chitinase mechanism (Scheme 1), family 18 chitinase (Scheme 3) ,and lysozyme (Scheme 2) mechanism.

molting and cell division (Kuranda and Robbins, 1991). Bacteria utilize chitinases for assimilation of chitin as a carbon source (Jones *et al.*, 1986). In addition, plants produce chitinases as a defense against fungal pathogens (Shinshi, Mohnen, and Meins, 1987). Because chitin is not found in vertebrates, it has been suggested that inhibition of chitinase may be used for the treatment of fungal infections and human parasitosis. Human macrophages can synthesize a functional chitinase, chitotriosidase (Boot *et al.*, 1995). This enzyme may play a role in the degradation of chitin-containing pathogens and can be used as a marker for specific disease states.

On the basis of amino acid sequence, the glycosyl hydrolases have been classified into 45 families (Henrissat, 1991; Henrissat and Bairoch, 1993).Using this classification method, the chitinases forms families 18 and 19 which are unrelated, differing in structure and mechanism. Family 19 chitinases (founds in plants) share he bilobal $\alpha+\beta$ folding motif of lysozyme, which forms a well-defined substrate binding cleft between the lobes (Hart *et al.*, 1995). In contrast family 18 chitinase, isolated from a wide range of eukaryotes and prokaryotes, shares two short sequence motifs which form the catalytic $(\beta\alpha)_8$ active site (Terwisscha van Scheltinga, Hennig, and Dijkstra, 1996).

Chitinase Mechanism

There are two general mechanistic pathways for acid-catalyzed glycosyl hydrolysis : (1) retention of the stereochemistry of the anomeric oxygen at C1' relative to the initial configuration or (2) inversion of the stereochemistry. Family 19 chitinases have been reported to yield hydrolysis products with inverse the anomeric configuration at C1' while Family 18 chitinases have produced hydrolysis products which retain the anomeric configuration at C1'.

Extensive studies of the mechanism of hen egg white lysozyme (HEWL) show that glycoside hydrolysis requires two acid residues (Glu36 and Asp52), one of which is protonated. The consensus view of the mechanism (Scheme 2, Figure 4) involves protonation of the β-(1,4)-glycosidic oxygen atom, leading to an oxocarbenium ion intermediate, which is stabilized by the secondary carboxylate group (either through

covalent or electrostatic interactions). Nucleophilic attack by water yields the hydrolysis product, which retains the initial anomeric configuration. This is commonly referred to as the double displacement mechanism of hydrolysis.

The X-ray crystal structure of a family 19 chitinase isolated from barley shows structural similarities with HEWL, suggesting an analogous double displacement mechanism (Hart *et al.*,1995). However, subsequent analysis of the anomeric products for two family 19 chitinases show that an inversion of the anomeric configuration accompanies these reactions (Fukamizo *et al.*, 1995; Iseli *et al.*, 1996). This observation rules out the double displacement mechanism of HEWL. A possible mechanism explaining inversion is a concerted single displacement reaction (Davies and Henrissat, 1995; McCarter and Withers, 1994) in which a bound water molecule acts as the nucleophile. Although this water molecule was not observed in the crystal structure, the second catalytic carboxylate group is at a sufficient distance to allow coordination of a water molecule, consistent with a single displacement mechanism shown in Scheme 1, Figure 4 (Hart *et al.*, 1995).

The crystal structure of a family 19 plant endochitinase isolated from barley (*Hordeum vulare* L.) seeds reveals two acidic residues (Glu 67 and Glu 89) in the active site (Brameld and Goddard III, 1998). By using molecular dynamics simulations, the binding of a hexaNAG substrate and two potential hydrolysis intermediates (an oxazoline ion and an oxocarbenium ion) to a family 19 barley chitinase have been examined. The hexaNAG substrate binds with all sugars in a chair conformation, unlike the family 18 chitinase which causes substrate distortion. Glu 67 is in a position to protonate the anomeric oxygen liking sugar residues D and E whereas Asn 199 serves to hydrogen bond with the C2′ N-acetyl group of sugar D, thus preventing the formation of an oxazoline ion intermediate. In addition, Glu 89 is part of a flexible loop region allowing a conformational change to occur within the active site to bring the oxocarbenium ion intermediate and Glu 89 closer by 4-5 A°. A hydrolysis product with inversion of the anomeric configuration occurs because of nucleophilic attack by a water molecule that is coordinated by Glu 89 and Ser 120.

