INTRODUCTION

Enzymes are highly efficient and specific biocatalysts which may be intracellular or extra cellular. Enzymes have found their way into many industrial processes with the advances in protein engineering techniques. The first completely enzymatic industrial process was developed in 1960 (Illanes et al., 2008). Thereafter, scientific research has led to increased usage of enzymes in industries. Moreover, increasing public awareness about the adverse effects of pollution and environment protection laws has been forcing industries to replace traditional chemical processes with green chemistry processes involving microorganisms and enzymes, wherever possible.

In the above context, increased usage of enzymes is justified because they are natural and biodegradable. The compatibility of enzymes with the environment as well as their ability to increase process efficiency and product specificity has led to expansion of the global enzyme market. The industrially important enzymes include pectinase, xylanase, lipase, laccase, cellulase, phytase, mannanase, β-galactosidase and so on. Pectinases have 25% share in the global sale of the food enzymes (Jayani et al., 2005). The application of enzymes in industries has grown as a large 'Global Enzyme Industry'. The global industrial enzymes market was valued at $4.91 billion in 2015 and is expected to reach $9.74 billion by 2022 growing at a compound annual growth rate of 10.3% between 2015 and 2022 (www.einnews.com). The factors contributing to the market growth include growing demand for biofuels, investments in research and development, and demand for consumer products. Moreover, increasing pressure to reduce pollution and advancements in biotechnology are important factors driving the market growth. Some of the key players in the global industrial enzyme market include Amano Enzyme, Codexis, MP Biomedicals, Genencor International, Dyadic International, Novozymes, Advanced Enzyme Technologies Ltd., BASF SE, Dupont Danisco, BioResource International, Inc., Adisseo France Sas, Associated British Foods PLC, Buckman Laboratories, Chr. Hansen A/S, Guangdong VTR BioTech, ICC Industries, Koninklijke Dsm N.V and Lonza Group. Microorganisms represent the most common source of industrial enzymes due to their broad biochemical diversity and feasibility of large scale
production by exploiting cheap carbohydrate sources. Moreover, these can be subjected to genetic and environmental manipulations to increase the enzyme yield.

The enzymes that hydrolyze pectic substances are broadly known as pectinases or pectinolytic enzymes, which may be classified as polygalacturonase (EC 3.2.1.15), pectin esterase (EC 3.1.1.11), pectin lyase (EC 4.2.2.2) and pectate lyase (EC 4.2.2.2) on the basis of their mode of action (Dinu et al., 2007). In the industrial arena, pectinase refers to mixtures of primarily all the above three different enzymatic activities. Polygalacturonase catalyzes the cleavage of α-(1, 4)-glycosidic linkages in unesterified polygalacturonic acid molecules by hydrolysis. The pectinase catalyzing the cleavage of α-(1, 4)-glycosidic linkages in pectin molecules by hydrolysis is termed is polymethyl galacturonase. Pectin esterase (or pectin methyl esterase) catalyzes the de-esterification of pectin into pectate and methanol. Pectin lyase cleaves α-(1, 4)-glycosidic linkages in pectin by β-elimination mechanism to form galacturonides with a double bond between C4 and C5 at the non-reducing end without affecting the polymer chain ester content, which is responsible for specific aroma of fruits (Yadav et al., 2009). Pectate lyase catalyzes the cleavage of pectate by β-elimination mechanism to form oligogalacturonates with a double bond between C4 and C5 at the non-reducing end. Among all the pectinases, pectin lyases are the only enzymes capable of depolymerizing highly esterified pectin into small molecules without prior action of other enzymes. Exo-polygalacturonases (EC 3.2.1.67) liberate monomers or dimers from the non-reducing side of the chain, whereas exopectate lyases (EC 4.2.2.9) discharge unsaturated dimers from the reducing end. The pectin-degrading enzymes are classified into the family 28 of glycoside hydrolases.

Pectinases can also be categorized into acidic and alkaline types depending on their pH optima. Acidic pectinases find applications in the extraction, clarification and liquefaction of fruit juices and wines. Most of the microbial pectinases produced by the industry are dedicated to this purpose. Pectins are responsible for the turbidity and consistency of juices causing an increase in their viscosity which hinders their clarification, filtration and concentration (Jayani et al., 2005). The degradation of pectic substances in mashed fruit purees is achieved through the addition of pectinolytic enzymes resulting in an increase in juice yield and its clarification as well as a decrease in viscosity. Alkaline pectinases are used in textile industry for
degumming of plant fibers (such as ramie, sun hemp, jute, flax and hemp) and for bioscouring of cotton fibers since the enzymatic processing causes no damage to the fibers and saves energy. They are also used for in the treatment of pulp and paper mill effluents and for improving the quality of black tea (Kohli and Gupta, 2015).

Pectinases have been produced by microbes (Chiliveri et al., 2016; Demir et al., 2014; Sethi et al., 2016), plants (Amid et al., 2014) and insects (Evangelista et al., 2015). But microorganisms are considered to be prospective pectinase producing sources because these are not influenced by climatic and seasonal factors as compared to plants, and can be subjected to genetic and environmental manipulations to increase the enzyme yield. Among the microorganisms, bacteria and fungi are the common producers of pectinase. A survey of the literature revealed that much of the work on pectinases has been focused on fungi and less reports are available on bacterial pectinases as compared to fungal pectinases. Pectinases from bacteria exhibit several advantages over fungal pectinases. Bacterial pectinases are often stable over a wide range of temperature and pH while fungal pectinases exhibit lower stability at high temperature and high pH values. Therefore, bacterial pectinases are also useful in various industrial applications that require high temperature and/ or high pH (Selim et al., 2016). Pectin lyases have been produced mainly from fungal genera such as Aspergillus, Penicillium and Fusarium. Since only a few reports are available on bacterial pectin lyases (Nadaroglu et al., 2010; Demir et al., 2011; Gopinath and Suneetha, 2012; Li et al., 2012; Demir et al., 2014), it was considered worthwhile to isolate, produce and characterize these industrially important enzymes from bacterial sources.

