Chapter I

Review of literature

Ever increasing population and industrialization has resulted in sudden increase in pollution. Because of the detrimental effects of pollution on humans, animals and plants, the ever increasing pollution is causing concern all over the world (Gao et al., 2010). One of the major environmental concerns in urban areas today is the issue of Solid Waste Management. In India, the collection, transportation and disposal of solid waste is normally done in an unscientific and chaotic manner (Carlsson et al., 2012). Uncontrolled dumping of wastes on outskirts of towns and cities has created overflowing landfills, which are not only impossible to reclaim because of the haphazard manner of dumping, but also have serious environmental implications in terms of ground water pollution and contribution to global warming (Manigat et al., 2010).

There is an increasing energy demands worldwide towards the utilization of renewable resources, from agricultural and forest residues. The major components of the residues are cellulose, lignin and pectin (Antranikian 1992). These materials have paid more attention as an alternative feed stock and energy source, since they are abundantly available. Several microorganisms are capable of using these substances as carbon and energy sources by producing a vast array of enzymes in different environmental conditions (Kaur, 2004). Solid wastes are perceived as undesirable matter that is generated from human and animal activities. The sources of solid waste include various sectors such as residential, industrial, commercial, institutional and agricultural premises (Carlsson et al., 2012).

The composition and characteristics of municipal solid waste (MSW) vary throughout the world, with variations noted in factors such as social and customary norms, standard of living, geographical location and climate (Arena, 2012). Solid
wastes can be treated by using various methods such as biological treatment and physical treatment (Kiely, 2007).

Microorganisms are widely used in agricultural biotechnology, environmental protection and in treatment of waste. Natural organic fertilizer prepared from fermentation of fruit waste has many benefits on plant and soil. Fermented fruit waste is useful in organic farming because the nutrient level and microbial diversity will be high that helps to supply nutrients to crops (Alkorta et al., 1998).

The fastest growing segment of research is enzymes for feed and fuel production. Abundant amount of waste materials are produced by agricultural and fruit processing industries, which pose considerable disposal problems and ultimately leads to pollution (Dhingra and Gupta, 1984). Vast varieties of micro-organisms are present in the environment which can be exploited for the utilization of waste material. For example in the processing of fruits like mango or citrus, a large proportion of the produce goes waste in the form of peel, pulp and seeds (Bauman, 1981). Of the many microorganisms, Bacillus spp. are known to produce variety of extracellular enzymes and they have a wide range of industrial applications (Annamalai et al., 2011).

**Fruit production in India:**

The current fruit production of India is around 32 million metric tonnes (MMT), accounts for about 8% of the world’s fruit production (Ravi et al., 2007). India is the second largest producer of Fruits after China. A large variety of fruits are grown in India, of which mango, banana, orange, guava, grape, pineapple and apple are the major ones. Apart from these, fruits like papaya, sapota, annona, phalsa, jackfruit, ber, pomegranate grown in tropical and sub-tropical areas and peach, pear,
almond, walnut, apricot and strawberry in the temperate areas (Fig.2). Although fruit is grown throughout the country, the major fruit growing states are Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Uttar Pradesh and Gujarat.

**Fruits and vegetable processing in India:**

The Food Processing Industry in India is one of the largest in terms of production, consumption, export and growth prospects. In India only 2% of fruits and vegetables produced are processed as against 65% in the USA, 70% in Brazil etc (Table 1).

![Pie chart showing share of India's fruit and vegetable production in world.](Source: FAO STAT, 2007)
Chapter II

Review of literature

Table 1: Level of processing of Fruits and Vegetables in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage of processing</th>
</tr>
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<tbody>
<tr>
<td>USA</td>
<td>65%</td>
</tr>
<tr>
<td>France</td>
<td>70%</td>
</tr>
<tr>
<td>Brazil</td>
<td>70%</td>
</tr>
<tr>
<td>Malaysia</td>
<td>83%</td>
</tr>
<tr>
<td>Philippines</td>
<td>78%</td>
</tr>
<tr>
<td>Thailand</td>
<td>30%</td>
</tr>
<tr>
<td>India</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Source: FAO STAT, 2007

Mango:

It is a matter of astonishment to many that mango (*Mangifera indica* L.), one of the most celebrated fruits of tropical part of India, is a member of the family Anacardiaceae, which is notorious for embracing a number of highly poisonous plants. It has rich luscious, aromatic flavor and a delicious taste in which sweetness and acidity is delightfully blended. Mango production has experienced continuous growth in the last decades of the twentieth century (Baisya, 2004).

The world’s total annual mango fruit production was estimated at 22 MMT. Global production of mangoes is concentrated mainly in Asia and more precisely in India that produced 12 MMT per annum. Mangoes are cultivated in 85 countries (FAO STAT, 2007). Asia and the oriental countries produced around 80% of the world’s total production. Major mango producing countries are India, Mexico, China and Pakistan (Loeillet, 1994; Mahayothee, 2005).
Chapter I

Review of literature

Mango Cultivation in India:

Mango fruit is utilised at all stages of its development both in its immature and mature state. Raw fruits are used for making chutney, pickles and juices. The ripe fruits besides being used for desert are also utilised for preparing several products like squashes, syrups, nectars, jams and jellies. The mango kernel also contains 8-10 percent good quality fat which can be used for soap and also as a substitute for cola in confectionery (Pott et al., 2005; Vazquez-Caicedo et al., 2004).

It is the most cultivated area occupied crop in India with 60% of the total area under fruits. More than 25 mango cultivars are available in India that are widely cultivated (Anon 1962). Negi et al. (2000) presented information on area and production, cultivars, hybrids and clone, agrotechniques, disorders, insect pests and diseases, harvest and postharvest management, export, problems and prospects of growing mango in India.

The export of mango pulp as a raw material for these products was estimated to be about 62% of total exports of fruits and vegetables in India (Sreenath et al., 1995). Though there are nearly 1000 varieties of mango in India, only following varieties are grown in different states: Alphonso, Bangalora, Banganpalli, Bombai, Bombay Green, Dashehari, Fazli, Fernandin, Himsagar, Kesar, Kishen Bhog, Langra, Mankhurd, Mulgoa, Neelam, Samarbehist, Chausa, Suvarnarekha, Vanaraj and Zardalu. Recently some mango hybrids have been released for cultivation by different institutes/universities. These varities included: Mallika, Amrapali, Mageera, Ratna, Arka, Aruna, Arka Puneet and Arka Anmol.
Chapter II

**Mango cultivation in Andhra Pradesh:**

Andhra Pradesh, being blessed with varied soil types and agro-climatic conditions, is better placed for cultivation of large varieties of fruit crops. It is one of the largest fruit producing states in India as it possess slightly acidic soils (pH 5.5 to 7.5). The major fruit crops grown in Andhra Pradesh are mango, sweet orange, banana, grape, pomegranate, coconut, and cashew. Mango occupying an area of 3.7 lakh hectares, with an annual production of 32 lakh MT, has placed the state in first position with a share of 20% of the India’s production coupling with highest productivity (Baisya, 2004).

**Components of Mango:**

Mango contains a high concentration of sugar (16–18% w/v) and acids with desirable organoleptic properties, and also contains antioxidants like carotene (as Vitamin A, 4,800 IU). Sucrose, glucose and fructose are the principal sugars in ripened mango, with small amounts of cellulose, hemicellulose and pectin. Mango juice along with aromatics is recommended as a restorative tonic; which contains good concentrations of vitamin A and C, and is useful in heat apoplexy. Mangoes with higher initial concentration of β-carotene are helpful as cancer-preventing agents (Sandhu and Joshi, 1995).

Fruits like mango are highly perishable commodities. In developing countries like India, 20–30% of fruits produced undergo spoilage due to lack of proper utilization and undeveloped post-harvest technology (Sandhu and Joshi, 1995). The edible pulp makes up 33–85% of the fresh fruit, while the peel and the kernel amount to 7–24% and 9–40%, respectively (Wu et al., 1993).
Biodegradation of fruit waste:

There is an increasing energy demands worldwide towards the utilization of renewable resources, from agricultural and forest residues. The major components of these residues are cellulose, lignin, starch, xylan and pectin (Antranikian, 1992). These materials have paid more attention as an alternative feedstock and energy source, since they are abundantly available. Several microorganisms are capable of using these substances as carbon and energy sources by producing a vast array of enzymes in different environmental conditions (Kaur et al., 2004).

Fruit sources of pectin:

Fruit peel is rich in carbohydrate, protein and pectin. Pectin, a major component peel of these fruit, is a polymer of galacturonic acid residues connected by α-1, 4 glycosidic linkages (Rombouts and Pilnik, 1986). The degradation of organic wastes by the bacterial populations is highly significant. It reduces the time span of degradation and produces no fowl odour (Solis et al., 1990). The use of microbial
consortium generated through natural selection or improvement of the performance of these microorganisms in waste degradation through genetic manipulation, may be the best option for the efficient treatment of organic waste or domestic waste in the near future (Arena, 2012). The pretreatment of fruit waste can be used for biological solubilization and mineralization in garbage disposal system which is a novel approach. The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact (Coker, 2006). This process of biological treatment of wastes is also known as Composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting.