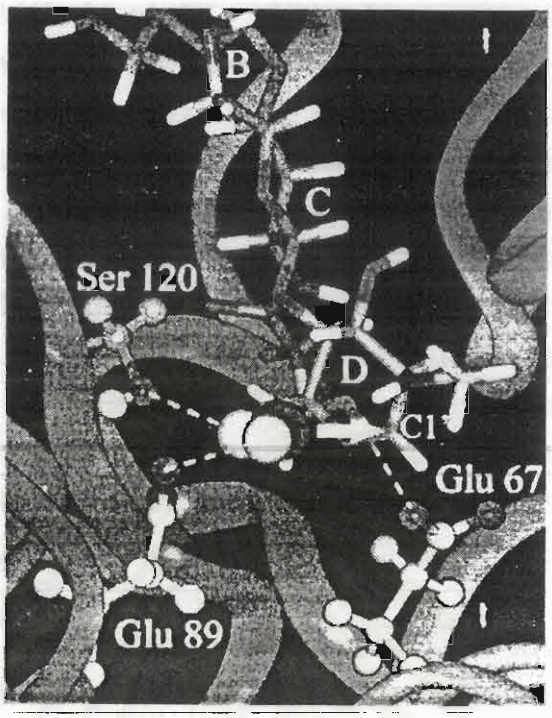


Figure 5 A snapshot from the dynamics simulations of a tri-NAG oxocarbenium ion intermediate bound to barley chitinase (Brameld and Goddard III, 1998)
An arrow marks the α face of the oxocarbenium ion which will result in inversion of the anomeric configuration upon nucleophilic attack by water. Ser 120 and Glu 89 are positioned to coordinate with this water molecule as shown with dashed lines. Glu 67 forms a hydrogen bond with H06′.

Family 18 chitinases have been reported to yield hydrolysis products which retain the anomeric configuration at C1′ (Brameld et al., 1998; Brameld and Goddard III, 1998). However, the X-ray crystal structure of two family 18 chitinases reveals no second acidic residue in the active site capable of stabilizing the oxocarbenium ion. Thus, neither the single nor double displacement mechanism is consistent with the observed structure and hydrolysis products. An increasing body of experimental and theoretical evidence points to an oxazoline ion intermediate formed through anchimeric assistance by the neighboring N-acetyl group (Scheme 3) as being the likely mechanism for family 18 chitinases (Scheme III, Figure 4). Such an intermediate alleviates the need for a second acidic residue. In solution, spontaneous acid-catalyzed hydrolysis of 2-acetamido-substituted polysaccharides have been reported to occur through anchimeric assistance.

Chitinase A from *Serratia marcescens* have investigated. Initial substrate binding and the possible resulting hydrolysis intermediates has been studied by molecular dynamics (MD) simulations. Combining these simulations results with reported experimental observations, some predictions regarding the hydrolysis mechanism have been made. The first step of the acid-catalyzed hydrolysis mechanism of Chitinase A is proton transfer from Glu 315. Evidence in support of this includes the observation that Glu 315 is completely conserved in family 18 chitinases. In addition, site-directed mutagenesis of the corresponding Glu residue in other family 18 chitinase, such as *Bacillus circulans* chitinase, to a Gln was reported to essentially eliminate chitinase activity. The likehood of proton transfer in these reactions primarily depends on the distance between the proton donor and acceptor. For chitinase A of *Serratia marcescens* (Figure 7), it is evident that the extended N-acetyl geometry of the -1-chair conformation places the glycosidic oxygen too far from Glu 315 (7 A°) for efficient proton transfer. In contrast, the -1-boat geometry of the sugar residue places the proton between 3 and 4 A° away from the glycosidic oxygen and occasionally much closer when a direct hydrogen bond is made. From this evidence we may concluded that binding of a chitin substrate in the -1-boat geometry is compatible with protonation. Glu 315, Asp 313 and Asp 311 were predicted as important amino acid residues in active sites chitinase A of *Serratia marcescens*. These studies were done in parallel with studies on hevamine, a plant

Figure 6 Residues that are involved in catalytic activity of hevamine, a plant chitinase (Terwisscha van Scheltinga et al., 1995) Glu 127, Asp 125 and Asp 123 were predicted as important amino acid residues in active sites.

