REVIEW ON CHARACTERIZATION AND PURIFICATION OF INDUSTRIALLY IMPORTANT ENZYMES FROM MICROORGANISMS ISOLATED FROM SOIL AND DECOMPOSED FOOD MATERIALS

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Abstract:
This article overviews the enzymes produced by microorganisms, which have been extensively studied worldwide for their isolation, purification and characterization of their specific properties. All enzymes which have been purified are protein in nature, and may or may not possess a non protein prosthetic group. Enzymes occur in every living cell, hence in all microorganisms. Each single strain of organism produces a large number of enzymes. But the absolute and relative amounts of the various individual enzymes produced vary markedly between species and even between strains of the same species. Hence, it is customary to select strains for the commercial production of specific enzymes which have the capacity for producing highest amounts of the particular enzymes desired. The microbial enzymes act as bio-catalysts to perform reactions in bio-processes in an economical and environmentally-friendly way as opposed to the use of chemical catalysts. The special characteristics of enzymes are exploited for their commercial interest and industrial applications, which include: thermo tolerance, thermophilic nature, tolerance to a varied range of pH, stability of enzyme activity over a range of temperature and pH, and other harsh reaction conditions. Such enzymes have proven their utility in bio-industries such as food, leather, textiles, animal feed, and in bio-conversions and bio-remediations, etc.

Index Terms: Microbial-enzymes, Thermophilic, Alkalophilic, Thermostable, Protease, Keratinase, Amylase; Xylanase, Lipase, Decomposed.

I. INTRODUCTION

Enzymes are the bio-catalysts playing an important role in all stages of metabolism and biochemical reactions. Certain enzymes are of special interest and are utilized as organic catalysts in numerous processes on an industrial scale. Microbial enzymes are known to be superior enzymes obtained from different microorganisms, particularly for applications in industries on commercial scale. Though the enzymes were discovered from microorganisms in the 20th century, studies on their isolation, characterization of properties, production on bench-scale to pilot-scale and their application in bio-industry have continuously progressed, and the knowledge has regularly been updated. Many enzymes from microbial sources are already being used in various commercial processes. Selected microorganisms including bacteria, fungi and yeasts have been globally studied for the bio-synthesis of economically viable preparations of various enzymes for commercial applications [1].

In conventional catalytic reactions using biocatalysts the use of enzymes, either in free or in immobilized forms, is dependent on the specificity of enzyme. In recent advancement of biotechnology, according to the requirements of a process, various enzymes have been and are being designed or purposely engineered. Various established classes of enzymes are specific to perform specialized catalytic reactions and have established their uses in selected bio-processes. A large number of new enzymes have been designed with the input of protein-engineering, biochemical-reaction engineering and metagenomics. Various molecular techniques have also been applied to improve the quality and performance of microbial enzymes for their wider applications in many industries [2]. As a result, many added-value products are being synthesized in global market with the use of established bioprocess-technology employing purposely engineered biocatalyst-enzymes.

Most of the commercially applicable proteases are alkaline and are bio-synthesized mainly by bacteria such as *Pseudomonas*, *Bacillus*, and *Clostridium*, and some fungi are also reported to produce these enzymes [3]. The xylanases with significant applications in bio-industries are produced by the fungal species belonging to genera *Trichoderma*, *Penicillium* and *Aspergillus*; the xylanases produced by these microorganisms have been found to possess high activity over a wide range of temperatures (40°–60°C) [4].

II. OBJECTIVE OF THE STUDY

This review is based on study of microbes isolated from different sources and their screening and optimization for production of enzymes that will be characterize, purify and widely being used in industries. Productions of enzymes are optimized to get high yield in optimum culture conditions. The selected strains used for enzyme production and optimization can be used for further studies and their bioprospecting use in future.

III. HISTORICAL REVIEW OF MICROBIAL ENZYMES

Jokichi Takamine (1894 and 1914) was the first person to realize the technical possibility of cultivated enzymes and to introduce them to industry. He was mainly concerned with fungal enzymes, whereas Boidin and Effront (1917) in France pioneered in the production of bacterial enzymes about 20 years later. Technological progress in this field during the last decades has been so great that, for many uses, microbial cultivated enzymes have replaced the animal or plant enzymes. For example, in textile desizing, bacterial amylase has largely replaced malt or pancreatin.

