1.1 Actinomycetes

Actinomycetes are Gram positive bacteria having characteristics common to both bacteria and fungi. They were initially considered to have high G+C nucleotide content usually more than 55% (Goodfellow and Williams, 1983) but now it is realised that many fresh water actinomycetes actually have low G+C content (about 42%) (Ghai et al., 2012). The first edition of Bergey’s manual of Systematic Bacteriology divides actinomycetes into seven sections based on phenotypic properties. The second edition of Bergey’s manual classify them on the basis of 16S rRNA sequences and places them in volume 4. There is only one class, Actinobacteria, five subclasses, six orders, nine suborders and 35 families. These actinobacteria have been reported from diverse terrestrial and aquatic habitats.

Morphologically actinomycetes range from unicellular coccoid, rod-coccoid, fragmenting filamentous forms to highly differentiating non fragmenting branched mycelial forms. Filaments are called as hyphae and their network as mycelium resembling that of fungi (although hyphae are always of small diameter normally not exceeding one micron) which may fragment to produce asexual spores. Most of actinomycetes on culture medium form slow growing, powdery colonies that sticks to agar surface. Truly filamentous actinomycetes forms both aerial mycelium (AM; hyphae on the surface) and substrate mycelium (SM; hyphae in to surface) when grown on a solid substrate like agar. Sometimes a tissue like mass called as thallus may form. Aerial mycelium of many actinomycetes extends above the substrate and forms asexual spores, conidia or conidiospores at the end of filaments. Sometimes the spores are located in a sporangium called sporangiospores. Spores are formed in response to decreased nutrient conditions by septal formation at filament tips. Generally the spores are not heat resistant but can withstand desiccation. Mostly actinomycetes are non motile, if motility is present, it is limited to flagellated spores. Morphology of aerial and substrate filaments, mycelium colour, spore chain morphology and pigments produced are of taxonomic value that assists in their classification and identification (Prescott et al., 2005).
Although the cell wall of actinomycetes is made up of peptidoglycan but its composition varies greatly among different groups and is of taxonomic importance. Some of the important features of their peptidoglycan are presence of diaminopimelic acid (DAP) isomeric forms, presence or absence of glycine in interpeptide bridge and presence of unique characteristic sugars (like arabinose, galactose, xylose, and madurose) in some of the groups. Other taxonomically valuable properties are phospholipid composition of cell membrane lipids and spore heat resistance. Now 16S rDNA sequencing is considered to be a valuable tool in actinomycete taxonomy (Prescott et al., 2005).

1.2 Saline Environments

Many reports available on the ecology of actinomycetes show that they are prevalent in nature and also occur in extreme environments and this has led to the discovery of many new species and genera of actinomycetes, which produced bioactive compounds (Jiang and Xu, 1993).

One of the examples of extreme habitat is saline environment that harbours enormous microbial diversity. It is present in the coastal and offshore regions, in water surface and abyssal depths, in specialized ecosystems like hydrothermal vents, tropical coral reef ecosystems, estuaries, lagoons, salt lakes, salt pans, backwaters and mangroves. Such saline waters are characterized by presence of dissolved solutes and alkaline pH where native microbes are under a number of intense ecological pressures. Microorganisms found in these saline sites require high salt (NaCl) concentrations for their growth and are referred as halophiles. They are classified on the basis of their salt requirement for growth. According to Ollivier et al., 1994 they are divided into three groups as slight halophiles (optimum growth at 0.2-0.85 M NaCl (2-5 %), Moderate halophiles (rapid growth at 0.85-3.4 M NaCl (5-20 %) and extreme halophiles (optimum growth at 3.4-5.1 M NaCl (20-30 %). Halotolerant microorganisms do not show an absolute requirement for salt but can tolerate very wide range of salinity (Xiang et al., 2011). In contrast non-halophiles show optimum growth at NaCl concentrations below 2 % (0.2 M). These halophiles harbour distinguishing physiological properties that can be exploited for production of enzymes, bioactive compounds, osmoprotectants and many polymers (Ventosa et al., 1998).
Salt lakes form a unique ecosystem and its native micro flora is expected to produce many new and potential compounds. Hyper saline waters of salt lakes can be divided in two types. They are called as thalassohaline, if their composition is similar to that of sea water. However, if their composition emulates the surrounding geology, topography and climatic conditions and is chiefly influenced by the dissolution of mineral deposits then the water is called as athalassohaline. Examples of thalassohaline environments are solar salterns, used for salt production by evaporation of sea water whereas examples of athalassohaline waters are the Dead Sea, Great Salt Lake, few cold hypersaline lakes in Antarctica or alkaline lakes, Lake Magadi or the lakes of Wadi Natrun and soda lakes (Rodriguez-Valera et al., 1985; Grant et al., 1990).