Figure 7 Residues that are involved in catalytic activity of chitinase A from *Serratia marcescens* (Perrakis *et al.*, 1994) Glu 315, Asp 313 and Asp 311 were predicted as important amino acid residues in active sites.

family 18 chitinase, which has an enzyme-substrate co-crystallized structure.

Purification and Molecular Cloning of Chitinase

Chitinases have been isolated and purified from various kinds of microorganisms and plants, such as *Streptomyces* sp. J-13-3 (Okazaki *et al.*, 1995), *Serratia marcescens* BJL200 (Brurberg *et al.*, 1995), *Enterobacter* sp. G-1 (Park *et al.*, 1996) and *Aeromonas* sp. No.10S-24 (Ueda *et al.*, 1995). Chitinase can be purified from a total homogenate, from the intercellular fluid or from latex (Flach, Pilet, and Jolles, 1992). Affinity chromatography using chitin or regenerated colloidal chitin is the most specific method. It was used successfully for the purification of the chitinases from various organisms. This method cannot always be used, as problems in the binding or the release of chitinase may occur. Most chitinases have a very high or very low isoelectric point. This characteristic has often been used to purify chitinase. For example, Bernasconi, Jolles, and Pilet (1986) described a one-step purification of *Rubus* chitinase from culture medium, using ion exchange chromatography. However, this step is generally inadequate for obtaining a pure protein. Additional steps such as gel filtration may be used. Alternatively, all steps can be performed by HPLC. Some chitinases are summarized in Table 2. Molecular weights of enzymes secreted by those organisms are distributed within a range of 31,00 to 115,000. Most of the chitinases are active at a wide pH range of 4.0-8.0 and a temperature range of 40-55 °C.

Many chitinase genes derived from different organisms were analyzed. Until recently, chitinase gene from organisms such as *Alteromonas* sp. Strain O-7 (Tsujibo *et al.*, 1993), *Bacillus circulans* (Alam *et al.*, 1995), *Serratia marcescens* (Brurberg, Eijsink, and Nes I, 1994), and *Streptomyces griseus* HUT 6037 (Ohno *et al.*, 1996), and others have been cloned and sequences (Table 3). Chitinase gene expression in microorganisms has been reported to be controlled by a repressor inducer system in which chitin or other products of degradation act as inducers (Felse and Panda, 1999). In general, chitinase gene could be induced by either chitin, chitobiose or N-acetylglucosamine. For example, high levels of chitinase were found in cultures of *Metarhizium*

Table 2 Comparison of the Characteristics of Purified Chitinase from several Microorganisms.

Species	Molecular weight (MW)	Optimum pH	Optimum temp(°C)	pI	Reference
Aeromonas sp. 10S-24					
Chitinase I	112,000	4.0	50	7.9	Ueda, M. *et al.*, 1995
Chitinase II	115,000	4.0	50	8.1	
Alteromonas sp. strain O-7	70,000	8.0	50	3.9	Tsujibo, H. *et al.*, 1992
Enterobacter sp. G-1:					
Chitinase A	60,000	7.0	40	6.6	Park, J. *et al.*, 1996
Bacillus circulans WL-12					
Chitinase A1	74,000	5.0	N.D.	4.7	Watanabe, T. *et al.*, 1990
Chitinase A2	69,000	N.D.	N.D.	4.5	
Chitinase B1	38,000	N.D.	N.D.	6.6	
Chitinase B2	38,000	N.D.	N.D.	5.9	
Chitinase C	39,000	N.D.	N.D.	8.5	
Chitinase D	52,000	N.D.	N.D.	5.2	

N.D., not determined

Table 2 (continued)

Species	Molecular weight (MW)	Optimum pH	Optimum temp(°C)	pI	Reference
Streptomyces sp. J13-3	31,000	6.0	45	3.9	Okazaki, K. *et al.*,1995
Streptomyces erythraeus	30,000	5.0	N.D.	3.7	Hara, S., *et al.*, 1989
Streptomyces olivaeoviridis	47,000	7.3	45-55	4.0	Blaak, H., *et al.*, 1993
Streptomyces thermoviolaceus OPC-520 Chi 30	30,000	4.0	60	3.8	Tsujibo, H., *et al.*, 2000
Serratia marcescens QMB1466 Chitinase A	58,000	4.0-7.0	N.D.	7.9	Roberts, R., *et al.*,1982
Serratia marcescens BJ2000 Chitinase A	61,000	4.0-7.0	N.D.	6.4	Brurberg, M., *et al.*, 1982