At present, only a relatively small number of microbial enzymes have found commercial application, but the number is increasing, and the field will undoubtedly be much expanded in the future. The search for a new enzyme or a biological mechanism for a desired industrial process can start by examining soil samples from the far corners of the world (or the far corners of East Lansing) Just one microorganism can contain over 1,000 different enzymes and a long period of trial and error in the laboratory can be needed to isolate the best microorganism for producing a particular type of enzyme. When the right microorganism has been found, the work is still not over. The microorganism has to be characterized and maybe even genetically-modified to produce the desired enzyme or process at high yields. Commercially, the microorganisms are grown in huge (four-story) tanks where they produce the desired product. This technique, referred generically to as fermentation (not to be confused with metabolic fermentation), has made it possible to produce enzymes, vitamins and other products economically and in virtually unlimited quantities. The end product of fermentation is a culture broth from which the desired products are extracted. The fermentation broth is centrifuged or filtered to remove all solid particles. The resulting biomass, or sludge, contains the residues of microorganisms and raw materials and is often used as fertilizer.
Akhilesh Kushwaha et al [5] have reported the amylase Production and Purification from bacteria isolated from a waste potato dumpsite in district Farrukhabad, UP, India. The most effective precipitation of enzyme took place at 60% w/v salt concentration. *Bacillus subtilis* was found to be most frequently occurring amylolytic bacteria followed by *Bacillus cerus* and *Bacillus megaterium*. The mean zone of amylolytic activity for the isolates ranged between 1.8mm for Bacillus subtilis and 0.9 mm for Bacillus cereus. The activity of the purified enzyme was 17 IU/ml. Shyam Sunder Alariya et al [6] has studied the amylase activity of a starch degrading bacteria isolated from soil. The results on different factors reveals that of all isolates evaluated, the highest protease activity was obtained from *Pseudomonas florescens* followed by *Bacillus subtilis* and *E.coli*. Iraj Rasooli et al [7] have isolated *Bacillus sp* in Iran soil. Mishra BK et al [8] has isolated 15 fungal isolates from soil of Rajastan. Among them two fungal isolates exhibited high enzymatic potential. *Aspergillus niger* RJ4 produced the highest extracelluar amylase activity (196.4 U g-1). *Tricoderma sp* RJ2 produced the highest levels of extracellular xylanase (140.8 U g-1) activity. Kanokphorn Sangkharak et al [9] has isolated five novel extracellular cellulase-producing bacteria from agriculture soil and identified through 16S rRNA sequence as *Cellulomonas sp*. Then, wastepaper hydrolysate was utilized as substrate for ethanol production using *S.cerevisiae*, the highest ethanol production was 12.5 g L-1 after 48 h of cultivation under separate hydrolysis and fermentation (SHF) process. Muhammad Irfan et al [10] has isolated seven different bacterial strains and screened for cellulase production in submerged fermentation process. Among these seven tested bacterial strains; *Cellulomonas sp*. ASN2 showed maximum yield for cellulase production. Supplementation of glucose, peptone and cysteine to the fermentation medium are favoured enzyme secretion. The optimum pH and temperature for the activity of crude enzyme was 7.5 and 600C, respectively. Barman et al [11] was described the effective cellulytic bacteria for biodegradation of solid kitchen and agricultural wastes as organic manure or compost. Bacterial strains of *Moraxella sp., Cellulomonas sp. and Planococcus sp.* were isolated from soil and overall findings of the nvestigation demonstrate the effectiveness of Moraxella sp. as a useful strain for bioconversion of solid organic waste. Similarly Miranda L Maki et al [12], Karin Vega et al [13], Katsuhiko Fujii et al [14], Silvina Ghio et al [15], Rahna Rathnan et al [16] were also studied on the cellulolytic microorganisms.