Microbes adapted to these extreme environments have gained significant attention in past few years. Many of the studies on extremophilic organisms are reported on extremophilic bacteria like Bacillus, Halobacillus, Marinococcus, Salinicoccus, Nesterenkonia, Tetragenococcus. Actinomycetes are rather less examined group in this regard. Since saline environments differ greatly from normal terrestrial habitats, the biological characteristics of halophilic actinomycetes and their distribution are expected to be unusual when compared to those of terrestrial Actinomycetes. They can form metabolites of different chemical structure which can lead to the synthesis of new drugs (Solanki et al., 2008). They can also act as source of enzymes better adapted to function in extreme conditions of temperature, pH and salt.

1.3 Enzymes

Enzymes are biological catalyst that performs many chemical reactions necessary to sustain life. They had now-a-days completely replaced chemical catalyst in various industries and are preferred over inorganic catalyst because of their highly specific and efficient catalytic action, capability to carry out reactions under mild conditions that save energy and resources and biodegradable in nature (Kirk et al., 2002).
From traditional times enzymes are used for synthesis of food products like beer, wine, vinegar, cheese and sourdough. They are also used for the production of leather, indigo and linen. Microbial enzymes or enzymes from calves’ rumen or papaya fruit were used for all such processes.

In the later part of last century selected microbial strains were used for enzyme production on a large scale using fermentative processes. This has permitted the use of enzymes in detergent, textiles and starch based industries. Enzyme manufacturing processes were further improved with the use of recombinant gene technology that enabled their commercialization. Developments in protein engineering have further improved the production of industrially important enzymes (Beilen and Li, 2002; Kirk et al., 2002).

Although enzymes are produced by animals, plants and microorganisms but microbial sources are extensively used in industrial processes today because of following reasons:

- Enormous microbial diversity present in environment
- Large scale production capacity
- Low production cost
- Easy downstream processing
- More stable then plant and animal enzymes
- Capacity to perform under wide variety of environmental conditions (extreme temperatures, pH, salt concentration etc.)
- Optimization of process conditions more feasible
- Biodegradability
- Ease of genetic manipulation to obtain high yielding microbial strain (Burhan et al., 2003; Mishra and Behera, 2008)

Today many industries such as food, detergent, pharmaceuticals, diagnostics and fine chemicals make use of microbial enzymes. Almost 75 % of all commercially used enzymes are hydrolytic in nature, of which proteases, carbohydrases, and lipases dominate the enzyme market, responsible for more than 70 % of all enzyme sales. It is estimated that this technical enzyme market will grow
at a 6.6% compound annual growth rate (CAGR) and will be near to $1.5 billion in 2015 (BCC Research report, 2011). This increasing demand attracted interest in search of new sources for enzymes apt for industrial usage and their cost effective production technologies (Burhan et al., 2003).

Initially actinomycetes were believed as one of the predominant group of soil population (Kuster, 1968) playing a significant role in degradation of organic matter, made up of chitin and cellulose and thus contributing to the organic matter turnover and in carbon cycle. They are beneficial not only to pharmaceutical industries but also for agriculture. They have the potential to use polysaccharides such as starch, xylan, pectin and cellulose as carbon sources which is accomplished through the production of extracellular hydrolytic enzymes that can be used for biotechnological applications. This biocatalytic potential of actinomycetes is now been exploited worldwide (Ramesh et al., 2009).

The ever increasing industrial demand for enzymes that can function under extreme conditions has led to the study of actinomycetes producing extracellular enzymes. Due to their stability, such enzymes offer new opportunities for biocatalysis and biotransformation.