It has clearly been established that composts have the potential to protect the soil against erosion (Bazzoffi et al., 1973), to enhance the soil water retention (Bengston, and Cornette, 1973), to reduce soil compatibility, to decrease soil acidity (Duggan and Wiles, 1976), to enhance soil biochemical and biological activity (Pfotzer and Schuler, 1997) and to establish a sound soil ecological equilibrium (Stickelberger, 1977). Additionally, composts can protect plants from soil or seed borne pathogens (Alvarez et al., 1995; Schuler et al., 1993). Hence, compost can be considered as a much-needed soil conditioner with generally positive crop yield effects (He et al., 2000; Gallardo-Lara and Nogales, 1987).

Citrus waste and apple pomace are the two most important sources of pectin in the technologically advanced countries of the world (Francis and Bell 1975). However, the availability of these raw materials in India is not enough to manufacture pectin sufficient enough to meet the local demand and hence a significant quantity of it is being imported from other countries every year.
Chapter I

Review of literature

Pectin is hydrolysed by pectinase enzymes produced extracellularly by microflora available in our natural environment (Bhat et al., 1968). With the help of the pectinase enzyme, micro-organisms can convert fruit wastes into sugars which can be used for food and value added products (Kumar et al., 1985). These micro-organisms can also be exploited for production of pectinase which is an industrially important enzyme and have potential applications in fruit, paper, textile, coffee and tea fermentation industries. In order to isolate a potential pectinase producer the microbial populations have to be screened. Because of the ever growing cost for energy enzymatic technologies will stay in focus of science and technology, and its relevance will increase significantly in the future (Kircher, 2006).

Enormous quantities of industrial waste residues are generated throughout the world from processing raw agricultural materials for foods. These, in turn, impose a high BOD burden on the environment when dumped. Thus industrial residues from the processing of sugarcane, fruit and vegetable waste, coffee and rice present suitable feed stocks for bioconversion into chemicals, including enzymes by fermentation processes, there by adding value to what normally constitute a waste product.

Mango fruits constitute an important group of fruit crops produced all over the world. In the Indian fruit processing industry, mango products occupy the first place and at present its peels are being discarded as waste. Mango peels, which constitute nearly 15% of the total weight of the fruits, were reported to contain appreciable amount of pectin (Beerh et al., 1976; Srirangarajan and Shrikhande 1976, 1979). According to Larrauri et al. (1996), byproducts of industrial mango processing may amount to 35–60% of the total fruit weight. Because these byproducts represent a serious disposal problem, ways for a sustainable agricultural production have been
searched. From the 9.4 million tons of mangoes produced annually in India about 14,000 tons of fresh peels would be available taking into account that about 1% mango fruits are processed. Even if 50% of these peels are utilized for production of pectin about 141 tons of pectin can be produced annually in India (Tandan et al., 1991). The industrial utilization of mango peels for manufacturing pectin would not only solve the problem of waste disposal but also save valuable foreign exchange by reducing the pectin imports. With the increase in the price of world pectin a country like India, with free access to abundant supplies of quality raw material may also find it feasible in setting up pectin factories more favorable than before in order to meet the internal demand.

While the utilization of the mango kernels has extensively been investigated as a source of fat, natural antioxidants, starch, flour and feed (Arogba, 2002; Kaur et al., 2004; Moharram and Moustafa, 1982; Puravankara et al., 2000; Ravindran and Sivakanesan, 1996). Their use for the production of biogas (Madhukara et al., 1993; Mahadevaswamy and Venkataraman, 1990) or dietary fiber with a high antioxidant activity (Larrauri et al., 1996 and 1997) has been described in the past.

The main bio wastes produced when processing mangos are the peel and the seed, which represent approximately 35% to 60% of the fruit (Larrauri et al., 1996). The disposal of mango bio waste is a growing problem due to increasing production of this material at is estimated amount of 75000 MT worldwide. From an environmental perspective, it is vital to reuse the plant byproducts produced by the agro-food industry (Ravi et al., 2007).
Chapter II  Review of literature

Importance of mango fruit waste:

Mango peel and seed has very high antioxidant activity, a fact attributed to its high phytochemical content (Berardini et al., 2005; Soong and Barlow 2006; Abdalla et al., 2007; Ajila et al., 2007; Barreto et al., 2008). Therefore, mango byproducts have been studied as a safer natural alternative to synthetic food antioxidants in biscuits, buffalo ghee, vegetable oils, and potato chips (Puravankara et al., 2000; Abdalla et al., 2007). Most of the developments made over the past few years in the evaluation of antioxidants from mango bio wastes have focused on the identification of phytochemical compounds (Berardini et al., 2004, 2005; Barreto et al., 2008).

Most studies on the exploitation of mango peels are dealing with their use as a source of pectin, which is considered a high quality dietary fiber (Beerh et al., 1976; Pedroza-Islas and Aguilar- Esperanza, 1994; Srirangarajan and Shrikhande, 1976; Tandon and Garg, 1999; Tandon et al., 1991). Berardini et al., (2005) demonstrated the screening of 14 mango cultivars that the content and the degree of esterification of mango peel pectins to range from 12% to 21% and from 56% to 66%, respectively. Furthermore, mango peels have been shown to be a rich source of flavonol O- and xanthone C-glycosides (Berardini et al., 2005; Schieber et al., 2003), gallotannins and benzophenone derivatives (Berardini et al., 2004). Sugar beet pulp, a by-product of sugar extraction, also contains pectin. Fruit processing industries produce a large amount of waste material in the form of peel, pulp, seeds, etc. Some fresh mango peel is, however, used in shredded form in the preparation of juices. This waste material presents considerable disposal problems and ultimately leads to pollution. Dried mango peel is rich in carbohydrates, proteins and pectin; the fat content, however, is low.
Various microbial transformations have been proposed for the utilization of food processing waste for producing valuable products like biogas, ethanol, citric acid, chemicals, various enzymes, volatile flavouring compounds, fatty acids and microbial biomass.

Mango peel contains an appreciable amount of pectin and thus can be used as a substrate for the production of pectinolytic enzymes by micro-organisms. Pectin acts as the inducer for the production of pectinolytic enzymes by microbial systems. The advantage of using micro-organisms for the production of enzymes is that these are not influenced by climatic and seasonal factors, and can be subjected to genetic and environmental manipulations to increase the yield. Highly productive strains of micro-organisms are required at the industrial level to reduce the production costs. Different types of micro-organisms have been exploited for the production of enzymes.

Pectic substances are present in the primary plant cell wall and the middle lamella. Besides these, other fruits like Citrus, Avocado Pear (*Avocado* *avocado*), Guava (*Psidium guajava*), Banana (*Musa sapientum*), Papaya (*Carica papaya*), Cashew Apple (*Anacardium occidentale*), Garden-egg (*Solanum nigrum* Linn.), Star Apple (*Crysophyllum albidium*), and Tomato (*Lycopersicum esculentum*) also contain substantial amounts of pectin having a high gelling grade. Pectin was first isolated in 1820 and shown to be the key substances in making jams and jellies (Torres-Fanela *et al.*, 2003). Jam and jellies have been produced for many years, at least since the 18\(^{th}\) century. Pectinases play a very important role in various biological process across the whole spectrum of life (Sprockett, 2009). Pectinases are a heterogeneous group of enzymes that degrade pectin. These are widely used in the food industry for the

<table>
<thead>
<tr>
<th>Chapter II</th>
<th>Review of literature</th>
</tr>
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production and clarification of fruit juices, to improve the cloud stability of fruit and vegetable juices and nectars; for depectinization in order to produce high density fruit juice concentrates, and for haze removal from wines. Pectic enzyme preparations are also used for the production of low methoxy pectin for diabetic foods, in the degumming of natural fibers in the textile industry, and in making commercial softwoods, such as Sitka and Norway spruce, more permeable to preservatives. Purified pectinases have also been developed specifically for use in plant protoplast culture studies. When used with cellulase, purified pectinases have been found to be very useful for generating good yields of viable protoplast in several plant systems, e.g. corn, soybean, red beet, sunflower, tomato, citrus etc.

Pectic substances are classified into four main types based on the type of modifications of the backbone chain which are; protopectin, pectic acid, pectinic acid and pectin (Kashyap et al., 2001). Protopectin is the water insoluble parent pectin substance found in the middle lamella of plant tissues. It yields soluble pectic substances such as pectin or pectinic acid upon restricted hydrolysis. Pectic acid is a group designation applied to pectic substances mostly composed of galacturonans containing negligible amounts of methoxyl groups. The salts of pectic acid are called pectates. Pectinic acids are the galacturonans containing various amounts of methoxyl groups (Fig 4). The salts of pectinic acids are either normal or acid pectinates. Under suitable conditions, pectinic acids are capable of forming gels with sugars and acids or if suitably low in methoxyl content, with certain metallic ions. Pectins are the soluble polymeric materials containing pectinic acids as the major component. They can form insoluble protopectins with other structural polysaccharides and proteins located in the cell wall (Kashyap et al., 2001). Pectin has gelatinized and concentrated characterization i.e., is used in different industries (Tripodo et al., 2007).
Fig. 4 The basic structure of pectin. Schematic representations of the conventional (A) and recently proposed alternative (B) structures of pectin

Pectinases share about 25% of global sale in the food enzymes. They are one of the most widely distributed enzymes in bacteria, fungi, and plants (Rombouts and Pilnik, 1986). Pectinase Production by microbes varies according to the composition of growth medium and the cultivation conditions i.e., pH, temp, aeration, agitation and incubation time (Thakur et al., 2010). Pectinolytic enzymes have been reported to be produced by a large number of bacteria and fungi such as Bacillus spp., Clostridium spp., Pseudomonas spp., Aspergillus spp., Monilla laxa, Fusarium spp., Verticillium spp., Penicillium spp., Sclerotinia libertiana, Coniothyrium diplodiella,
Chapter II

Review of literature

*Thermomyces lanuginosus*, *Polyporus squamosus* etc. Pectic enzymes are widely distributed in nature. They mainly occur in plants, bacteria, fungi, yeasts, insects, nematodes and protozoa.