Chakravarthy et al [17] has isolated a Lipase-producing bacterial strain *Acinetobacter calcoaceticus* by employing enrichment culture techniques from oil mill. Mukesh Kumar et al [18] have described the Production, optimization and purification of lipase from *Bacillus sp*. MPTK 912 isolated from oil mill effluent. The enzyme was purified to homogeneity by Sephadex G-100 column chromatography and revealed molecular weight of 66kDa. Wei Feng et al [19] has Isolated and characterized six lipase-producing bacteria (*Bacillus sp., Brevibacterium sp., Corynebacterium sp., Staphylococcus sp., Klebsiella sp.,* and *Stenotrophomonas sp.*) in the intestine of the silkworm, Bombyx mori, reared on different forage. Lavanya et al [20] isolated the Lipase producing bacterial strains (*staphylococcus sp, bacillus sp, micrococcus sp* and *Pseudomonas sp*) from Agricultural lands and Oil Mill Waste. Likewise Tembhurkar et al [21], Jeyaraman Prasanna et al [22], Bhavani et al [23], Serdar Ulker et al [24] were also studied on the lipolytic microorganism from different sources. Soundra Josephine et al [25] have described the isolation, production and characterization of protease from *Bacillus Sp* isolated from soil sample. The specific activity of partially purified protease obtained from ammonium sulphate fractionation is found to be 10.32 and the fractionation is 1.32 fold purified from the crude enzyme preparation yielding 75.75% from the crude protein. Kuberan et al [26] has isolated the Protease producing bacteria from Halophilic soil. Swapna Vadlamani et al [27] Studied on Industrially Important Alkaline Protease Production from locally Isolated Superior microbial Strain from soil microorganisms. 50 microbial strains were isolated from the soil samples from different regions of Andhra Pradesh. Among the isolates hyper producing strain namely, *Bacillus clausii* was selected for alkaline protease. Gitishree Das et al [28] has described the Isolation, purification and mass production of protease enzyme from *Bacillus subtilis* from four different soil samples collected from various places in Bangalore. Pradeep Palsaniya et al [29], Vaishali Choudhary et al [30], Kalaiarasi et al [31], Zabin Bagewadi et al [32] were also studied on different microorganisms for their protease producing activity.

Mohamed Fathallh Eida et al [33] Clarify the identity and enzymatic activities of microorganisms associated with the decomposition of organic materials is expected to contribute to the evaluation and improvement of composting processes. In this study, he examined the cellulolytic and hemicellulolytic abilities of bacteria isolated from sawdust compost (SDC)

and coffee residue compost (CRC). DNA analysis indicated that the isolates from SDC and CRC belonged to the genera Streptomyces, Microbispora, and Paenibacillus, and the genera Streptomyces, Microbispora, and Cohnella, respectively. Microbispora was the most dominant genus in both compost types. All isolates, with the exception of two isolates lacking mannanase activity, showed cellulase, xylanase, β-glucanase, and mannanase activities. Based on enzyme activities expressed as the ratio of hydrolysis zone diameter to colony diameter, it was suggested that the species of Microbispora (SDCB8, SDCB9) and Paenibacillus (SDCB10, SDCB11) in SDC and Microbispora (CRCB2, CRCB6) and Cohnella (CRCB9, CRCB10) in CRC contribute to efficient cellulolytic and hemicellulolytic processes during composting.
Kiran Kumar Doddapaneni et al [34] isolated a protease producing bacterium from slaughter- house waste samples, Hyderabad, India. It was related to Bacillus cereus on the basis of 16SrRNA gene sequencing and biochemical properties. The protease was purified to homogeneity using ammonium sulfate precipitation, and ion exchange chromatography with a fold purification of 1.8 and a recovery of 49%.The enzyme had a relative molecular weight of 28kDa, pH and temperature optima for this protease were 10 and 60° C. The activity was stable between a pH range of 7.0 and12.0.The activity was inhibited by EDTA and enhanced(four-fold) by Cu2+ ions indicating the presence of metalloprotease. The enzyme showed extreme stability and activity even in the presence of detergents and anionic surfactants. The enzyme also showed stability in the presence of organic solvents.