In Nature a wide variety of microorganisms exist that secrete extracellular enzymes to degrade many polymer compounds. Many different enzymes available for commercial applications are identified to date but still they are not sufficient to meet the increasing industrial demands. Most of the enzymes were isolated from mesophilic environment which has limited stability at the extreme temperature, pH, ionic strength hence, they were not able to meet the harsh industrial processes. In order to fulfill this need now the focus is on study of extreme environments and their microbial products. These extremophiles are a potential source of extremozymes, structurally modified at the molecular level to endure extreme conditions and thus are stable in such conditions. The adaptation of extremophiles towards extreme pH and temperature attracted the attention towards their valuable products as enzymes (Gomes and Steiner, 2004). In this regard enzymes from halophilic habitats can be exploited as a potential source of enzymes.
Variety of biotechnological products and processes makes use of microbial enzymes especially food, beverages, textiles, paper, pharmaceutical and biofuel industries. Two of the most important enzymes that meet the industrial demands are amylase that degrades starch and cellulase that degrades cellulose.

1.3.1 Amylase

Amylase is the enzyme that catalyzes the hydrolysis of starch and related compounds as pullulan, glycogen to form dextrins and small polymers of glucose units (Windish and Mhatre, 1965). They contribute to approximately 25-30% of world’s enzyme consumption (Rao et al., 1998; Van der Maarel et al., 2002). Starch, the main substrate of amylase is one of the most abundant carbohydrate polymer found in nature. Chemically, it is a heteropolymer of glucose units made up of two polysaccharides, amylose and amylopectin. Amylose is made up of about 6000 glucose residues arranged in a linear manner by α-1, 4 glycosidic bonds and generally forms 17-30% of the total starch composition. Amylopectin is a branched polymer made up of small linear chains of 10-60 glucose units (linked by α-1, 4 bonds) which are connected to α-1, 6 side chains of 15-45 glucose units (Fig. 1.1). About 2 million glucose units are present in amylopectin and it accounts for 75 to 85% of most starches (Godfrey and West, 1996).

Fig. 1.1: Chemical Structure of Starch
Starch was always used as food ingredient and considered to be staple diet but its low cost, easy availability and good performance attracted its use as a raw material in various industries. About 57% of starch produced is used in food sector and 43% in non food sectors that requires the use of amylase enzyme.

Kirchhoff in 1811 discovered the first starch hydrolysing enzyme that was followed by numerous studies on malt and digestive amylases. On the basis of type of anomeric sugars formed, Ohlsson in 1930, classified starch degrading enzymes present in malt as α and β amylases (Gupta et al., 2003).

1.3.1.1 Types of Amylase

Starch hydrolysing enzymes can be classified into four groups on the basis of their mode of action.

(a) **Endoamylase**: They randomly cleave internal α 1, 4 glycosidic bonds in starch and related polysaccharides forming variable length oligosaccharides with alpha-configuration on the C1 of the reducing glucose unit produced (Van der Maarel et al., 2002; Reddy et al., 2003). α-amylase (E.C. 3.2.1.1) are the prominent endoamylase widely distributed in nature (Sivaramakrishnan et al., 2006). On the basis of percentage hydrolysis of glycosidic linkages of starch they are further classified in two groups, saccharifying (50-60%) and liquefying α amylases (30-40%) (Vihinen and Mantsala, 1990).

(b) **Exoamylase**: they act on the nonreducing end of starch and preferentially catalyze the hydrolysis of the second α-1, 4 glycosidic bonds. Based on bond and substrate preference as well as products formed they can be grouped into different types. β-amylase (E.C. 3.2.1.2) that cleaves α-1,4 glycosidic bonds and produces maltose and β limit dextrins. Glucoamylase (E.C. 3.2.1.3) and α-glucosidase (E.C. 3.2.1.20) that cleaves both α-1,4 and α-1,6 glycosidic bonds forming glucose as end products (Sivaramakrishnan et al., 2006). These enzymes change the anomeric configuration of end product from α to β (Pandey et al., 2000).

(c) **Debranching Enzymes**: they hydrolyze α-1,6 bonds forming long polysaccharide chains. They include isoamylase (E.C.3.2.1.68) that acts on α
1, 6 linkages in amylopectin and pullanase (E.C. 3.2.1.41) that act on α 1, 6 linkages in starch, amylopectin, pullulan, and related oligosaccharides (Israelides et al., 1999; Sivaramakrishnan et al., 2006).