Pectinase accounts for 10% of global industrial enzymes produced and their market is increasing day by day (Stutzenberger, 1992). Pectinase is an enzyme that breaks down pectins. They form the major components of the middle lamella and primary plant cell wall. Pectinases are produced by a large number of organisms, such as bacteria, fungi, actinomycetes and yeast. Pectinases have been used in processes and industries where the elimination of pectin is essential; fruit juice processing, coffee and tea processing, macerating of plants and vegetable tissue, degumming of plant fibers, treatment waste water, extracting vegetable oil, bleaching of paper, adding poultry feed and in the textile, alcoholic beverages and food industries.

Fungi can produce both intracellular as well as extracellular enzymes. All fungi are heterotrophic, and rely on carbon compounds synthesized by other living organisms. Small molecules like mono, di saccharides fatty acids and amino acids can easily pass through but for breaking down of larger complex compounds like pectin, fungi secrete extracellular enzymes. It is well known that as compared to intracellular enzymes, the extra cellular enzymes are easier to be extracted. Intracellular enzymes require more time and costly chemicals for extraction. Till date, substrates used for solid-state fermentation are materials of plant origin like grains such as rice, corn, root, tubers, and legumes. Apart from these, pomace, mango peels, orange waste like peels and other fruit and vegetable industry waste are also being in much use (Khalid et al., 2011).
Pectinase are the group of enzymes, which cause degradation of pectin that are chain molecules with a rhamnogalacturonan backbone, associated with other polymers and carbohydrates (Farooqi, 2012). Pectinases are frequently used in fruit and vegetable industry, and pectin is also employed widely in food industry. Peel oil finds many useful applications in both food and pharmaceutical industry. It is good for the skin. Citrus solvent is a biodegradable solvent occurring in nature as the main component of citrus peel oil. Pectinase production by different organisms in submerged state fermentation has received more attention and is found to be cost-prohibitive because of high cost of process engineering. Pectinolytic enzymes are commonly used during processing of fruits and vegetables for juices and wine. Pectinase group of enzymes include PGs, PME, PL. These pectinase enzymes act in different ways on the pectins.

Pectinase are extensively used in fruits juices processing (extraction and clarification) vegetable oil extraction, processing of alcoholic beverages and a variety of applications in food industries. Pectinase have an optimum temperature and pH at which they are most active. The commercial pectinase might typically be activated at 45° to 55°C and work optimally at a pH of 4 to 5. Pectinolytic micro-organism are widely distributed in soil, spoiled fruits, vegetables, decayed leaves and wood and can also be seen in water samples taken from decaying coconut husks, especially in Coastal areas. Flora of human intestine also includes pectinolytic microorganisms, mainly bacteria, since pectin the dietary fibre is the substrate for them. Traditionally, commercial source of pectin has been citrus peel and apple pomace. Citrus peel has often been the preferred material for pectin manufacture due to its high pectin content and good colour properties. Most recently other sources of pectin are sugar beet pectin.
and sunflower pectin. The amount of pectin from different sources varies considerably.

Pectinases are mainly produced by plants and microorganisms. Acidic pectinases are widely used in the production and clarification of fruit juices. They are also very important in maceration and solubilization of fruit pulps (Naidu and Panda, 1998). Alkaline pectinases have been used in several areas, including retting and degumming of fiber crops, textile processing, coffee and tea fermentations, paper and pulp industry, and oil extraction (Hoondal et al., 2002).

Pectic substances are naturally degraded by pectinases. The classification of pectic enzymes is based on their attack on the galacturonan backbone of the pectic substance molecule. Basically, there are three types of pectic enzymes; de-esterifying enzymes (pectin esterase), depolymerizing enzymes and protopectinases (Kashyap et al., 2000). Pectin esterases catalyze the hydrolysis of methyl to produce pectic acid and methanol. Depolymerizing enzymes consist of hydrolases and lyases. Lyases are also called transeliminases, which split the glycosidic bonds of either pectate (polygalacturonate) or pectin (polymethylgalacturonate). Commercial enzymes are generally obtained from fungal sources since the pH optima of these enzymes are in the range found naturally in materials to be processed and the enzymes are secreted into the culture media, making the downstream processing easier (Jayani et al., 2005). Keeping in view the importance of enzyme pectinases in the food processing industry and the problems associated with the disposal of food processing industry waste, the present study has been attempted.
Sources of Pectinolytic and Pectolytic Enzymes

Most pectin-degrading organisms are associated with raw agricultural products and with soil. Up to 10% of the organisms in soil have been shown to be pectinolytic (Hankin and Anagnostakis, 1975). These include bacteria in the genera *Achromobacter, Aeromonas, Arthrobacter, Agrobacterium, Enterobacter, Bacillus, Clostridium, Erwinia, Flavobacterium, Pseudomonas, Xanthomonas*, (Rombouts and Pilnik, 1986; Voragen, 1972) and many yeasts, molds, protozoa, and nematodes. Many of these organisms are plant pathogens. Pectolytic activity was also found in a strain of *Leuconostoc mesenteroides* which is the first report of pectolytic activity in lactic acid bacteria (Juven *et al.*, 1985). Studies have reviewed the role of pectic enzymes, their regulation, and their molecular genetics in plant pathogenesis by plant pathogenic *Erwiniae* (Collmer *et al.*, 1988; Collmer and Keen, 1986; Kotoujansky, 1987). However, the detection of anaerobic pectinolytic bacteria also has been described (Lund, 1972; Nagel and Vaughn, 1961).

Screening of pectinolytic and pectolytic organisms

The basic method used to detect pectinolytic or pectolytic organisms is to grow the organisms on a gel medium that contains pectin or pectate as substrate. Production of enzymes by a culture is detected either by observing clearing jones around the colony where the substrate has been degraded or by flooding the plate with a precipitant solution. Around producer colonies a clear zone will appear where the substrate has degraded to the point that precipitation does not occur. While non-producing colonies will be surrounded by opaque gel containing the non-degraded pectin or pectate substrate (Jayasankar and Graham, 1970).
Sources of commercial pectic enzymes

No pectic enzymes commercially available are free of other classes of pectic enzymes. The ability to clone pectate lyase genes from *E. chrysanthemi* into *E. coli* makes available clones with sequenced genes that are good producers of individual pectate lyases (Keen and Tamaki, 1986). In addition, *E. coli* clones containing *E. carotovora* genes for endo- and exo-pectate lyases are also available (Roberts *et al.*, 1986). Niture (2008) described an yeast, *Kluyveromyces fragilis*, that reliably secretes large amounts of PG into the growth medium where PG was estimated to be about 95% pure in the culture filtrate. The organism does not produce either pectinesterase or pectate lyase. Recent papers have demonstrated that three or more polygalacturonases are present in the preparation (Call and Emeis., 1983; Inoue *et al.*, 1984). All of the enzymes appear to be endo-splitting with similar tissue-macerating properties. Despite the presence of multiple enzymes, this is a source for easily produced polygalacturonases that are free of other classes of pectic enzymes (Lim *et al.*, 1980).

Enzyme Production

Currently, enzymes are becoming increasingly important in sustainable technology and green chemistry. In the opinion of many experts and based on different studies, 20% of all chemical products in a dimension of 300 billion US dollar will be produced using biotechnology by 2010. This would represent a ten fold increase compared to 2001. Micro-organisms are considered to be prospective enzyme producing sources as they have a number of advantages: like the specific selection methods; ease of biosynthesis via the conditions of cultivation; in-depth interaction on various substrates; wide spectrum of available enzyme complexes and
their application in gene engineering via gene cloning technology (Laing and Pretorius, 1993).

The utilization of microbial enzymes has found broad technological application in different industrial processes. Among the various commercialized enzymes, many are products of fermentation of filamentous fungi (Piccoli-valle et al., 2001). The genus *Penicillium* is worldwide known for production of secondary metabolites and extracellular enzymes of commercial value, including pectinases, which was used as the first commercial application (Kertesz, 1930).

Enzyme production is a growing field of biotechnology and the world marked for enzyme is over 1.5 billion and it is anticipated to double by the year 2008. The majority of the industrial enzymes are of microbial origin. In developing nations, there is noticeable growth of food and feed processing industries, leading to an increase in the demand for pectic enzymes (Lowe, 2002). Unfortunately, many of these nations depend largely on imported pectinase, which has become more expensive because of higher cost of foreign exchange. Therefore, with a view of improving the utilization of horticultural or agricultural product/waste in the depressed economy of a nation like India, it is necessary to carry out research on the production of pectic enzymes locally.