Sangeetha et al. [35] investigated for potential bacterial strains for protease and lipase production, isolated from Effluents of meat processing industry, dairy industry, food processing industry and oil industry. Only four strains had shown both protease and lipase activity but among them only one bacterial strain was selected for further studies according to plate assay and enzyme assay (protease activity 52U/mL, lipase activity 38U/mL). According to Bergey's manual of systematic microbiology the strain was identified as *Bacillus pumilus*. Different parameters were used for optimization of protease production like temperature, pH, incubation time and carbon and nitrogen sources and also added different additives to the production medium to influence enzyme production. The optimum temperature for production of both enzymes was 37°C and pH was 8 and 9 in 36 hrs and 63 hrs of culture respectively for protease and lipase. Glucose and yeast extract was found to be best carbon and nitrogen source for enzyme production. Different crude substrates were also used in production media to improve enzyme production whey was found to be best crude substrate for protease production and castor oil, ginger oil and olive oil for lipase production.

P. Manivasagan et al. [36] isolated a total of twenty nine strains from the Kodiyakarai coastal sediments (Bay of Bengal) and ten of them exhibited multiple enzyme activity. Of these, the best (GK-22) was selected based on zone formation (amylase, cellulase and protease) and its growth conditions were standardized for better production of multiple enzymes. Present study on production of multiple enzymes (amylase, cellulose and protease) by GK-22 showed higher enzyme levels at the end of the stationary phase after incubation for 72 h at pH 7.0. Maximum activity of amylase, cellulase and protease (84, 88 and 89 IU/ml, respectively) was obtained at pH 7.0, temperature 45°C, sodium chloride concentration 2%, carbon compound sucrose, nitrogen compound beef extract, amino acid L-asparagine for amylase and cellulase and L-tyrosine for protease. The multiple enzymes were purified by precipitation with ammonium sulphate and ion exchange chromatography and the SDS-PAGE showed a single band for the purified enzyme, with an apparent molecular weight of 80 (amylase), 66 (cellulase) and 97 KDa (protease). The strain, GK-22 which showed higher multiple enzyme activity was tentatively identified as *Streptomyces alboniger*. These findings suggest that the strain can effectively be used in large scale production of multiple enzymes for commercial purposes, after testing and ascertaining the strain's capability in large scale fermentations.

IV. APPLICATION OF MICROBIAL ENZYMES

Uses of microbial enzymes in food, pharmaceutical, textile, paper, leather, and other industries are numerous and are increasing rapidly.

Protease :

Proteases prepared from microbial systems are of three types: acidic, neutral and alkaline. Alkaline proteases are efficient under alkaline pH conditions and consist of a serine residue at their active site. Alkaline serine proteases have the largest applications in bio-industry. Alkaline proteases are of particular interest being more suitable for a wide range of applications, since these possess high activity and stability in abnormal conditions of extreme physiological parameters. Alkaline proteases have shown their capability to work under high pH, temperature and in presence of inhibitory compounds. The alkaline protease produced from *Bacilli* and proteases from other microorganisms have found more applications overall in bio-industries such as: washing powders, tannery, food-industry, leather processing, pharmaceuticals, for studies in molecular biology and in peptide synthesis.

Keratinase :

Keratin is an insoluble and fibrous structural protein that is a constituent of feathers and wool. The protein is abundantly available as a by-product from keratinous wastes, representing a valuable source of proteins and amino acids that could be useful for animal feeds or as a source of nitrogen for plants. However, the keratin-containing substrates and materials have high mechanical stability and hence are difficult to be degraded by common proteases. Keratinases are specific proteolytic enzymes which are capable of degrading insoluble keratins. The importance of these enzymes is being increasingly recognized in fields as diverse as animal feed production, textile processing, detergent formulation, leather manufacture, and medicine. Proteolytic enzymes with specialized keratinase activity are required to degrade keratins and for this purpose the keratinases have been isolated and purified from certain bacteria, actinomycetes, and fungi.