N.D., not determined

Table 3 Molecular cloning of Chitinase genes

Species	Host	Plasmid	DNA insert sizes (kb)	ORF (kb)	Reference
Aeromonas sp. No.10S-24	*E.coli*	pUC18	8.0	1.6, 1.4, 1.6, 2.8	Shiro, M., *et al*, 1996
Aeromonas caviae	*E.coli*	pBluescript II SK	4.5	2.6	Sitrit, Y., *et al*, 1995
Alteromonas sp. strain O-7	*E.coli*	pUC18	5.0	2.5	Tsujibo, H., *et al*, 1993
Bacillus circulans WL-12					
Chitinase A1	*E.coli*	pKK223-3	4.0	2.1	Watanabe, T., *et al*., 1990
Chitinase C	*E.coli*	pUC19	2.8	1.5	Watanabe, T., *et al*., 1995
Clostridium paraputrificum					
Chitinase B	*E.coli*	pMW119	5.1	2.5	Morimoto, K. *et al*., 1997

Table 3 (continued)

Species	Host	Plasmid	DNA insert sizes (kb)	ORF (kb)	Reference
Streptomyces thermoviolaceus OPC-520 Chi 30	*E.coli*	pUWL219	3.4	1.0	Tsujibo, H., *et al*, 2000
Serratia marcescens BJL200 Chitinase B	*E.coli*	pBR325	9.2	1.5	Brurberg, M.*et al.*, 1995
Streptomyces griseus HUT 6037 Chitinase C1	*E.coli*	pUC119	1.7	0.9	Ohno, T.*et al.*, 1996

anisopilae supplied with chitin but not with pectin, xylan or cellulose. Studies with restricted cultures (a carbon-deficient medium with slow feeding of sugars) to prevent catabolic repression revealed that the most efficient inducers of chitinase and chitobiase were N-acetylglucosamine and glucosamine ,respectively. In batch cultures it was found that inducing sugars accumulated to repressive level even when the extracellular chitinase activity was very low. This leads to the conclusion that the accessibility and amount of chitin relative to other available nutrients (including lipids and proteins) may control the level of chitinase through the repressor/inducer system. Chitobiase was always produced along with chitinase in all media and was not affected by catabolite repression. Chitobiase was very effective in degrading the dimers and trimers of N-acetylglucosamine thus contributing to the release of chitinase inducers. This explains the large constitutive levels of chitinase present and the accumulation of reducing sugar even at low chitinase activity. This is an interesting contrast to the usual mode of regulation, where the expression of one enzyme affects the regulation of another gene.

In addition, Monreal and Reese (1969) found that, in batch cultures of *S. marcescens*, a larger amount of chitinase was produced in the presence of chitin than in the presence of N-acetylglucosamine. At low concentrations of N-acetylglucosamine (0.2%), a reasonable large amount of chitinase was produced, indicating induction and repression of the chitinase gene. Similarly Young and Carroad (1981), working with the same strain of *S. marcescens* in restricted cultures (carbon concentration controlled by dilution rate), found that chitinase was produced at low concentrations of N-acetylglucosamine. The observation of Monreal and Reese (1969) along with Young and Carroad (1981) suggests that chitinase of *S. marcescens* is regulated by N-acetylglucosamine induction and catabolic repression in much the same way as the chitinase gene is regulated in *Metarhizium anisopilae.*

In other cases, Vasseur *et al.* (1990) found that chitinase gene expression in a chitinase-overproducing mutant *A.album* E3 was induced by N-acetylglucosamine while it was repressed by glucose. Chitin or other oligomers did not enhance chitinase expression in *A. album* E3. On the other hand, in the gene regulation of *T. harzianum*, high chitinase

activity was found only in cultures supplied with chitin but not with cellulose, chitosan or chitobiose. In *T. harzianum* , N-acetylglucosamine did not enhance the enzyme production but instead repressed its synthesis. Repression of the chitinase gene was observed in the presence of glucose.

In general transcription of the genes encoding chitinase is induced by their substrates and repressed by more readily assimilated sugar such as glucose. However, the regulatory mechanisms of genes remain to be elucidated.