There were two fermentation techniques for pectinases production which are Solid State Fermentation (SSF) and submerged fermentation (SmF) (Murad and Foda, 1992). In solid state fermentation the cultivation of microorganisms is on moist solid supports, either on inert carriers or on insoluble substrates that can be used as carbon and energy source. This process occurs in the absence or near absence of free water in the space between substrate particles (Lonsane *et al*., 1985; Pandey *et al*., 2001). In
contrast, in submerged fermentation (SmF) the nutrients and microorganisms are both submerged in water (Grigelmo-Migeul and Martin-Belloso, 1998). Approximately 90% of all industrial enzymes are produced in SmF, frequently using specifically optimized, genetically manipulated microorganisms. Almost all these enzymes could be produced in SSF using wild-type microorganisms (Filer, 2001; Pandey et al., 2001).

**Production of PG from microbial origin**

Polygalacturonases (PG) are the pectinolytic enzymes that catalyze the hydrolytic cleavage of the polygalacturonic acid chain with the introduction of water across the oxygen bridge. They are the most extensively studied among the family of pectinolytic enzymes. The PGs involved in the hydrolysis of pectic substances are endo-PG (EC 3.2.1.15) and exo-PG (EC 3.2.1.67) (Table 2). PGs have the biological, functional and technical applications in food processing and plant-fungal interactions.
Table 2: Properties of Some Purified polygalacturonases

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzyme</th>
<th>Optimum pH</th>
<th>Optimum temperature</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>Aspergillus carbonarius</strong></td>
<td>Endo – PG I</td>
<td>4.0</td>
<td>55</td>
<td>Devi and Rao, 1996</td>
</tr>
<tr>
<td></td>
<td>Endo – PG II</td>
<td>4.1</td>
<td>50-50</td>
<td>Devi and Rao, 1996</td>
</tr>
<tr>
<td></td>
<td>Endo-PG III</td>
<td>4.3</td>
<td>50-55</td>
<td>Devi and Rao, 1996</td>
</tr>
<tr>
<td><strong>Streptomyces lydicus</strong></td>
<td>Exo – PG</td>
<td>6.0</td>
<td>55-50</td>
<td>Jacob <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><strong>Aspergillus giganteas</strong></td>
<td>Exo-PG</td>
<td>6.0</td>
<td>55</td>
<td>Pedrolli, 2009</td>
</tr>
<tr>
<td><strong>Aspergillus kawaki</strong></td>
<td>Endo – PGI</td>
<td>4.5</td>
<td>50</td>
<td>Contreas-Esquivel <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Aspergillus niger</strong></td>
<td>PG</td>
<td>4.6</td>
<td>40</td>
<td>Dinu <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><strong>Bacillus spp.</strong></td>
<td>Exo- PG</td>
<td>7.0</td>
<td>60</td>
<td>Kobayashi <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Fusarium moliniforme</strong></td>
<td>Endo – PGI</td>
<td>4.8</td>
<td>45</td>
<td>Niture <em>et al.</em>, 2004</td>
</tr>
<tr>
<td></td>
<td>Endo – PGI II</td>
<td>5.3</td>
<td>40</td>
<td>Niture <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Mucor flavus</strong></td>
<td>Endo-PG I</td>
<td>3.5 -5.5</td>
<td>45</td>
<td>Driessche <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><strong>Pectinase CCM</strong>*</td>
<td>PG</td>
<td>40</td>
<td>50</td>
<td>Ortega <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Pastinex 3XL</strong></td>
<td>PG</td>
<td>4.7</td>
<td>50</td>
<td>Ortega <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Penicillium frequentians</strong></td>
<td>Exo-PG I</td>
<td>3.09</td>
<td>50</td>
<td>Chellelagatti, 2002</td>
</tr>
<tr>
<td></td>
<td>Exo-PG II</td>
<td>5.0</td>
<td>50</td>
<td>Barense <em>et al.</em>, 2001</td>
</tr>
<tr>
<td></td>
<td>Endo-PG III</td>
<td>5.8</td>
<td>50</td>
<td>Barense <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Rapides C800</strong>*</td>
<td>PG</td>
<td>4.0</td>
<td>55</td>
<td>Ortega <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Rhizopus oryzae</strong></td>
<td>Endo-PG</td>
<td>4.5</td>
<td>45</td>
<td>Saito <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><strong>Termoascus aurantiacus</strong></td>
<td>Endo- PG</td>
<td>5.5</td>
<td>60-35</td>
<td>Martins, 2007</td>
</tr>
</tbody>
</table>

**Source:** Pedrolli *et al.*, 2009

*commercial names
Brazilian studies had revealed that, 168 bacterial strains isolated from soil samples and vegetables in decomposition, were screened for the use of citrus pectin as the sole carbon source. The cultivation of these pectinolytic strains by submerged and semi-solid fermentation for PG production indicated that five strains of *Bacillus* spp produced high quantities of the enzyme. The physico chemical characteristics, such as optimum pH of 6.0 – 7.0, optimum temperatures between 45°C and 55°C, stability at temperatures above 40°C and in neutral and alkaline pH, were determined (Soares *et al.*, 1999).

In another study three extracellular pectinases were produced by *Aspergillus niger* CH4 by submerged and solid-state fermentation. The highest productivities of endo and exo-pectinase and pectin lyase were obtained with solid state fermentation. All activities were very different in terms of pH and temperature optima, stability at different pH and temperature values and affinity for the substrate ($K_m$ values). In solid-state fermentation, all pectinase activities were more stable at extreme pH and temperature values but the $K_m$ values of endo pectinase and pectin lyase were higher with respect to those activities obtained by the submerged-culture technique (Acuna-Arguelles *et al.*, 1995)

**Endo polygalacturanase**

Endo PGs are widely distributed among fungi, bacteria and many yeasts (Luh and Phaff, 1951). They are also found in higher plants and some plant parasitic nematodes (Sakai *et al.*, 1993). They have been reported in many microorganisms, including *Aureobasidium pullulans* (Sakai and Takaoka, 1984), *Rhizoctonia solani Kuhn* (Marcus *et al.*, 1986), *Fusarium moniliforme* (De Lorenzo *et al.*, 1987), *Neurospora crassa* (Polizeli *et al.*, 1991), *Rhizopus stolonifer* (Manachini *et al.*, 1987).
Chapter I

Review of literature

1987), Aspergillus spp. (Nagai et al., 2000), Thermomyces lanuginosus (Kumar and Palanivelu, 1999), Peacilomyces clavisporus (Souza et al., 2003). Endo-PGases have also been cloned and genetically studied in a large number of microbial species (Laing and Pretorius, 1993; Naumov et al., 2001).

Exo polygalacturanase

In contrast, exo PGs occur less frequently. They have been reported in Erwinia carotovora (Palomaki and Saarilahti, 1997), Agrobacterium tumefaciens (Rodrigues-Palenzuela et al., 1991), Bacteroides thetaiotamicron E. chrysanthemi, Alternaria mali, Fusarium oxysporum, Ralstonia solanacearum, and Bacillus spp. (Kobayashi et al., 1999). Occurrence of PGs in plants has also been reported (Pressey and Avants, 1973; Alonso et al., 2003).

Polygalacturolase activity

PG activity is determined on the basis of measuring its activity, during the course of the reaction based on the rate of increase in number of reducing groups or the decrease in viscosity of the substrate solution (Rexova-Benkova and Markovic, 1976). The amount of reducing sugar can be readily measured by colorimetric methods like 3, 5-dinitrosalicylate reagent method (DNS Method, Miller, 1959) and the arsenomolybdate-copper reagent method. One unit of enzyme activity (U) is defined as the enzyme that releases 1 µmol/mL/min galacturonic acid under standard assay conditions (Soares et al., 1999).

The factors affecting production of microbial pectinases:

Environmental and nutritional factors are known to have marked effects on enzyme production by microorganisms. There are, therefore, variations in optimum conditions for pectic enzyme production. The selection of microbial source (wild
Chapter II

**Review of literature**

type, recombinant, mutagenized) along with various parameters like pH, metal ions, temperature are affecting the pectinase production (Table 3). Surfactants such as Tween-20, Tween-80 increase the enzyme production due to favourable effect on cell membrane permeability which leads to secretion of the enzyme. Pectinase synthesis is inhibited by SDS PAGE because of the denaturation of enzyme. The degrading ability of the enzyme is enhanced by agitation (Ahlawat et al., 2009).

**Substrate:**

The culture medium varies from organism to organism; grape pomace is nutrient medium for A. awamori (Botella et al., 2005; Suneetha and Zaved Ahmad Khan, 2010), potato dextrose agar medium for Mucor flavus (Ramchandra et al., 2003), yeast extract and wheat bran for Bacillus subtilis (Ahlawat et al., 2009), and sabouraud dextrose agar for P. viridicum RFC3 (Denis et al., 2005). Maximum pectinase yield was obtained in the media with PGA (230 U/ml), then with wheat bran (190 U/ml), cotton seed cake (160 U/ml), whereas glucose shows least enzyme production and acts as a repressor (Suneetha and Zaved Ahmed Khan, 2010; Sangeeta Yadav et al., 2005).