Amylase :
Amylases are significant enzymes for their specific use in the industrial starch conversion process. Amylolytic enzymes act on starch and related oligo- and polysaccharides. The global research on starch hydrolyzing enzymes based on the DNA sequence, structural analysis and catalytic mechanism has led to the concept of one enzyme family—the alpha amylase. The amylolytic and related enzymes have been classified as glycoside hydrolases. The enzymes have been produced by a wide range of microorganisms and substrates and categorized as exo-, endo-, de-branching and cyclodextrin producing enzyme. The application of these enzymes has been established in starch liquefaction, paper, food, sugar and pharmaceutical industries. In the food industry amylolytic enzymes have a large scale of applications, such as the production of glucose syrups, high fructose corn syrups, maltose syrup, reduction of viscosity of sugar syrups, reduction of turbidity to produce clarified fruit juice for longer shelf-life, solubilisation and saccharification of starch in the brewing industry. The baking industry uses amylases to delay the staling of bread and other baked products, the paper industry use amylases for the reduction of starch viscosity to achieve the appropriate coating of paper. Amylase enzyme is used in the textile industry for warp sizing of textile fibers, and used as a digestive aid in the pharmaceutical industry.

Xylanase :
Hemicellulose is one of main constituents of agricultural residues and plants along with cellulose, lignin and pectin. Xylan is the major component of hemicellulose consisting of β-1,4-linked D-xylopyranosyl residues. The hydrolysis of xylan in plant materials is achieved by the use of a mixture of hydrolytic enzymes including endo-β-1,4-xylanase and β-D-xylosidase. The importance of xylanase has tremendously increased due to its biotechnological applications for pentose production, fruit-juice clarification, improving rumen digestion and the bioconversion of lignocellulosic agricultural residues to fuels and chemicals. Xylanases have established their uses in the food, pulp, paper and textile industries, agri-industrial residues utilization, and ethanol and animal feed production.

Laccase/Ligninase :
Ligninolytic enzymes are applicable in the hydrolysis of lignocellulosic agricultural residues, particularly for the degradation of the complex and recalcitrant constituent lignin. This group of enzymes is a mixture of synergistic enzymes, hence they are highly versatile in nature and can be used in a range of industrial processes. The complex enzyme system consists of three oxidative enzymes: lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. These enzymes have established their applications in bio-remediation, pollution control and in the treatment of industrial effluents containing recalcitrant and hazardous chemicals such as textile dyes, phenols and other xenobiotics.

Cellulase :
Cellulase enzymes are the third most important enzyme for industrial uses: world-wide research has been focused on the commercial potential of cellulolytic enzymes for the commercial production of glucose feedstock from the agricultural cellulosic materials. The significance of cellulose hydrolyzing thermophilic enzymes in various industries includes the production of bio-ethanol and value-added organic compounds from renewable agricultural residues. Cellulose is the most abundant natural resource available globally for bioconversion into numerous products in bio-industry on a commercial scale. For efficient bioconversion a strategy of efficient saccharification using cellulolytic enzymes is required.

Lipase :
Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are part of the family of hydrolases that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. These enzymes are widely found throughout the animal and plant kingdoms, as well as in molds and bacteria. Of all known enzymes, lipases have attracted the most scientific attention. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification, interesterification, and transesterification reactions in nonaqueous media. This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. The most significant industrial applications of lipases have been mainly found in the food, detergent, and pharmaceutical sectors. Limitations of the industrial use of these enzymes have mainly been owing to their high production costs, which may be overcome by molecular technologies, enabling the production of these enzymes at high levels and in a virtually purified form.

V. PURPOSE OF THE STUDY
Use of bacteria and the enzymes have been in practice for thousands of years (e.g. cheese making). The importance of microorganisms as a source of commercially useful chemicals, antibiotics and enzymes has been recognized for long time. A search began for better, less expensive and more readily available sources of such enzymes. It was found that certain microorganisms produce enzymes similar in action to the amylases of malt and pancreas, or to the proteases of the pancreas and papaya fruit. This led to the development of processes for producing such microbial enzymes on a commercial scale.