(d) **Transferases**: They cleave α-1, 4 glycosidic bond of the donor molecule and then transfer a part of the donor to a glycosidic acceptor thereby forming a new glycosidic bond. Amylomaltase (E.C.2.4.1.25) and cyclodextrin glycosyltransferase (E.C.2.4.1.19) are the examples of such enzymes (van der Maarel et al., 2002).

### 1.3.1.2 Sources of Amylase

Starch can be used as a source of carbon and energy by number of organisms that produces extracellular amylase to degrade this polymer. Amylases are produced by humans, plants, bacteria, fungi and actinomycetes. It is present abundantly in human saliva that breaks some of starch in mouth to sugars. It is also secreted by pancreas where it hydrolyses dietary starch in to di and tri saccharides which are then acted on by other enzymes to supply energy in the body. Brewing industry makes use of Barley amylases. From traditional time’s amylase from microbial sources and plants have been used as food additives.

Many bacteria produces amylase but industrial applications are dominated by the members of genus *Bacillus*. Enzymatic liquefaction and saccharification of starch occurs at high temperatures (100–110 °C) requiring the action of thermostable amylase which is produced mainly by *B. subtilis*, *B. licheniformis*, *B. stearothermophilus* and *B. amyloliquefaciens* (Prakash and Jaiswal, 2010). Halophilic bacteria as *Chromohalobacter sp.*, *Halobacillus sp*, *Halomonas meridian*, *Haloarcula hispanica* and *Bacillus dipsosauri* have been reported to produce halophilic amylases (de Souza and Magalhaes, 2010).

Starch utilizing capacity is widely present in *Streptomyces* and few of them are also capable of hydrolyzing raw starch, releasing maltose as main end product (Andrews and Ward, 1988; Goldberg and Edwards, 1990). Besides them amylase activity is also reported in *Nocardiopsis, Thermomonospora* and *Thermoactinomyces* (Kuo and Hartman, 1966; Upton and Fogarty, 1977; Stamford et al., 2001).
Amylases from fungi are widely used for preparing oriental foods. α-amylase production by fungi is limited to mesophilic, terrestrial isolates and mainly to *Aspergillus* and *Penicillium* (Kathiresan and Manivannan, 2006). α-amylase produced by *Aspergillus oryzae* and *Aspergillus niger* are most widely used for industrial applications (Jin et al., 1998). As compared to bacterial amylases fungal enzymes are acidic to neutral in nature and less thermostolerant. *Aspergillus niger* can produce α-amylase even at acidic conditions of pH < 3 (Djekrif-Dakhmouche et al., 2006).

1.3.1.3 Applications of Amylase

- **Starch Processing**

  Starch processing industry utilizes amylase for the production of glucose and fructose. It involves gelatinization, liquefaction and saccharification of starch and is completed by a combination of bacterial α-amylase and fungal glucoamylase (Gupta et al., 2003). The resulting glucose syrup is used in many sweet and bakery products (Arasaratnam and Balasubramaniam, 1993). Starch is also used to form high fructose corn syrups (HFCS, 42% fructose) in which glucose formed during saccharification is converted into fructose by use of glucose isomerase. HCFS are used in large quantities as sweeteners for soft drinks, candies, baking, jams and jellies (Ramachandran et al., 2004).

- **Textile Industry**

  Starch is used in textile industry as a sizing agent that prevents breaking of the warp thread during the weaving process. α-amylase are used for desizing of these starch sized textiles where they break the starch into water soluble dextrins that can be removed by washing without attacking the fibers (Gupta et al., 2003).

- **Detergent Industry**

  Enzymes are now used as a regular ingredient of new detergent formulations. They make detergent milder, eco friendly, lower the washing temperatures and improve the detergents ability to remove tough residues of starchy foods like custard, gravies, chocolate, potato, and smaller oligosaccharides. α-amylase is a regular component of about 90% of all liquid detergents (Mitidieri et al., 2006;
Hmidet et al., 2009) and its need for automatic dishwashing detergents is also increasing. Novozyme and Genencore International have marketed their amylase preparations under the trade names Duramyl® and Purafect OxAm® respectively (Gupta et al., 2003).