Carbon sources also effect the production of pectinase as observed, lactose, pectin increased pectinase production (Kashyap et al., 2003). In Aspergillus fumigatus sucrose yielded maximum pectinase production (Urmila et al., 2005).
### Table 3: Characterization of microbial pectinases

<table>
<thead>
<tr>
<th>Producer</th>
<th>Type of pectinase</th>
<th>Opti. pH for activity</th>
<th>Opti. Temp. for activity (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidic pectinases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger CH 4</em></td>
<td>Endo - pectinase</td>
<td>4.5-6.0</td>
<td>Below 50</td>
<td>Acuna–Arguelles et al., 1995</td>
</tr>
<tr>
<td><em>Penicillium frequentans</em></td>
<td>Endo - PG</td>
<td>4.5-4.7</td>
<td>50</td>
<td>Borin et al., 1996</td>
</tr>
<tr>
<td><em>Sclerotium rolfsii</em></td>
<td>Endo - PG</td>
<td>3.5</td>
<td>55</td>
<td>Channe and Shewal, 1995</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em></td>
<td>Endo - PG</td>
<td>4.8</td>
<td>50</td>
<td>Marcus et al., 1986</td>
</tr>
<tr>
<td><em>Mucor pusillus</em></td>
<td>PG</td>
<td>5.0</td>
<td>40</td>
<td>Al-Obaidi et al., 1987</td>
</tr>
<tr>
<td><em>Clostridium thermosaccharolyticum</em></td>
<td>Polygalacturonate hydrolase</td>
<td>5.5-7.0</td>
<td>30-40</td>
<td>Rijssel et al., 1993</td>
</tr>
<tr>
<td><strong>Alkaline pectinases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus sp. RK9</em></td>
<td>PGL</td>
<td>10.0</td>
<td>-</td>
<td>Fogarty and Kelly, 1983</td>
</tr>
<tr>
<td><em>Bacillus sp. NT-33</em></td>
<td>PG</td>
<td>10.5</td>
<td>75</td>
<td>Cao et al., 1992</td>
</tr>
<tr>
<td><em>Bacillus polymyxa</em></td>
<td>PG</td>
<td>8.45-9.4</td>
<td>45</td>
<td>Nagel and Vaughn, 1961</td>
</tr>
<tr>
<td><em>Bacillus pumilis</em></td>
<td>PATE</td>
<td>8.0-8.5</td>
<td>60</td>
<td>Dave and Vaughn, 1971</td>
</tr>
<tr>
<td><em>Amucola sp.</em></td>
<td>Pectatelyase (PAL)</td>
<td>10.25</td>
<td>70</td>
<td>Bruhlman et al., 1994</td>
</tr>
<tr>
<td><em>Xanthomonas compestris</em></td>
<td>PATE</td>
<td>9.5</td>
<td>25-30</td>
<td>Nasumo and Star, 1967</td>
</tr>
<tr>
<td><em>Bacillus No. P-4-N</em></td>
<td>PG</td>
<td>10-10.5</td>
<td>65</td>
<td>Horikoshi, 1990</td>
</tr>
<tr>
<td><em>Bacillus steatothermophilus</em></td>
<td>PATE</td>
<td>9.0</td>
<td>70</td>
<td>Karbassi and Vaughn, 1980</td>
</tr>
<tr>
<td><em>Penicillium italicum CECT 22941</em></td>
<td>Pectinlyase</td>
<td>8.30</td>
<td>50</td>
<td>Alana et al., 1990</td>
</tr>
<tr>
<td><em>Bacillus sp. DT 7</em></td>
<td>Pectinlyase</td>
<td>8.0</td>
<td>60</td>
<td>Kashyap et al., 2000</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>PAL</td>
<td>8.5</td>
<td>60-65</td>
<td>Chesson and Conder, 1978</td>
</tr>
<tr>
<td><em>Poseudomonas syringae pv. Glycinea</em></td>
<td>PAL</td>
<td>8.0</td>
<td>30-40</td>
<td>Magro et al., 1994</td>
</tr>
</tbody>
</table>

Chapter II

Among the nitrogen sources for pectinase production the maximum yield of pectinase was shown by yeast extract. Pectinase production is inhibited by glycine, urea, ammonium nitrate while wheat bran, peptone, ammonium chloride, yeast extract enhance pectinase production (Kashyap et al., 2003). The induction of pectate lyases is higher with pectin than with polygalacturonic acid (Suneetha and Zaved Ahmed Khan, 2010). The PGL activity increases as concentration of reducing sugars in the culture broth decreases (Ramchandra et al., 2003).

**pH:**

Except for exo PGase from *Fusarium oxysporum* and endo PG from *Bacillus licheniformis*, all PG have acidic optimum pH between 3.3-7. Xylano-pectinolytic enzymes find a wide application in biobleaching industry, the optimal efficiency is at pH 8.5 (Amanjot kaur et al., 2010). The optimum pH of the three PME isoforms that is 6.5-9.0 depends on the salt concentration. Salts, mask the carboxylic charged groups from those involved in the enzyme substrate recognition thus effecting the PME activity (Bruna et al., 2008). Pectate lyases have an optimum pH of 8.5 (Suneetha and Zaved Ahmed Khan, 2010). Fungi and yeast produce PG with acidic pH. For pectinase production by *Bacillus subtilis*, the highest pectinase production is observed at pH 9.5. The optimum pH for growth and pectinase production for most of the bacteria is 7-10 (Sonia et al., 2009). Some bacterial strains; *Streptomyces* QG-11-3 and *Aspergillus aculeatus* produced PG active at pH 3.0 (Ernesto et al., 2006).

**Temperature:**

The activity of pectinases depends on thermal stability. In fruit juice industries and wine processing the property of PG of *M. rouxii* being efficient at 20°C and sensitive to 30°C is used (Saad et al., 2007; Suneetha and Zaved Ahmad Khan, 2010).
Chapter II

Review of literature

The optimum temperature was reported as 37°C in pectinase production by *Bacillus subtilis* (Ahlawat *et al.*, 2009). Pectate lyase optimal temperature under standard assay was 70°C (Suneetha and Zaved Ahmed Khan, 2010). Pectinase from *Streptomyces* spp QG-11-3 have optimal activity at 60°C. PG from fungi have optimum activity at 50°C while from yeast the temperature varies from 40°C to 60°C (Ernesto *et al.*, 2006).

**Metal ions:**

The endo PG activity was reported as reduced due to Cu$^{2+}$ and Hg$^{2+}$ (Saad *et al.*, 2007). Metal ions Hg$^{2+}$, Zn$^{2+}$, Mg$^{2+}$ inhibit enzyme production due to inhibition by thiol group blocking agents as there is possible involvement of the thiol group in the enzyme active site (Suneetha and Zaved Ahmad Khan, 2010). Mn$^{2+}$ increases the PG activity, however Li$^{2+}$, Fe$^{2+}$, Rb$^{2+}$ have no effect on the activity (Sonia *et al.*, 2009). At high concentration of metal ions the enzyme production is low due to blockage of secretion of protein into external medium. Bacterial pectate lyases need Ca$^{2+}$ for growth, fungal pectinase do not need Ca$^{2+}$ (Suneetha and Zaved Ahmed Khan, 2010).

**Initial pH of growth medium:**

According to Shoichi *et al.*, (1985) the initial pH of the medium has a great effect on the growth of the organism, on the membrane permeability, also on the biosynthesis and stability of the enzymes (Murad, 1998; Murad and Salem, 2001). Optimum production of pectic enzymes from many moulds has been reported to be within the acidic pH range (Zetelaki-Horvath, 1980; Shin *et al.*, 1983). Zheng and Shetty (1999) had reported that, PG produced from *Lentinus edodes* has a relatively lower optimum pH (pH 5.0). Piccoli-Valle *et al.* (2001) had observed a high PG and
pectin esterase activity in more acid pH of 4.5 and 5 by *P. griseoroseum*. Also, Silva *et al.*, (2002) found that *P. viridicatum* produced maximum production of polygalacturonase and pectin lyase at a pH of 4.5 and 5, respectively.

Phutela *et al.*, (2005) concluded that the thermophilic fungi *A. fumigatus* expressed maximum pectinase (1116 Ug⁻¹) activity at pH 4.0 while polygalacturonase was active at pH 5.0 (1270 Ug⁻¹). Also, Debing *et al.*, (2005) found that the pH 6.5 was optimal for pectinase production by *A. niger* in solid state fermentation. Reda *et al.*, (2008) found that the PG productivity by *Bacillus firmus*-1-10104 reached its maximum at initial pH 6.0 and 6.2. Rasheedha *et al.*, (2010) found that *P. chrysogenum* exhibited maximum PG production at initial pH of 6.5.

**Incubation period:**

The time of fermentation had a profound effect on microbial product formation (Murad and Foda, 1992; Murad, 1998; Murad and Salem, 2001). Maximum production of pectic enzyme from different moulds varies from 1 to 6 days (Ghildyal *et al.*, 1981). Castilho *et al.*, (2000) reported that the highest polygalacturonase activities were obtained by *A. niger* after 70 h of fermentation period. In addition, Fawole and Odunfa (2003) reported that optimum production of pectin methylesterase was obtained after 4 days of fermentation under submerged fermentation condition. Moreover, Sarvamangala and Dayanand (2006) observed a gradual increase in the production of pectinase from de-seeded sunflower head by *A. niger* after 72 h of fermentation period in submerged and up to 96 h in solid-state conditions. Reda *et al.* had reported the level of PG increased with increase in the incubation period up to a maximum of 96 h by *Bacillus firmus*-1-10104 under solid state fermentation conditions.
Nitrogen source:

The effect of organic and inorganic nitrogen sources on the production of pectinase was extensively studied. Galiotou-Panayotou and Kapantai (1993) observed that ammonium phosphate and ammonium sulphate did influence production of pectinase positively but also recorded the inhibitory effects of ammonium nitrate and potassium nitrate on pectinase production. Moreover, Sarvamangala and Dayanand (2006) revealed that both ammonium phosphate and ammonium sulphate did influence production of pectinase positively in both submerged and solid-state conditions.