VI. CONCLUSION
Biotechnology is utilizing a wide range of enzymes synthesized on a commercial scale employing purposely screened microorganisms. Selected microorganisms have been characterized, purposely designed and optimized to produce a high-quality enzyme preparation on large scales for industrial applications. Different industries require enzymes for different purposes; hence microbial enzymes have been studied for their special characteristics applicable in various bio-processes. Recent molecular biology techniques have allowed to tailor a specific microorganism, to produce not only the high yields of an enzyme, but also enzyme with desired special characteristics such as thermostability, tolerance at high temperature and its stability in acidic or alkaline environment, and retaining the enzyme activity under severe reaction conditions such as in presence of other metals and compounds.

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References:

1. Pandey, A.; Selvakumar, P.; Soccol, C.R.; Nigam, P. Solid-state fermentation for the production of industrial enzymes. *Curr. Sci.* **1999**, *77*, 149–162.

2. Chirumamilla, R.R.; Muralidhar, R.; Marchant, R.; Nigam, P. Improving the quality of industrially important enzymes by directed evolution. *Mol. Cell. Biochem.* **2001**, *224*, 159–168.

3. Kumar, C.G.; Takagi, H. Microbial alkaline proteases: From a bioindustrial viewpoint. *Biotechnol. Adv*. **1999**, *17*, 561–594.
4. Ahmed, S.; Riaz, S.; Jamil, A. Molecular cloning of fungal xylanases: An overview. *Appl. Microbiol. Biotechnol*. **2009**, *84*, 19–35.

5. Akhilesh Kushwaha, Amylase Production & Purification from Bacteria Isolated from a Waste Potato Dumpsite in District Farrukhabad U.P State India, European Journal of Experimental Biology, 2011, 1 (3):107-113.

6. Shyam Sunder Alariya, Amylase activity of a starch degrading bacteria isolated from soil, Archives of Applied Science Research, 2013, 5 (1):15-24.

7. Iraj Rasooli, A Thermostable □-Amylase Producing Natural Variant of Bacillus spp. Isolated From Soil in Iran, American Journal of Agricultural and Biological Sciences 3 (3): 591-596, 2008.

8. Mishra, BK, Production of Amylase and Xylanase Enzymes from Soil Fungi of Rajasthan, J.Adv.Dev.Res.,Vol-1(1) 2010.

9. Kanokphorn Sangkharak, Isolation of Novel Cellulase from Agricultural Soil And Application For Ethanol Production, International Journal Of Advanced Biotechnology And Research, Vol 2, Issue 2, June-2011, Pp 230-239.

10. Muhammad Irfan, Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity,Turkish Journal of Biochemistry– 2012; 37 (3) ; 287–293

11. D Barman, Isolation of Cellulolytic Bacterial Strains from Soil for Effective and Efficient Bioconversion of Solid Waste, Life Sciences and Medicine Research, Volume 2011.

12. Miranda L Maki, Characterization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizers, Int J Biochem Mol Biol 2011;2(2):146-154.

13. Karin Vega, Production of Alkaline Cellulase by Fungi Isolated from an Undisturbed Rain Forest of Peru, Biotechnology Research International, and Volume 2012.

14. Katsuhiko Fujii, Development of a simple cultivation method for isolating hitherto-uncultured cellulase-producing microbes, Appl Microbiol Biotechnol (2011) 91:1183–1192.

15. Silvina Ghio, Isolation of Paenibacillus sp. and Variovorax sp. Strains from decaying woods and characterization of their potential for cellulose deconstruction, Int J Biochem Mol Biol 2012;3(4):352-364.

16. Rahna K. Rathnan, Isolation, Identification and Characterization of Efficient Cellulolytic Fungi from Natural Resources, International Journal of Microbial Resource Technology, Vol.1, No. 4 (Dec. 2012).

17. Kalyana Chakravarthy Bonala, Screening, isolation and identification of extracellular lipase producing bacteria from oil mill waste, Journal of Chemical, Biological and Physical Sciences, Feb-Apr.2012, Vol.2.No.2, 814-819.

18. Mukesh Kumar DJ, Production, optimization and purification of lipase from Bacillus sp. MPTK 912 isolated from oil mill effluent, Advances in Applied Science Research, 2012, 3 (2):930-938.

