Enzymes are proteins and are nature's own biocatalysts. Enzymes have many advantages over their chemical counterparts in that they are more specific, and generally possess high catalytic properties. Almost all processes in a biological cell need enzymes to occur at significant rates. More than 4000 enzymes catalyzing a wide array of reactions are known to exist. Since the 1950s, Enzyme technology has really taken off. It is the basis of the new industry called biotechnology. There are great benefits in using enzymes as catalysts to make products. They can be some 10,000 times more efficient than ordinary inorganic catalysts used in industry. One enzyme molecule can catalyze 10 million reactions in a single second!

Chitinase, a group of enzymes capable of degrading chitin directly into low molecular weight products and found in a broad range of organisms, including bacteria (Bacillus, Aeromonas, Pseudomonas, Serratia, Enterobacter, Actinomycete), fungi (Trichoderma, Aspergillus), higher plants, insects, crustaceans, invertebrates and some vertebrates (Yong et al., 2005). “Chitinase was described for the first time in 1911 by Bernard who found a thermosensitive and diffusible antifungal factor in orchid bulbs and in 1992 by Karrer and Hofmann in snail” (Gokul et al., 2000). The physicochemical properties and reaction mechanisms of chitinases in different organisms vary in their metabolic roles (Martinou et al., 1995). Chitinases can be classified as endochitinases or exochitinases on the basis of mode of action of enzyme on chitin or chitioligomers. Endochitinases cleave chitin at internal sites to generate multimers of GlcNAc. Exochitinases catalyze the hydrolysis of chitin progressively to produce GlcNAc, chitobiose or chitotriose. Chitinases belong to two major families of carbohydrate enzymes, family 18 and family 19, depending on their sequence similarities, structure and mechanism of action. Family 18 includes chitinases found in bacteria, fungi, viruses, and animals, and class III or V of plant chitinases. Family 19 includes class I, II and IV chitinases of plant origin only, with exception of chitinase C from Streptomyces griseus HUT 6037 (Ohno et al., 1996) and chitinases F and G from Streptomyces coelicolor (Saito et al., 1999). Chitinolytic enzymes have wide-ranging applications such as preparation of pharmaceutically important chitooligosaccharides and N-acetyl D-glucosamine, preparation of single-cell protein, isolation of protoplasts from fungi and yeast, control of pathogenic fungi, treatment of chitinous waste, and control of malaria transmission (Dahiya et al., 2006). The production of inexpensive chitinolytic enzymes is important in the use of chitin containing waste particularly in the sea food industry, which not only can solve environmental problem but also do with added value in certain cases.
Chito-oligomers produced by enzymatic hydrolysis of chitin is useful for applications in various fields like in medical, agricultural and industrial applications, such as antibacterial, antifungal, hypocholesterolemic, antihypertensive activity, and as a food quality enhancer (Bhattacharya et al., 2007). In recent year, efforts have been going on throughout the world to enhance the production and isolation of gene(s) encoding for the chitinase enzyme to be utilized as candidate gene(s) to combat fungal pathogens (Oppenheim and chet, 1992; Lorito et al., 1994; Tsujibo et al., 2000; Viterbo et al., 2001).

Chitin \((\text{C}_8\text{H}_{13}\text{O}_5\text{N})_n\) is the second most abundant unbranched glycopolymer on the Earth after cellulose. It was first isolated by French chemist Henri Braconnot from mushrooms in 1811 (Domard and Domard, 2002), having high molecular weight, non-toxic, biodegradable, strong positively charge, biocompatible along with antibacterial activity. Annually \(10^{10}\) tonnes of chitin are estimated to be produced is considered as waste, yet it is not synthesize by mammals (Goodday, 1990). The shrimp bio-waste in the tropical region contains 10-20% calcium, 30-65% protein content and 8-10% chitin on a dry basis (Zhu et al., 2004). Approximately, 75% of the total weights of shellfish, such as shrimp, crab and krill is considered as waste, and chitin comprises 20 to 58% of the dry weight of the said waste (Wang and Chang, 1997). In comparison with marine sources, chitin production from fungal waste is negligible. A lot of chitineous substances contained like shell of shrimp, crabs, lobsters and others are accounting for about 10% of global landings of the aquatic products (Nopakarn et al., 2002). However, these substances are discarded which may create a serious disposal problem leading to environmental pollution. Its degradation is of great importance as it can contribute to both carbon and nitrogen cycles in the biosphere (Reguera and Leschine, 2003) (Fig. 1.1). However, several strategies have been developed for converting chitin into small soluble oligomers (chitooligosaccharides), which are more useful for application in the various fields of medicine, agriculture, and industry (Stevens, 2005). Chitin oligomers were also examined for their inhibitory effects on human leukemia and have been reported to possess antitumor activity (Wang et al., 2006). Derivatives of chitin, including polysaccharides, oligosaccharides and monosaccharide’s, have performed several therapeutic activities such as immunomodulation (Shibata et al., 1997; Shibata et al., 2001), antitumor (Suzuki et al., 1986) osteoarthritis treatment (Creamer, 2000), and so on. Also, in the recent decade, N-acetyl-D-glucosamine (NAG), the end hydrolytic product of chitin, has become an attractive biomaterial as food supplements and cosmetics (Talent and Gracy, 1996). Naturally occurring chitin varies in its degree of deacetylation and in its crystalline form (\(\alpha, \beta, \gamma\)).
most organisms, chitin is found cross-linked with specific proteins and glucans to form structural units (Blackwell and Weih, 1984).