In contrast, Sapunova (1990) found that ammonium salts stimulated the pectinolytic enzyme production in *A. alliaceus* BIM-83. Moreover, Sapunova *et al.*, (1997) has also observed that (NH₄)₂SO₄ stimulated pectinase synthesis, as in its absence fungus displayed a slight proteolytic activity and did not produce extracellular pectinases.

In addition, Fawole and Odunfa (2003) found that ammonium sulphate and ammonium nitrate were good nitrogen sources for pectic enzyme production from *A. niger* while glycine and tryptophan did not support enzyme production. Also, Phutela *et al.*, (2005) reported that (NH₄)₂SO₄ stimulated pectinase production, as in its absence fungus displayed a slight proteolytic activity and did not produce extracellular pectinases and also reported that the presence of yeast extract (NH₄)₂SO₄ in growth medium supported maximal production of pectinase (925 U g⁻¹) followed by malt sprouts (NH₄)₂SO₄(785 U g⁻¹), which also supported maximal PG activity (938 U g⁻¹). In addition, Rasheedha *et al.*, (2010) found that ammonium sulphate has enhanced the production of *P. chrysogenum* pectinase.
On the other hand, report of Aguilera et al., (2008) showed yeast extract (organic nitrogen source) as the best inducer of exopectinases by Aspergillus spp. Moreover Kashyap et al., (2003) found that, yeast extract, peptone and ammonium chloride were found to enhance pectinase production up to 24% and addition of glycine, urea and ammonium nitrate inhibited pectinase production. Also, Reda et al., (2008) found that the maximum value of PG productivity by Bacillus firmus-1-10104 reached up to 350 U mL⁻¹ in the presence of peptone as a nitrogen source in the growth medium. In addition, Vivek et al., (2010) found that organic nitrogen sources showed higher endo, exo pectinases activities than inorganic nitrogen sources. Also the increasing trend in the enzyme activity with the increase in nitrogen source content was observed in the case of organic nitrogen sources while decreasing trend observed for inorganic nitrogen sources. Soybean meal (4%) showed the maximum Exopectinase activity of 5128 IU g⁻¹ and endo-pectinase activity of 793 IU g⁻¹.

**Carbon source:**

An adequate supply of carbon as energy source is critical for optimum growth of organism and its metabolism. Aguilar and Huitron (1987) reported that the production of pectic enzymes from many moulds is known to be enhanced by the presence of pectic substrates in the medium. Fawole and Odunfa (2003) found that pectin and polygalacturonic acid promoted the production of pectic enzyme and they observed the lack of pectolytic activity in cultures with glucose as sole carbon source from the strain of A. niger. However, when different concentrations of glucose was added to the medium containing pectin, production of pectic enzymes was inhibited at high glucose concentration while low glucose concentrations (0.5% w/v) stimulated enzyme production. Also, the reducing sugar content of the culture filtrate increased with increase in the amount of glucose added to the growth medium. The ability of
high concentrations of glucose in the medium to meet growth requirement of the organism probably made the breakdown of pectin in the medium unnecessary or minimal and thus the low pectic activities observed in cultures. Phutela et al., (2005) stated that wheat bran supported maximum pectinase production (589 U g\(^{-1}\)) while pure pectin give the maximum production of polygalacturonase (642 U g\(^{-1}\)). Sarvamangala and Dayanand (2006) reported that glucose (4-6%) increase the production of pectinase in submerged condition whereas 6-8% sucrose gives better yield of pectinase in solid-state condition. Reda et al., (2008) reported that Solanum tuberosum (ST) peels was the best carbon source for polygalacturonase production by Bacillus firmus-1-10104 under solid state condition (Danielle, 2009).

Purification of pectinolytic enzymes

The analysis of enzyme activity in the crude extract does not indicate either an isolated action or the presence of a multienzymic system working in synergy on the substrate degradation. The characterization of purified enzymes is an important research line since it provides discrimination between the enzymic complex components about substrate degradation mechanism, optimum activity conditions and enzyme synthesis regulation. Purification of an exo-PG and an exo-PMG was achieved in a series of three separation steps: 1. Ammonium sulphate precipitation; 2. gel filtration and 3. cation or anion exchange chromatography. The specific activities of purified exo PG and exo-PMG represented purities 66 and 50 fold greater than that of the crude extract. The molecular masses assessed by SDS-PAGE were 68 kDa for the exo-PG and 140 kDa for the exo-PMG. This indicates that the exo-PG is likely to be a single polypeptide protein, while the native exo-PMG is composed of 2 identical sub units. The pH optima of the enzymes were about pH 5 and their optimum temperature was 45°C. This enzyme activity was inhibited by Hg\(^{+2}\), Zn\(^{+2}\),
Cu$^{+2}$ and p-chloromercurybenzoate. The activity was stimulated by Mn$^{+2}$ and Co$^{+2}$ (Christine et al., 1992). The PG from *Thermoascus aurantiacus* was isolated with 21 fold increase in specific activity with a recovery of 24.6 % by Sephadex G-75 gel filtration followed by SP-Sepharose ion exchange chromatography (Martin et al., 1995). Celestino et al., (2006) purified pectinase produced by *Acrophialophora nainiana* was 9.37 fold which has exo-PG and pectin lyase activity. The enzyme was recovered upto 60.6% after three steps: Sephacryl S-100 gel filtration, DEAE-Sepharose ion exchange and another gel filtration on Sephadex G-50. Kashyap et al., (2000) developed a purification strategy for the isolation of the pectin lyase from *Bacillus* spp. DT7. The enzyme was precipitated with ammonium sulphate followed by DEAE-Sephacel and Sephadex G-150 column chromatographies. The pectin lyase produced by *Aspergillus flavus* was purified 58 fold with a recovery of 10.3 % of the initial activity in three steps: ammonium sulphate fraction, DEAE-Cellulose ion exchange and Sephadex G-100 gel filtration. Semenova et al. (2009) isolated five pectinases produced by *Aspergillus japonicus*, PGI, PGII, PEI, PEII and PL, by hydro-phobic and ion exchange column chromatographies. The polygalacturonase from *Streptomyces lydicus* was purified with 57.1 fold increase in the specific activity and with a yield of 54.9% after ultrafiltration followed by CM-Cellulose and Sephadex G-100 column chromatographies (Yadav et al., 2009). As shown by several researchers, the conventional chromatography techniques have been efficiently used to purify pectinolytic enzymes.

**Applications of pectinases:**

Pectinases are used in the textile industry as they are capable of depolymerising the pectin breaking it into low molecular water soluble oligomers improving absorbency and whiteness of textile material and avoiding fiber damage.
(Sonia Ahlawat \textit{et al.}, 2009). There is use of xylan-pectinolytic enzymes in paper and pulp industry. Pectinases are effective in biobleaching of mixed hard wood and bamboo kraft pulp, as pretreatment of kraft pulp with xylan-pectinolytic enzymes from alkalo thermostolerant isolate facilitating adaptation of environment friendly technology in paper pulp industry (Amanjot kaur \textit{et al.}, 2010). Pectinase also find application in the degumming of plant fibres and retting of plant fibres.

Pectinase from \textit{Bacillus} species are used in waste water treatment. Pectinase are further used in coffee and tea fermentation by breaking pectins present in tea leaves and in oil extraction by avoiding emulsification formation. Pectinases posses biological applications in protoplast fusion technology and plant pathology (Emma \textit{et al.}, 2010). These pectinases have wide applications in fruit juice industry and wine industry. Pectins are the major polysaccharide compounds present in the fruits (Voragen \textit{et al.}, 2004). In fruit juice industry, it is used for clarification, where reduction in viscosity is caused, which ultimately leads to formation of clear juice. They increase the yield of juices by enzymatic liquefaction of pulps; these pectinase also helps in formation of pulpy products by macerating the organized tissue into suspension of intact cells (Farooqui, 2012). Pectinase inspite of their usage in other industries are endowed with promising applications in fruit processing industry as juice clarifies, color and yield enhancers and in fruit mash treatment (Chawanit \textit{et al.}, 2007). Abundant waste from agriculture and fruit processing industrial waste becomes an appreciable section for biological utilization of fruit processing waste in juice industry (Farooqui, 2012). Practically different steps notably; washing, sorting and crushin Pectinase play a crucial role in clarification (Jose \textit{et al.}, 2008), extraction, in reduction of viscosity, to remove off the peels and to increase the yield (Maria Teresa \textit{et al.}, 2008). In apple, pear and grape Pectinase are used during pressing and straining
stages. In orange, mango, guava, pineapple and papaya, pectinase are employed to remove the cloudiness. Pectinase are of prompt application in maceration, liquefaction, extraction and clarification processes.

In the industrial sector, acidic pectinases are used in the extraction and clarification of fruit juices (Rombouts and Pilnik 1986), whereas alkalophilic pectinases are finding immense use in the degumming of ramie fibers (Cao et al., 1992), plant protoplast formation and treatment of effluents discharged from fruit processing units (Tanabe et al., 1987). In wine industry pectinase are mainly used for improvement of chromaticity and stability of red wines, to improve the wine characteristics of colour and turbidity, decreasing astringency by solubilizing anthocyanins without leaching out procyadin polyphenols, and pectinase also increase pigmentation by extracting more anthocyanins (Tucker and Woods, 1991).