- **Paper Industry**
  α-amylase is used in paper industry for reducing the high viscosity of natural starch used for the sizing process. Starch coating (sizing) protects the paper against mechanical injury during processing and also enhances stiffness, strengths and quality of finished paper. Pulp and paper industry makes use of number of commercially available enzymes as α-amylase G9995® (Enzyme Biosystems, USA), Amizyme® (PMP Fermentation Products, Peoria, USA) and Termamyl®, Fungamyl, BAN® (Novozymes, Denmark) (Gupta et al., 2003).

- **Baking Industry**
  α-amylase are used widely in the baking industry. They degrade flour starch to dextrins which are further acted on by yeast. Its action increases the fermentation rate and decreases the dough viscosity thus causing improvement in texture and volume of the product. It also increases the sugar content of bread thereby enhancing the crust colour, taste and toasting qualities (Van Dam and Hille, 1992). α-amylases also prevent staling of baked products thereby increasing the shelf life (Gupta et al., 2003).

- **Ethanol Production**
  The growing environmental concern and rising crude oil prices has developed interest in fuel ethanol. It can be derived from renewable resources such as agricultural crops and by products. Starch is the most commonly used substrate for ethanol production because of its less price and easy availability in many regions of the world. α-amylase are used extensively for bioconversion of starch into simple sugars. This in turn is followed by fermentation, where ethanol is formed from sugar by *Saccharomyces cerevisiae* (De Moraes et al., 1999). Microbial amylases are also used in beer industries the production of fermentable sugars.
1.3.2 Cellulase

Cellulase are multicomponent enzyme systems that hydrolyze $\beta$-1,4 glycosidic linkages in cellulose. They are accountable for approximately 20% of the world’s enzyme market (Mantyla et al., 1998). Cellulose is considered to be most abundant renewable biopolymer on earth and is believed to be an inexhaustible source of raw material for variety of product formation (Bhat and Bhat, 1997). It forms the rigid skeleton of cell wall of woods and annual plants. It is also produced by some animals (e.g., tunicates), bacteria (e.g., Acetobacter xylinum) and by algae too. Plants are the major producers of cellulose. About 35-50% plant dry weight is made up of cellulose. (Lynd et al., 1999). Chemically, cellulose is a linear homopolymer of anhydroglucose units in chair configuration linked to one another by $\beta$-1,4 glycosidic bonds. Adjacent glucose units alternate in orientation by 180° that makes the cellobiose as basic repeating unit (Fig. 1.2). The degree of polymerization and the number of glucose residues varies in different sources. In plant cellulose it is 14000, in bacteria about 35000 and in commercial glucose it ranges from 50 to 5000 glucose units (Sharrock, 1988; Fogarty and Kelly, 1990).

![Fig. 1.2: Chemical Structure of Cellulose](image)

1.3.2.1 Types of Cellulase

On the basis of their catalytic action and structural properties three main components of cellulase are recognized (Henrissat et al., 1998).

(a) **Endo-(1,4)-$\beta$-D-glucanase** (E.C.3.2.1.4) $(C_x)$: They act on internal amorphous region and randomly hydrolyse long chains of cellulose producing oligosaccharides and new chain ends. They act synergistically with exo-(1,4)-$\beta$-D-glucanase to hydrolyse cellulose. Major substrates on which it is active are cellulose, cellobextrins, modified cellulose as carboxy
methyl cellulose (CMC) and hydroxymethyl cellulose. It cannot act on native cellulose alone.

(b) **Exo-(1,4)-β-D-glucanase** (E.C.3.2.1.91): they can act on both reducing and non-reducing ends of cellulose chains generating two major end products cellobiose and glucose. It can hydrolyze crystalline regions of cellulose and act on substrates as avicel, cellobiose, and also on amorphous cellulose but is not active on CMC and cellobiose.

(c) **β-glucosidases** (E.C.3.2.1.21): they act on cellobiose and cellodextrins producing glucose. It is not active against crystalline or amorphous cellulose (Teeri, 1997; Wood, 1989).