Burkholderia cepacia

Burkholderia cepacia (previously known as *Pseudomonas*), a nutritionally versatile, gram-negative organism, was first described in 1949 by Walter Burkholder of Cornell University, as the phytopathogen responsible for a bacterial rot of onion (Burkholder, 1950). Ironically, *B. cepacia* is now being considered by agricultural microbiologists as an agent to promote crop growth. *B. cepacia* is inherently resistant to multiple antibiotics, can metabolize diverse substrates, and is found in soul and in moist environments. The organism has a particular predilection for the lung in patients with cystic fibrosis (CF) and has emerged as an important opportunistic human pathogen in hospitalized and immunocompromised patients (Thomassen *et al.*, 1985).

***B. cepacia* as a Human Pathogen**

B. cepacia was first reported as a human pathogen causing endocarditis in the 1950s. Since then the organism has caused numerous catheter-associated urinary tract infections, wound infections, and intravenous catheterassociated bacteremias. In 1971, it was reported as the causative organism of "foot rot" in U.S. troops on swamp training exercise in northern Florida; it was also isolated from troops serving in the Mekong Delta, Vietnam.

B. cepacia is associated with increased illness and death among CF patients. CF is a recessively transmitted genetic disease that occurs in approximately 1 in 1,2500 Caucasians (carrier frequency of 1 in 25). The

condition is characterized by a generalized dysfunction of the exocrine glands, giving rise to a broad spectrum of clinical syndromes, especially malabsorbtion due to pancreatic insufficiency and chronic progressive lung disease giving rise to bronchiectasis.

In the early 1980s, the organism emerged as a major threat, causing superinfection in as many as 40% of patients in some CF centers. While in some patients indolent pulmonary infection occurs with only gradual deterioration in lung function similar to that associated with *Pseudomonas aeruginosa*, approximately 35% of *B. cepacia*-infected patients contract accelerated pulmonary deterioration or fulminant, necrotizing pneumonia with rapidly fatal bacteremia, sometimes referred to as "*cepacia* syndrome". Unlike infection with *P. aeruginosa*, *B. cepacia* infection significantly increases death rates among CF patients at all levels of lung infection.

Numerous CF-associated *B. cepacia* epidemics have now been described, and the epidemic strains have been characterized. One particular highly transmissible strain, epidemically spread within and between CF centers on both sides of the Atlantic, carries the *cblA* gene. This gene encodes for the major structural subunit of unique mucin binding cable pili. These enormously long pili (radiating 2 to 4 microns) are peritrichously arranged and are intertwined to form cablelike structure that adhere to CF mucin. This *cblA*+ strain has spread across Canada and has now been isolated in 50% of CF centers in the united Kingdom. Another strain of *B. cepacia* has spread among CF centers in four regions of France.

However, it has become clear that transmissibility varies markedly from strain to strain, and that most strains are not involved in epidemics, but appear to be independently acquired and show no cases of transmission were detected at the University of North Carolina CF center, despite clinical and social contact between patients and the absence of stringent infection control measures. Independent acquisition of *B. cepacia* with no evidence of transmission between CF patients was also reported from Denmark. Lack of transmission of some strains has also been observed between sibling with CF.

B. cepacia is rarely found in the non-CF patient; however, when it is found the organism can spread to and from CF patients. Transmission between CF and non-CF patients has been associated with serious illness and death and presents a greater nosocomial threat than previously recognized.

B. cepacia as an Agricultural Agent

While emerging as a human pathogen, *B. cepacia* has attracted intense interest from the agricultural industry as a possible biological control agent (Holmes, Govan, and Goldstein, 1998; LiPuma and Mahenthiralingam, 1999). The organism, which has been shown to have remarkable potential as an agent for both biodegradation and biocontrol, is being considered as a plant-growth-promoting rhizobacterium (McLoughlin, 1992; Homma, 1989; Sangodkar, Chapman, and Chakrabarty, 1988; Bowers and Parke, 1996; King and Parke, 1996; Cartwright, Chilton, and Benson, 1995).

B. cepacia has extraordinary metabolic versatility and can degrade chlorinated aromatic substrates (toxic compounds found in complex pesticides and herbicides, some with carcinogenic potential) for use as carbon energy sources. One important toxic compound degraded by *B. cepacia* is 2,4,5 chlorophenoxy acetic acid (2,4,5-T), a potent herbicide that is not easily biodegradable and persists for long periods in the environment.