Using byproducts for pectinase extraction is still more profitable (Aravantinos-Zafiris et al., 1994). In the world market, pectinases accounts for about 10% of total enzyme production. (Stutzenbergn, 1992) Pectinolytic enzymes are produced by many organisms like bacteria, fungi, yeasts, insects, nematodes, protozoan and plants. Of that, microbial pectinases possess more advantages as it involves in the phytopathologic process, plant–microbe symbiosis and the decomposition of dead plant materials (Danielle et al., 2009).

Pectinolytic enzymes having great industrial importance are required for food processing industries, especially for extraction and clarification of fruit juices, extraction of oils, flavors and pigments from plant materials, textile, pharmaceutical, leather, detergent and paper (Phugare et al., 2011). Microbial enzymes are routinely
Microbes are the best source of enzymes as they allow an economical technology with low resource consumption and low emission involving no social and political issues, as in the case of animal and plant sources (Dalvi et al., 2007). Such enzymes may be discovered by screening microorganisms sampled from diverse environments or developed by modification of known enzymes using modern methods of protein engineering or molecular evolution. As a result, several important food-processing enzymes such as amylases and lipases with properties tailored to particular food applications have become available (Olempska-Beer et al., 2006).

New enzymes for commercial applications with desirable biochemical and physico-chemical characteristics and a low cost production have become a focus of research (Silva et al., 2002; Malvessi and Silveira, 2004; Phutela et al., 2005).

Pectinase is an enzyme group that catalyzes pectic substance by degradation reactions through depolymerization (hydrolases and lyases) and deesterification (esterases) reactions. These polysaccharide degrading enzymes are suitable tools to study the structure of pectin. The main reason is the specificity of these enzymes in comparison to chemical methods, which are less-specific. Most of the plant cell wall degrading enzymes are encoded by a large multigenic family showing diverged expression pathways suggesting functional specialization (Coutinho et al., 2003).

Pectinases are classified according to the mode of attack on their specific structural element of the pectin molecule (Benen et al., 2002). Alkaline pectinases are generally produced by bacteria, but are also made by some filamentous fungi and yeasts (Kapoor et al., 2001). They may be used in the pretreatment of waste water
Chapter II
Review of literature

from vegetable food processing that contains pectin residues; the processing of textile fibers such as flax, jute and hemp, coffee and tea fermentation, vegetable-oil extraction and the treatment of paper pulp (Zhang et al., 2000).

Production of pectinases by Bacillus species

Pectinases constitute a unique group of enzymes which catalyze the degradation of pectic polymers present in the plant cell walls (Fogarty and Kelly 1983). Pectinases are produced by many organisms such as bacteria (Horikoshi 1972; Karbassi and Vaughn 1980), fungi and yeasts. Although the major source of acidic pectinases is fungi, alkaline pectinases are produced from alkalo-philic bacteria, mainly Bacillus spp.

Of the many micro organism, Bacillus Spp. are known to produce variety of extracellular enzymes and they have a wide range of industrial applications. (Annamalai et al., 2011)

The genus Bacillus includes aerobic or facultatively anaerobic, rod shaped, Gram positive to Gram variable, endospore forming bacteria that are widely distributed in the environment (Goto et al., 2000; Slepecky and Hemphill., 1991; Holt et al., 1994). There are many kinds of species which have thermophilic, psychrophilic, acidophilic, alkalophilic and halophilic properties in the genus. The reclassification of genus Bacillus began in 1991 and yielded eight genera: Alicyclobacillus, Aneurinibacillus, Bacillus, Brevibacillus, Gracilibacillus, Paenibacillus, Salibacillus and Virgibacillus (Goto et al., 2000). These eight genera include more than 100 species that have similar phenotypic characteristics. Thus identification of them is not easy. In the past Bacillus spp. have been identified mainly by morphological and physiological criteria. However the discrimination power of
Chapter II

Review of literature

phenotypic methods is limited. Randomly amplified polymorphic DNA (RAPD) method and the hybridization method were effective for detection of a small number of *Bacillus* sp. Over the years, a data base of 16S rRNA gene has been constructed and it was successfully used in the differentiation of bacteria (Goto *et al.*, 2000).

**Paenibacillus Spp**

*Paenibacillus* is a genus of facultative anaerobic, endospore-forming bacteria, originally included within the genus *Bacillus* and then reclassified as a separate genus in 1993 (Ash *et al.*, 1993). Bacteria belonging to this genus have been detected in a variety of environments such as: soil, water, rhizosphere, vegetable matter, forage and insect larvae, as well as clinical samples (Lal and Tabacchioni, 2009; Gardener, 2004). The name reflects this fact: Latin paene means almost, and so the *Paenibacilli* are literally almost *Bacilli*. The genus includes *P. larvae*, which is known to cause American foulbrood in honeybees, the *P. polymyxa*, which is capable of fixing nitrogen and therefore is used in agriculture and horticulture, the *Paenibacillus* sp. JDR-2 which is known to be a rich source of chemical agents for biotechnology applications and pattern forming strains such as *P. vortex* and *P. dendritiformis* were discovered in the early 90s.

There has been a rapidly growing interest in *Paenibacillus* spp. since many were shown to be important for agriculture and horticulture (e.g. *P. polymyxa*), industrial (e.g. *P. amylolyticus*), and medical applications (e.g. *P. peoriate*) (Choi *et al.*, 2004; Konishi and Maruhashi, 2003; Nielsen and Sorensen, 1997). These bacteria produce various extracellular enzymes such as polysaccharide-degrading enzymes and proteases, which can catalyze a wide variety of synthetic reactions in fields ranging from cosmetics to biofuel production. Various *Paenibacillus* spp. also produce
antimicrobial substances that affect a wide spectrum of micro-organisms (Girardin et al., 2002; Piuri et al., 1998; von der Weid et al., 2003) such as fungi, soil bacteria, plant pathogenic bacteria and even important anaerobic pathogens as Clostridium botulinum.

More specifically, several Paenibacillus species serve as efficient plant growth promoting rhizobacteria (PGPR). PGPR competitively colonize plant roots and can simultaneously act as biofertilizers and as antagonists (biopesticides) of recognized root pathogens, such as bacteria, fungi and nematodes. They enhance plant growth by several direct and indirect mechanisms. Direct mechanisms include phosphate solubilization, nitrogen fixation, degradation of environmental pollutants and hormone production. Indirect mechanisms include controlling phytopathogens by competing for resources such as iron, amino acids and sugars, as well as by producing antibiotics or lytic enzymes (Kloepper et al., 1980; Ryu et al., 2003). Competition for iron also serves as a strong selective force determining the microbial population in the rhizosphere. Several studies show that PGPR exert their plant growth-promoting activity by depriving native microflora of iron. Although iron is abundant in nature, the extremely low solubility of Fe\(^{3+}\) at pH 7 means that most organisms face the problem of obtaining enough iron from their environment. To fulfill their requirements for iron, bacteria have developed several strategies, including (i) the reduction of ferric to ferrous ions, (ii) the secretion of high-affinity iron-chelating compounds, called siderophores, and (iii) the uptake of heterologous siderophores. The genome of P. vortex is an example, harbors many genes which are employed in these strategies, in particular it has the potential to produce siderophores under iron limiting conditions.
Chapter II

Despite the increasing interest in *Paenibacillus* spp. genomic information of these bacteria is lacking. More extensive genome sequencing could provide fundamental insights into pathways involved in complex social behavior of bacteria, and can discover a rich source of genes with biotechnological potential. *Paenibacillus polymyxa* is an endospore-forming bacterium that is non-pathogenic and found in environments such as plant roots in soil and marine sediment (Timmusk *et al.*, 2005; Ravi *et al.*, 2007).

The biosorption of several toxic heavy metals (Pb, Cd, Co, Ni, Zn and Cu) by the exopolysaccharide (EPS) produced by *Paenibacillus jamilae* is a potential biosorption for metal remediation. The production of a heavy metal-binding EPS by *Paenibacillus jamilae*, a bacterium that is able to use toxic olive-mill wastes as the fermentation substrate for the production of the polymer (Morillo *et al.*, 2006). The growth of *P. jamilae* was reported to its association with a decrease in the toxicity of olive-mill waste waters (Aguilera *et al.*, 2008).

*Bacillus megaterium*

*Bacillus megaterium* is a virgate, Gram-positive, endospore forming, aerotolerant species of bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillus form. *Bacillus megaterium* is a rod shaped bacterium and one of the largest eubacteria found in soil. Groups of the bacteria are often found in chains where the cells are joined together by polysaccharides on the cell walls. *Bacillus megaterium* is able to survive in some extreme conditions such as desert environments due to the spores it forms. Where there are favourable conditions the spores can survive. Sometimes this particular
bacteria can be found on common surfaces that are frequently touched (Suga et al., 1990).