Different cellulase degrading mechanisms are found in microorganisms to hydrolyze cellulose. Filamentous fungi and actinomycetes can penetrate cellulosic substrates by their hyphae thus directly presenting their enzyme in confined cavities within cellulosic particles. The enzyme system of such organisms is referred as noncomplexed system. However anaerobic bacteria lack hyphae and thus cannot penetrate cellulosic material. Their cellulase is present in stable high molecular weight complex, cellulosomes which bring them at the site of hydrolysis (Kuhad et al., 1997; Bayer et al., 2004).

### 1.3.2.2 Sources of Cellulase

The capacity to degrade cellulose is found in many fungi, bacteria and actinomycetes that be either aerobic or anaerobic, mesophilic or thermophilic. Fungal cellulase are best characterized system dominating the industrial applications. They have the presence of all three components i.e. endoglucanases, exoglucanases and β-glucosidase that act together to degrade cellulose completely (Mawadza et al., 2000). Cellulase system of *Tricoderma ressei* is most extensively studied. It is composed of at least two exoglucanases, five endoglucanases and two β-glucosidase (Takashima et al., 1999; Nogawa et al., 2001).

The cellulase system of the *Aspergillus oryzae*, *Phanerochaete chrysosporium* and thermophilic fungus *Humicola insolens* has also been reviewed (Lynd et al., 2002) and are among the other studied fungal systems.
Among aerobic bacteria best studied system is of *Cellulomonas* and *Thermobifida* (formerly *Thermomonospora*). Cellulase systems of *Cellulomonas* are made up of at least six endoglucanases and at least one exoglucanase (Chaudhary *et al.*, 1997). Among thermophilic aerobic bacteria *Acidothermus cellulolyticus* (an actinomycete) and *Rhodothermus* produces cellulases (Sakon *et al.*, 1996; Halldorsdottir *et al.*, 1998). Among actinomycetes members of *Cellulomonas* (*C. fimi, C. uda, C. bioazotea*), *Streptomyces* (*S. lividans, S. drozdowiczii*) and *Thermomonospora* (*T. Curvata, T. fusca*) are the well known for their cellulolytic activity (Kuhad *et al.*, 2011). The process of cellulose breakdown in aerobic bacteria is similar to aerobic fungi, but anaerobic bacteria functions by a different mechanism (Carvalho *et al.*, 2003; Zhang *et al.*, 2006). In anaerobic environments, cellulolytic bacteria produce cellulosomes (complexed systems) on the cell wall in the form of protuberances that can tightly bind to microcrystalline cellulose. Examples of such cellulolytic bacteria are *Clostridium thermocellum, Clostridium cellulovorans, Clostridium cellulolyticum* and *Clostridium josu* and *Ruminococcus* species in the rumen (Schwarz, 2001). Extracellular cellulase is produced by bacteria in lower quantities than fungi and most of them are also not able to degrade crystalline cellulase as they produce only endoglucanases rather than a cellulase complex.

### 1.3.2.3 Applications of Cellulase

- **Pulp and Paper Industry**

  The woody raw material used in paper industry is mechanically processed by refining and grinding that generates pulp with high content of fines, stiffness and bulk and also consumes high amounts of energy. Cellulase are used in biomechanical pulping process that reduces the energy used (20-40 %) during refining and also enhances fibre qualities and tensile strength (Bhat, 2000; Pere *et al.*, 2001; Singh *et al.*, 2007).

  Cellulase in combination with hemicellulase has been used for modification of fibre properties. Endoglucanases act on bleached kraft pulps to decrease pulp
viscosity and improve pulp beatability and other properties of the paper (Oksanen et al., 1997).

Cellulase along with xylanase and pectinase are used to release ink from fibre surfaces (Prasad et al., 1992, 1993; Jeffries et al., 1994) which prevents the alkaline yellowing and reduces the environmental pollution thereby improving the fibre brightness and strength properties (Prasad et al., 1992). These enzymes are also used for the preparation of paper towels, sanitary paper and biodegradable cardboard (Kuhad et al., 2011).