B. cepacia can also antagonize and repress many soilborne plant pathogens. It can prevent leaf and stem blight caused by the fungus *Alternaria* by inhibiting spore germination. Economically important crop diseases such as blight due to *A. solani* and the blight caused by *A. brassicae* and *A. brassicola*, which affects the oil-producing plants rape and canola, can be controlled by *B. cepacia*. The organism is also being used to prevent the blight of ginseng plant due to *A. panax* and is effective against the fungus *Aphanomyces euteiches*, which causes root rot in peas, alfalfa, and snap beans (Bowers and Parke, 1996; King and Parke, 1996). It can prevent *Pythium* diseases of cucumber and peas, and *Rhizoctonia solani* stem rot of poinsettia (Cartwright *et al.*, 1995). To prevent these plant diseases, *B. cepacia* provides a seemingly

environmentally friendly alternative to potent and toxic fungicides, which cannot be broken down in the environment.

The forestry industry also sustains large economic losses from the pathogenic effects of fungi such as *Fusarium*, *Pythium*, *Rhizoctonia*, *Cylindrocarpum*, and *Botrytis*. These widespread fungal pathogens cause seedling loss in nurseries and may kill or stunt transplanted seedlings. A strain of *B. cepacia* has been developed as a successful seed and root inoculant , which can suppress these fungi on a variety of conifer.

Numerous patents are being sought for specific agricultural applications of different strains of *B. cepacia*. The ecologic and economic benefits could be enormous if the organism's antifungal activity is used to enhance crop yields and reduce the need for pesticides and its ability to degrade complex herbicides and pesticides is harnessed for bioremediation.

Commercial use of *Burkholderia cepacia*

B. cepacia was focused on the biopesticidal uses in agriculture. By virtue of its ability to antagonize a number of soilborne plant pathogens, *B. cepacia* is an attractive natural alternative to currently used chemical pesticides, such as captan, mancozeb, and metalaxyl. The replacement of these highly toxic agents, which are among the mainstays of crop protection chemicals, by safer products is a laudable goal. However, despite being nonpathogenic to healthy humans (and thus classified as a Biosafety Level 1 species), *B. cepacia* can cause life-threatening pulmonary infection in persons with cystic fibrosis. The deliberate widespread environmental application of strains of this organism should be considered carefully.

Perhaps of greater concern than agricultural use is *B. cepacia*'s use as a bioremedial agent. This species is capable of degrading chlorinated aromatic substrates such as those found in certain pesticides and herbicides. By virtue of its extraordinary metabolic versatility, *B. cepacia* can use such compounds as nutrient carbon energy sources. In addition, some strains produce enzymes capable of degrading nonnutritive substrates, such as trichloroethylene (TCE), a major ground

water contaminant used in the dry cleaning industry and in degreasing solvents.

The degree to which *B. cepacia* is being used in bioremediation products is unknown; however, the species has been used extensively to degrade ground water TCE contamination in at least one large U.S. city. A number of environment-friendly bioremediation products containing only naturally occurring, nonpathogenic bacteria are being marketed for use in drain opening and grease eradication systems. Because their formulations are proprietary, it is not known if these products contain *B. cepacia*; however, franchises that distribute such totally natural, noncorrosive, nontoxic products specifically target fast-food restaurants, photo processing facilities, and hospital radiology departments.

In this study, *Burkholderia cepacia* was found to produce high chitinase activity. Chitinase production and some properties of crude chitinase such as optimum pH, optimum temperature and substrate specificity was determined. Chitinase from *B. cepacia* was partially purified and the molecular weight was obtained from SDS-PAGE with chitinase activity staining. Cloning of DNA fragment containing chitinase gene from *Burkholderia cepacia* was focused on. Chromosomal DNA of *B. cepacia* was digested with restriction enzyme and cloned into *E. coli* DH5α by using pBluescript II KS^+ as a cloning vector. Southern blot analysis and phenotype screening are two main procedures used to screen for colonies of *E.coli* containing chitinase gene. DNA probe for detection of chitinase gene was designed from conserved amino acid sequenced of chitinase. Recombinant plasmid in positive colonies was mapped with restriction enzymes and sequenced.