*B. megaterium* grows at temperatures from 3 °C to 45 °C, with the optimum around 30 °C. Some isolates from an Antarctic geothermal lake were found to grow at temperatures up to 63 °C. *B. megaterium* has been recognized as an endophyte and is a potential agent for the biocontrol of plant diseases. Nitrogen fixation has been demonstrated in some strains of *B. megaterium*. *B. megaterium* has been an important industrial organism for decades. It produces penicillin amidase used to make synthetic penicillin, various amylases used in the baking industry and glucose dehydrogenase used in glucose blood tests. Further, it is used for the production of pyruvate, vitamin B12, drugs with fungicidal and antiviral properties, etc. It produces enzymes for modifying corticosteroids, as well as several amino acid dehydrogenases. *B. megaterium* is known to produce poly-γ-glutamic acid. The accumulation of the polymer is greatly increased in a saline (2–10% NaCl) environment, in which the polymer comprises largely of L-glutamate (L-isomer content up to 95%). At least one strain of *B. megaterium* can be considered a halophile, as growth on up to 15% NaCl has been observed. Phylogenetically, based on 16S rRNA, *B. megaterium* is strongly linked with *B. flexus*, the latter distinguished from *B. megaterium* a century ago, but only recently confirmed as a different species. *B. megaterium* has some phenotypic and phylogenetic similarities with pathogens *B. anthracis* and *B. cereus*, although itself being relatively harmless (Vary, 2007).

*Bacillus megaterium* has fascinated microbiologists since it was first described over 100 years ago. It is interesting especially, because of its physiology producing,
unusual and useful enzymes and products, and ability to grow in a wide range of ecological habitats. It is also capable of sporulation, a simple cell differentiation cycle that serves as a model system for understanding gene regulation during temporal and morphological development. Moreover, the large size of its vegetative cells and spores (the source of its name) make it especially amenable to morphological analysis. Although it is generally considered a soil organism, it is found in diverse environments from rice paddies to dried food, seawater, sediments, fish, normal flora, and even in bee honey. Strains are often isolated on unusual substrates (such as herbicides) in the company of *pseudomonads* and *actinomycetes* (Suneetha and Zaved Ahmed Khan, 2010).

*B. megaterium* has economic importance because of its commercially important enzymes such as penicillin amidase and steroid hydrolases. *Bacillus megaterium* produces penicillin amidase used for making penicillin. It produces enzymes for modifying corticosteroids, as well as several amino acid dehydrogenases. It is the major aerobic producer of vitamin B\textsubscript{12} and is one of the organisms involved in fish spoilage. An extensive review emphasizing its commercial applications has been published (Vary, 1992). During 1980s, genetic techniques of transduction, plasmid transformation, protoplast fusion and transposition became developed enough in *B. megaterium* to apply them to the study of many of its metabolic and developmental functions. Moreover, it is increasingly used as a host to produce foreign genes since it has been found to express, secrete and process foreign proteins without degradation.

*B. megaterium* has the ability to grow on many carbon sources including waste from the meat industry and corn syrups as well as a wide range of sugars; it has been found in petrochemical effluents and can oxidize thiosulfate compounds (Priest *et
al., 1988; Vary, 1992). Currently, one of the best characterized carbon source operons is the xylose operon. Some of the most interesting proteins of *B. megaterium* are a family of P-450 which is cytochrome monooxygenases. These have been of great interest since they have considerable similarity to eukaryotic P-450 important in many disease conditions (He, 2000). Fulco (1991) have characterized and sequenced these interesting enzymes. They have described one, P-450 that is induced by barbiturates and is catalytically self-sufficient, requiring only NADPH and oxygen to catalyse the hydroxylation of long-chain fatty acids. It is drawing considerable interest as a model system for the understanding of the eukaryotic enzymes.

*Bacillus megaterium* has been industrially employed for more than 50 years, as it possesses some very useful and unusual enzymes and a high capacity for the production of exoenzymes. It is also a desirable cloning host for the production of intact proteins, as it does not possess external alkaline proteases and can stably maintain a variety of plasmid vectors. Genetic tools for this species include transducing phages and several hundred mutants covering the processes of biosynthesis, catabolism, division, sporulation, germination, antibiotic resistance, and recombination. The seven plasmids of *B. megaterium* strain QM B1551 contain several unusual metabolic genes that may be useful in bioremediation.

Recently, several recombinant shuttle vectors carrying different strong inducible promoters and various combinations of affinity tags for simple protein purification have been constructed. Leader sequences-mediated export of affinity-tagged proteins into the growth medium was made possible. These plasmids are commercially available. For a broader application of *B. megaterium* in industry, sporulation and protease-deficient as well as UV-sensitive mutants were constructed.
The genome sequence of two different strains, plasmidless DSM319 and QM B1551 carrying seven natural plasmids, is now available. These sequences allow for a systems biotechnology optimization of the production host \textit{B. megaterium}. Altogether, a “toolbox” of hundreds of genetically characterized strains, genetic methods, vectors, hosts, and genomic sequences make \textit{B.megaterium} an ideal organism for industrial, environmental, and experimental applications.

\textit{B.megaterium} enzymes and products have been used in industrial applications for several years and are also effective in immobilized systems, making industrial processes even more efficient.

**Mango fruit waste used as a cost effective pectin substrate for the production of pectinases**

The use of various agricultural waste and agro-industrial by-products, in the present study suggested that mango fruit peel found to be the best substrate for PG production by \textit{B.megaterium}. The similar studies were also carried out by Silva \textit{et al.}, (2002) where orange bagasse and wheat bran gave higher yields of PG by the culture \textit{P. viridicatum} RFC3. In order to use enzyme from the isolates for commercial application, it must have desirable biochemical, physio-chemical characteristics and low cost of production. Mango peel is very cheap, abundantly available and could be easily stored after sun drying. This waste is generated after the extraction of juice and available in high quantity from fruit processing industries, but has a limitation of availability in only particular season. Its dumping in nature causes pollution problems; hence its eco-friendly utilization is essential which tempted to use agro waste for pectinase production by solid state fermentation (Afifi and Foaad \textit{et al.}, 2002).
Different natural substrates used as pectin instead of commercial pectin. Natural substrates like Rice bran (Farooqui, 2012), Wheat bran (Naderi et al., 2012), Fruit and vegetable waste (Soares et al., 1999), cassava waste (Mukesh Kumar, et al., 2012) etc, used for pectin substrate.

Conventional means of utilization of waste has been via biotransformation of Kinnow waste (mainly Kinnow peels and Kinnow Pomace) into humus. This is the 2nd important fruit crop after mango. Another useful way of exploitation of such waste could be by value addition whereby many useful products like pectinases, pectin, peel oil and dietary fibres can be obtained. Of these, pectin and pectinase have appreciable global importance (Pratima, 2003). Pectin’s are extracted by heating the plant materials in water (60-90°C) and at an acidic pH (2.5). The pectin are precipitated with ethanol and removed by centrifugation. (www.lifeviv.edu).

**Plant growth promoting activity of B.megaterium:**

Many plants have intimate relationships with soil microbes, which improve the plant’s growth and fitness through a variety of mechanisms. *Bacillus* spp. isolates are natural root-associated bacteria, isolated from *Nicotiana attenuate* plant roots growing in native soils shown a significant improvement (Meldau et al., 2012). *Bacillus megaterium* isolated from tea rhizosphere and tested for its ability to promote growth and cause disease reduction in tea plants. *In vivo* studies revealed the ability of this bacterium to promote growth of tea plants very significantly. Brown root rot disease, caused by *Fomes lamaoensis* was markedly reduced by application of the bacterium to the soil. Biochemical changes induced in tea plants were also examined. Root colonization by *B. megaterium* and subsequent inoculation with *F. lamaoensis* also led to an increase in polyphenolics, as well as in defense related enzymes-
peroxidase, chitinase, β-1,3-glucanase and phenyl alanine ammonia lyase.

Determination of mechanism of action of this bacterium revealed it to be able to solubilize phosphate, produce IAA, siderophore and antifungal metabolite. The plant growth promotion and reduction of disease intensity have been shown to be due to a combination of several mechanisms (Chakraborty et al., 2006).

### Table 4: Industrial products of B. megaterium and their applications

<table>
<thead>
<tr>
<th>Product/use</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylases</td>
<td>Can replace pullulanases</td>
<td>Takasaki 1989; Vihinen and Mantsala 1989</td>
</tr>
<tr>
<td>β-Amylases</td>
<td>Bread industry</td>
<td>Hebeda et al., 1988; Metz et al., 1988</td>
</tr>
<tr>
<td>Chitosanasesen</td>
<td>Yeast cell wall lysis</td>
<td>Pelletier and Sygusch 1990</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td>Generator of NADH/ NADPH, glucose blood test, biosensors</td>
<td>Kittsteiner-Eberle et al., 1989; Nagao et al., 1992</td>
</tr>
<tr>
<td>Neutral protease</td>
<td>Leather industry</td>
<td>Kühn and Fortnagel 1993; Meinhardt et al., 1994; Millet et al., 1969</td>
</tr>
<tr>
<td>Oxetanocin production</td>
<td>Inhibits HIV, hepatitis B virus, cytomegalovirus, herpes virus</td>
<td>Kohlbrenner et al., 1990; Morita et al., 1999; Shimada et al., 1986; Shiota et al., 1996; Tseng et al., 1991</td>
</tr>
<tr>
<td>Penicillin amidase</td>
<td>Construction of synthetic penicillins</td>
<td>Martin et al., 1995; Suga et al., 1990</td>
</tr>
<tr>
<td>Toxic waste cleanup</td>
<td>Herbicides, C-P bond lysis</td>
<td>Quinn et al., 1989</td>
</tr>
<tr>
<td>Vitamin B₁₂ production</td>
<td>Aerobic and anaerobic Vitamin B₁₂ producer</td>
<td>Raux et al., 1998; Wolf and Brey 1986</td>
</tr>
</tbody>
</table>

**Source:** Vary et al., 2007