**Textile and Laundry Industry**

Cellulase rank third among the various enzymes used in textile industry. They are used for bio-stoning of denims and bio-polishing of fabrics (Bhat, 2000). In bio-stoning process they break off the small fibre ends protruding on the yarn surface and weakens the indigo dye that is readily removed during wash cycle by mechanical action. Neutral cellulase preparations from *H. insolens* were used to eliminate the problem caused by back staining (released dye being again deposited on the garments). Mixture of acidic cellulase (from *Trichoderma reesei*) and microbial protease together also prevents back staining (Galante et al., 1998a).

The process of bio-polishing make use of cellulase to act on protruding fibre ends of fabrics so as to prevent fuzz formation (protruding of short fibres from the surface) and piling (attachment of loosened fuzz to surface). The mechanical action thereafter removes them and polishes fabric. It also improves softness and water absorbance property of fibres. Endoglucanase rich cellulase preparations are also used for enhancing fabric feel, look, and colour in an eco-friendly manner (Galante et al., 1998a; Bhat, 2000).

**Laundry and Detergent Industry**

Cellulase also used in formulation of laundry detergents where they remove the microfibrils and reinstate smooth surface and fabric original colour. It also softens the garment and aid in removal of dirt particles from microfibril network (Hebeish and Ibrahim, 2007; Ibrahim et al., 2011). Cellulase preparations from *H.*
Insolens are used commercially for this purpose as they are active under mild alkaline conditions and temperatures above 50°C in washing powders (Uhlig, 1998).

- **Animal Feed Industry**
  The forage diet of ruminants is a complex mixture of hemicelluloses, cellulose, lignin and pectin. Mixture of hemicellulase, cellulase and pectinase are used to improve the feed quality, body weight gain and milk yield in ruminants (Lewis et al., 1996). Cellulase preparations efficiently hydrolyse anti-nutritional factor (such as cellulose) in diet of few animals as swine to easily absorbent component thereby improving the animal health (Kuhad et al., 2011). They are also used to improve the quality of pork meat alone or in combination with proteases (Bhat, 2000).

- **Food Industry**
  A combination of cellulases, pectinases and hemicellulase collectively referred as macreating enzymes are used in processing of fruit and vegetable juices to increase both yield and process performance. These enzymes also improve the texture, cloud stability and help in concentration of nectars and purees of fruits (Grassin and Fauquembergue, 1996). Infusion of enzymes as β-glucosidases and pectinases are used to reduce excessive bitterness thereby improving flavour, texture and aroma of fruits and vegetables (Baker and Wicker, 1996). Macerating enzymes are also used for olive oil extraction that also improves the level of antioxidants and vitamin E (Galante et al., 1998b).

- **Beer and Wine Industry**
  The main raw material used for beer production is barley grain. Endoglucanase II and exoglucanase II from the Trichoderma hydrolyze the β-1,3 and β-1,4 glucan in low grade barley that reduces the polymerization and wort viscosity in brewing (Oksanen et al., 1985). In wine production process enzyme preparations containing glucanases, hemicellulases and pectinases are used for maceration of grape skins, colour extraction, must clarification and filtration thereby enhancing its quality and stability. Aroma of wines can also be improved by
modification of glycosylated precursors brought by action of \( \beta \)-glucosidases (Galante et al., 1998a).

- **Bioethanol Industry**

  One of most popular current application of cellulase is enzymatic saccharification of lignocellulosic materials (like rice straw, sugarcane bagasse, saw dust) for biofuel production. Cellulase are used to convert biomass to metabolizable sugars as glucose which is then used by other microbes for the formation of ethanol, other fermentative products and single cell proteins (Kuhad and Singh, 1993; Kuhad et al., 2011).

- **Agriculture**

  Cellulase and related hydrolase have the ability to degrade the cell wall of plant pathogens and thus they can be used for controlling plant diseases. Many cellulose utilizing fungi like *Trichoderma*, *Chaetomium*, *Penicillium* and *Geocladium* are known to enhance seed germination, improve root system and promote rapid plant growth (Bailey and Lumsden, 1998; Harman and Bjorkman, 1998; Harman and Kubicek, 1998).