More than 100,000 kinds of fungi exist, of which about 8,000 fungi can cause disease in plants, and a relatively small number of them cause disease in humans and livestock (Sweets and Baker, 1994). Almost all the agricultural and horticultural crop species suffer severe yield losses due to fungal diseases. In the Indian context, fungal diseases are rated as the most important factor contributing to yield losses in major cereal, pulse, fruits and oilseed crops. To minimize losses due to field crop diseases, one should identify the diseases and conditions that favor disease development and prepare management strategies that are effective, practical, safe, and economical. Pesticides and organic compounds are widely used to control plant pathogens in many countries. However, the non-degradable components of their compounds have accumulated over the years and entered the food chain causing higher toxicity in animals and environment (Cigdem and Merih, 2003; Chet, 1987; Lynch, 1990). It also led to build-up of resistance of the pathogens to these fungicides. Hence, to tackle this global problem, it is compelling to look for alternative and effective method for disease management practices, which include the use of antagonistic microbes as biocontrol agents and their metabolic products, management/quarantine of agricultural land and pathogen-resistant crop cultivars etc. The development of enzymatic and/or microbiological approaches for the control of plant pathogenic fungi is extremely urged as they offer a safe, cheap and ecofriendly method. The antagonistic activity against fungal pathogens is usually related to the production of antifungal compounds (Fguira et al., 2005) and extracellular hydrolytic enzymes (Mukherjee and Sen, 2006). Chitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of Fusarium oxysporum, Sclerotinia minor, and S. rolfsii (El-Tarabily et al., 2000; Benjaphorn et al., 2008). Potential uses of naturally occurring bacteria, actinomycetes and fungi replacement or supplements for chemical pesticides have been addressed in many studies (Kamil et al., 2007). Chitinases also confer broad resistance to other biotic and abiotic stresses, such as bacterial pathogens, salinity and heavy metals (Dana et al., 2006). It is a phytoprotectant biofungicide and can be used to control several seed-borne and soil-borne fungal pathogens. It is capable to control Fusarium, Verticillium, Pythium and Rhizoctonia both in vitro and in vivo condition (Marten et al., 2001). Expression of cloned genes in transgenic plants has provided evidence in plant defences (Kong et al., 2001).

Microbial degradation of chitin offers best solution for the problem leading to recycling of nutrients in the environment along with generating of useful products viz.
Chitinase, chitooligosaccharides, N-Acetyl-D-glucosamine and single cell protein (Gohel et al., 2007; Pichyangkura et al., 2002). The utilization of the shellfish waste solves not only environmental problems but also decreases the production costs of microbial chitinases. Bacterial and fungal chitinases are extremely important for maintaining a balance between the large amount of carbon and nitrogen trapped in the biomass as insoluble chitin in nature (Li, 2006; Aronson et al., 2003) (Fig 1.1). The production of inexpensive chitinolytic enzymes is an important element in the process (Wang et al., 2008). Chitinases are reported to dissolve cell walls of various fungi, a property that has been used for the generation of fungal protoplasts (Anjani kumari and Panda, 1992; Romaguera et al., 1993).

Other applications of chitinases are bioconversions of chitin waste to single cell proteins and ethanol and fertilizers. Industrial applications of chitinases have been governed mainly by key factors such as cost production, shelf-life stabilities and improvement in enzyme properties by immobilization (Bhushan, 2000). Most of the chitin is used as raw material for the production of the monosaccharide GlcN, which is the number one dietary supplement in the USA, used for pain relief of osteoarthritis (Sandford, 2002).

**Fig 1.1: Role of chitinase in maintaining the balance of carbon and nitrogen in nature**
Plants are equipped with a variety of defence mechanisms to protect themselves against the attack of pathogens. Some of these are constitutive while others are induced upon the attack by pathogens. The interaction between plants and pathogens induces a variety of defense mechanisms which includes cell wall strengthening (Bradley et al., 1992), de novo production of antimicrobial compounds (pathogenesis response proteins and secondary metabolites (Hammerschmidt, 1999; Misra and Gupta, 2009; Gupta et al., 2010) and rapid localized cell death etc. (Alverez, 2000). In the category of pathogenesis related proteins chitinase and glucanase (Sela-Buurlage et al., 1993) have very important role since they attack directly on the fungal and insect structural component. Besides these two, other enzymes of plant secondary metabolite pathway including Chalcone synthase and isomerase (Hahlbrock et al., 1981) Phenylalanine ammonia lyase (Cramer et al., 1985) are also significant due to antimicrobial nature of secondary metabolites.

Chitinolytic enzymes are synthesized by microorganisms as a means to hydrolyse chitinolytic material. This trait could be manipulated to offer a “greener” approach to the control of plant associated fungal as well as nematode pathogens. Chitinases are known to release oligo N-acetyl glucosamine’s which function in plants to elicit the activation of a defence related response at the cellular level in the plant cells (Kasprzewska, 2003). The form of expression, which can be systematic or local, is dependant on the infecting pathogen, its virulence, and the particular chitinase class (Meier et al., 1993). This two-fold strategy of pathogen elimination and plant protective stimulation could be the environmental strategy best able to satisfy both environmental and agricultural needs.

Therefore, the aim of the present study was to characterize extracellular chitinase and explore the biotechnological potential of microorganisms and enzymes along with the upcoming approaches for discovering and developing novel chitinase.
Objectives of the study:

- Isolation and identification of microbes for chitinase production
- Production optimization of the chitinase
- Extraction and purification of chitinase
- Characterization of chitinase
- Applications of chitinase