On an industrial scale, pectinases can be produced by both solid state fermentation (SSF) and submerged fermentation (SmF) systems. SSF involves the growth and fermentation of microorganisms on moistened solid substrate in the absence or near absence of free water whereas SmF involves the microbial growth and fermentation in the presence of sufficient water so as to dissolve the whole medium components in it. Each method has its own advantages and limitations. SSF is considered more suitable for higher enzyme yields, lower operation costs and energy requirements, simpler plant and equipment projects and less effluent generation as compared with SmF (Pandey et al., 1999; Couto and Sanroman, 2006;
SSF process mimics natural environment and offers the best possible use of agro-industrial wastes as substrate for enzyme production. Large amounts of agro-industrial wastes are generated every year which, if not managed, will cause environmental pollution. SSF processes are therefore, of special economic interest for countries like India producing abundant biomass and agro-industrial residues, as they can be used as cheap raw materials (Tunga et al., 2003). Agro-industrial residues being economical and ecofriendly are the prime choice of substrate for enzyme production. This will not only help in utilizing these agro-residues, but also result in cost effective production of enzymes. Several agro-residues including, wheat bran, sugar beet, sugar cane bagasse, corn cob, wheat straw and citrus wastes have been utilized for pectinolytic enzymes production (Bai et al., 2004; Maller et al., 2011; Khan et al., 2012; El-shishtawy et al., 2014). Orange peel, a rich source of pectin containing about 20-30% pectic substances (May, 1990), is a good inducer of pectinase. Coconut fiber, which contains 3-4% pectic content (Franck, 2000), has not been exploited for pectinase and pectin lyase production in SSF. Currently, the global annual production of coconut fiber is about 350,000 metric tons of which 90% is accounted for by India along with Sri Lanka. But still, this resource is underutilized and it rounds as a waste during coconut processing which can cause a threat to environment on disposing (Coir Board, India). So, this can be utilized in production of enzymes such as pectinase and pectin lyase. However, on a laboratory scale submerged fermentation system are more applicable due to their ease of operation.

Fermentation conditions and growth parameters need to be optimized for obtaining maximum enzyme yield as the enzyme production depends on several nutritional and physico-chemical factors. The optimization can be done by either one variable at a time approach or statistical methods. The latter approach is preferred since it also takes into consideration the interaction among the factors. In some applications like retting of bast fibers, bioscouring of cotton etc crude pectinase can be used whereas in fruit juice and oil processing industries, purified pectinase is generally required. The key to successful and efficient protein purification is to select the most appropriate techniques, optimize their performance to suit the requirements
and combine them in a logical way to maximize yield and minimize the number of steps required (Mesbah and Wiegel, 2014). Purification removes contaminants which are not required in the application of pectinases. It also concentrates the desired protein and transfers it to an environment where it is stable and is present in a form ready for the intended application. Moreover, for knowing the physico-chemical properties and characteristics, enzyme needs to be purified.

Though pectinases offer wide industrial applications with excellent catalytic properties but their uses get restricted in soluble form as it present some limitations such as poor stability under operational conditions, difficulty of product recovery and impossibility of multiple reuses in an industrial process (Sheldon et al., 2013). These constraints can be overcome by immobilizing the pectinase to some suitable support which offers commercial viability due to possible increase in stability, good catalytic activity, easier product and enzyme recovery, continuous operation of enzymatic processes, convenience in handling, reusability and reduced susceptibility to microbial contamination (Hanefeld et al., 2009; Sheldon et al., 2013). Immobilization of pectinase on suitable matrix can broaden its exploitation in industries.

In view of the wide utility of pectinases in the industrial sector, further research must be focused on the isolation of efficient microbial strains which are capable of synthesizing novel pectinases having desirable properties for their exploitation in industries. Availability of insufficient information on bacterial pectinases implied that more focus be diverted to this area and led us to plan the present study. Natural microbial diversity can be utilized for isolation of superior bacterial strains. Several pectinolytic bacterial strains have been isolated and characterized by several researchers from various natural sources (Klug-Santner et al., 2006; Bayoumi et al., 2008; Sharma and Satyanarayana, 2012; El-Shishtawy et al., 2014; Hanif et al., 2015; Sohail and Latif, 2016). However, as a consequence of the microbial activity in natural environments due to continuous evolution of bacteria at cellular and molecular levels, robust pectinolytic bacterial strains may be produced, which will secrete the enzyme of industrial utility.
Keeping in view the importance of microbial pectinases and the availability of less information on bacterial pectinases, the present investigation was planned with the following objectives:

- Isolation and screening of the pectinase producing bacterial strains from different sources
- Optimization of fermentation conditions for pectinase production in submerged and solid state fermentation from the selected bacterial strain
- Purification of extracellular pectinase from the selected bacterial strain
- Physico-chemical characterization of purified pectinase
- Immobilization of pectinase
- Applications of pectinase produced by the selected bacterial isolate