- **Research and Development**

  Combination of cellulase and hemicellulase are used for the production of fungal and plant protoplast fused to produce mutant or hybrid strains with desired features (Beguin and Aubert, 1994; Bhat and Bhat, 1997). Cellulose-binding domains (CBD) of fungal cellulase are utilized for protein purification and immobilization in the form of affinity tags (Bayer et al., 1994, 1995). Native enzymes, recombinant enzymes or cellulosome subunits can be used for production of designer cellulosomes improving the efficiency of specific applications (Bayer et al., 1994).

1.4 **Bioactive Compounds**

  Microbes in their natural environments interact with each other and also with other organisms including plants and animals. They have the tendency to produce variety of different compounds which can promote or inhibit the growth of other
organisms. These compounds possessing biological activity directly affecting the other living creatures are called as bioactive compounds. Depending on the chemical nature, the amount and the bioavailability of the produced compound their effects can be beneficial or adverse. Antimicrobials are one of the best characterized bioactive compounds that inhibit the growth of other microbes.

Many natural antimicrobial compounds are secondary metabolites of microbial origin having low molecular weight. They are not essential for normal growth of an organism and are not necessarily expressed continuously. Moreover their production is often limited to a narrow set of species within a phylogenetic group. On the basis of their chemical structure and physical properties they can be classified into one or more of the following groups like alkaloids, aliphatic, aromatic, terpenoids, polyketides, and heteroaromatic organic acids, steroids, saponins, resins, peptides, and phenols. Many of these secondary metabolites, including antibiotics serve survival functions for the organisms producing them (Demain and Fang, 2000).

Actinomycetes are well recognized for their ability to produce secondary metabolites and other bioactive substances (McCarthy and Williams 1992; Sanglier et al., 1996; Horan 1999; Lazzarini et al., 2001). They contribute to the production of about 50% of the discovered compounds as antibiotics (Berdy, 2005; Strohl, 2004), immunosuppressive agents (Mann, 2001) and antitumor agents (Cragg et al., 2005). *Streptomyces*, the largest genera of actinomycetes is alone responsible for 80% of the total antibiotics produced today and *Micromonospora* ranks second to it (Hopwood et al., 2000). Some of the examples of antibiotics produced by actinomycetes are streptomycin, erythromycin, chloramphenicol, rifamycin etc. Since, the incidence of microbial resistance to existing antibiotic are increasing day by day thus, there is a desperate need of screening actinomycetes for new antimicrobial compounds from unexplored or underexploited extreme habitats as sources of novel bioactive metabolites (Berdy, 2005).
OBJECTIVES

Programmes to select new microbes for enzyme production are increasing now. Enzymes from bacterial and fungal sources are most commonly used for industrial applications today (Pandey et al., 2000). Possibility of using actinomycetes for the enzyme production is now being explored on a large scale. They can be easily grown in submerged fermentations and downstreaming of products obtained from them is convenient (Jang and Chen, 2003). In the past few years also terrestrial actinomycetes has been proved to be a valuable source of enzymes (Mohamedin, 1999; Azeredo et al., 2001; Stamford et al., 2002; Sharma et al., 2005). Moreover, many pathogenic microbes have developed resistance to existing antibiotics demanding the need of new antibiotics to be discovered.

Therefore, it is essential that actinomycetes from unexplored or underexploited habitats like Sambhar Salt Lake, Rajasthan be pursued as sources of antibiotics and enzymes that are better adapted to harsh industrial conditions. Sambhar Salt Lake (SSL) and the salterns at Sambhar Salt Limited, Rajasthan, India, have already been studied for chemical composition of brines and extremely haloalkaliphilic archaebacteria (Upasani and Desai, 1990) and the biology of Blue green algae (Subbaramanian, 1972). Recently, this saline lake has drawn the interest of microbiologist for the investigation of microbial diversity (Jose and Jebakumar, 2013; Yadav et al., 2013). But so far no attempts have been made to study enzyme production by actinomycetes isolates of this lake and hence, the present study aims to isolate actinomycetes that produce enzymes and antibacterial compounds from halophilic and alkaline habitat of Sambhar Salt Lake, Rajasthan. Therefore, the main objectives of the study were:

- To isolate and characterize actinomycetes from waters of salterns of Sambhar salt Lake, Rajasthan.
- To study the production of amylase and cellulase by isolated actinomycetes.
- To optimize the culture conditions for production of amylase and cellulase.
- To screen the isolates for production of antibacterial compounds.