19. Wei Feng, Isolation and characterization of lipase-producing bacteria in the intestine of the silkworm, Bombyx mori, reared on different forage, Journal of Insect Science: Vol. 11 | Article 135.

20. M. Lavanya, Isolation of Lipase Producing Bacterial Strains from Agricultural Lands and Oil Mill Waste, Asian Journal of Biochemical and Pharmaceutical Research Issue 3 (Vol. 1) 2011.

21. Tembhurkar V. R, DAMA International, Production and Characterization Of Extracellular Lipases Of Staphylococcus Sp. Isolated From Oil Contaminated Soil, Vol. 1 No. 1 (2012).

22. Jeyaraman Prasanna, Isolation and optimization of Lipase producing organisms from Oil amended soil, J. B io s c i. Re s .,2012.Vol. 3(3):214-219.

23. Bhavani M, Screening, Isolation and Biochemical Characterization of Novel Lipase Producing Bacteria from Soil Samples, International Journal of Biological Engineering 2012, 2(2): 18-22.

24. Serdar ULKER, Isolation, production, and characterization of an extracellular lipase from Trichoderma harzianum isolated from soil, Turk J Biol, 35 (2011) 543-550.

25. F Soundra Josephine, Isolation, production and characterization of protease from Bacillus Sp isolated from soil sample, J. Microbiol. Biotech. Res., 2012, 2 (1):163-168.

26. T. Kuberan, Isolation and optimization of Protease producing Bacteria from Halophilic soil, J. Bisci. Res., 2010. Vol. 1(3):163-174.

27. Swapna Vadlamani, Studies On Industrially Important Alkaline Protease Production From Locally Isolated Superior Microbial Strain From Soil Microorganisms, International Journal of Biotechnology Applications, Vol. 3, Issue 3, 2011, pp-102-105.

28. Gitishree Das, Isolation, purification & mass production of protease enzyme from bacillus subtilis, International Research Journals of microbiology Vol. 1(2) pp. 026-031 April 2010.

29. Pradeep Palsaniya, Optimization of alkaline protease production from Bacteria isolated from soil, J. Microbiol. Biotech. Res., 2012, 2 (6):858-865.

30. Vaishali Choudhary, Isolation and identification of alkaline protease producing fungi from soils of different habitats of Sagar and Jabalpur District (M.P), J. Acad. Indus. Res. Vol. 1(3) August 2012.

31. K. Kalaiarasi, Optimization of alkaline protease production from Pseudomonas fluorescens isolated from meat waste contaminated soil, African Journal of Biotechnology Vol. 8 (24), pp. 7035-7041, 15 December, 2009.

32. Zabin K. Bagewadi, Production dynamics of extracellular alkaline protease from Neisseria sps. Isolated from soil, Research Article, Biotechnol. Bioinf. Bioeng. 2011, 1(4):483-493.

33. Mohamed Fathallh Eida,Toshinori Nagaoka, Jun Wasaki, and Kenji Kouno, Isolation and Characterization of Cellulose-decomposing Bacteria Inhabiting Sawdust and Coffee Residue Composts, Microbes Environ. Vol. 27, No. 3, 226–233, 2012.

34. Kiran Kumar Doddapaneni, Radhika Tatineni, Ravi Nagaraj Vellanki, Sangeetha Rachcha, Naveen Anabrolu, Venkanna Narakuti, Lakshmi Narasu Mangamoori, Purification and characterization of a solvent and detergent-stable novel protease from Bacillus cereus, Microbiological Research 164 (2009) 383—390

35. R Sangeetha, A Geetha, I Arulpandi (2007) Optimization of protease and lipase production by Bacillus pumilus SG 2 isolated from an industrial effluent. Internet Scientific Publications 5:1-13.

36. P. Manivasagan, S. Gnanam, K.Sivakumar, T. Thangaradjou, S. Vijayalakshmi and T.Balasubramanian, Isolation, identification and characterization of multiple enzyme producing actinobacteria from sediment samples of Kodiyakarai coast, the Bay of Bengal, African Journal of Microbiology Research Vol. 4(14), pp. 1550-1559, 18 July, 2010.