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Review Paper

A Critical Review of Production of Extracellular Enzymes

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Abstract
Microorganisms are present in different source; characterized microorganisms due to their capacity to produced different extracellular compound are biologically active molecules and enzymes. They compounds are very important reactions in other living organisms. It produce a large amount of extracellular enzymes are commercially interesting because many processes require. It is easily cultivated and limited space. Extracellular enzymes are used in different industries. Enzymes are potential biocatalysts for a large number of reactions. Microorganisms represent a viable alternative source of enzymes, as they may be cultured in large quantities within short time frames by fermentation are biochemically diverse, and amenable to genetic manipulation. Production of extracellular enzyme metabolites is a wide spread feature of microbial biology and biotechnology potential in the biological treatment and genetic engineering. In the present paper large number of published and available literature is reviewed.

Key words: *Microorganisms, Production, Extracellular, Characterization, Fermentation, Temperature, pH.*

Introduction

Fungi in general are well characterized microorganisms due to their capacity to produce different extracellular compound; some of them are biological very important causing severe reaction in other organisms (Alves, 1998, Dias, *et al*. 2008, Mustafa, *et al*. 2009,). These compounds include different types of enzymes and biological active molecules. St Leger *et al* (St .Leger, *et al*. 1986) reported that M. *anisopliae Var*. *anisopliae*, B. *bassiana* and *Vertcilium lecanii* produced a large amount of extracellular enzymes endoproteases, amino peptidases, lipases, esterase and chitinases when grown on cuticle of the locust *Schistocera gregaria*. The protease Pr1 from M. *anisopliae* was able to remove 25-30% of the cuticle proteins showing that protease were involved in the hydrolysis of the cuticle and thus facilitating the penetration through the tegument (St Leger *et al*. 1986). In their study observed large variations, in the levels of enzyme production among different isolate of entomopathogenic fungus. Thus all isolates showed high productions of endoproteases (St. Leger, *et al*. 1986b). Considering relevant to design strategies with a view in microbial control of insects, the aim of this study was to investigate the production of extracellular enzymes of the entomopathogenic fungi *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces sp* when grown on different substrates in the presence or absence of glucose on the media looking for the possibility to use these strains in insect control. Actinomycetes are excellent source of the biotechnologically important compounds and that still makes them one of the most sought after microbes to research on, despite decades of research dedicated to unravel their bioprospects. A new dimension to this research was added when habitats other then soil were searched for presence of actinomycetes and reports pointed towards a promising future.

The first step towards any detailed research into industrial prospects and applicability of a microbial product is screening of the microbes in question for their bioactivity. A preliminary investigation to gain insights into their extracellular enzyme production potential since the need to explore the aquatic habitats to uncover the presence and bioactive potential of actinomycetes has been expressed time and again (Cheng and Jiang, 2006; Jiang and Xu, 1986; Rifaat, 2003). Among a large number of non-pathogenic microorganisms capable of producing useful enzymes, filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential. These enzymes are applied in the industrialization of detergents, starch, drinks, food, textile, animal feed, backing, pulp, and paper, leather, chemical and biochemical products. The use of starch degrading enzymes was the first large-scale application of microbial enzymes in the food industry (Bennett, 1998, Dekker, *et al*.2003). Two enzymes carry out the conversion of starch to glucose, namely: (1) α-amylase, that cuts the large α-1, 4-linked glucose polymers into shorter oligomers and (2nd) glucoamylase that hydrolases that oligomers to glucose. Amylases have applications in food detergents, drinks, animal feed and baking (James, *et al*. 1997, Pandey, *et al*. 2000). Pectinases are used in the preparation of wine and fruits lamella and cell walls. Pectinases were classified according to its specificity to attack pectin, pectic acid and oligo-d-galacturonate. Pectin, pectinesterases, depolymerizing, enzymes and protopectinase are the three major types of pectinases, presently pectinases have been used in textile industries (Kashyap, *et al*. 2001, Sathyanarayana, *et al*.). Phosphatases hydrolyze esters and anhydrides of phosphoric acid (Wyckoff, 1987). These enzymes are involved in various biological processes e.g. in cell cycle differentiation and others. Xylanases have potential application in food, feed, paper pulp and textile industries (Polizeli, 2005). These enzymes degrade plant fibers made of xylan hemicelluloses producing xylose monomers one of the most important xylanase application is the pretreatment of pulps, prior to bleaching in pulp and paper industries (Subramaniyan, *et al*. 2002). These enzymes release lignin fragments by hydrolyzing residual xylan and the pretreatment with xylanase reduces the usage of chlorine as the bleaching agent. Not less important in the use of xylanases for bread-making and beer production (Harbak, *et al*. 2002, Dervilly, *et al*. 2002). On the other hand, in the industry of animal feed, the xylanases can be added to the ration increasing the -intestinal absorption of nutrients (Twomey, *et al*. 2003). Glucose oxidase catalyzes the oxidation of D-glucose to D-gluconic acid and hydrogen peroxide in the presence of oxygen and water its major use is in the quantification of free glucose (Marks, 1996). But it has also been used for lebeling antibodies used in the detection of tumor marker and viral antigens (Porter, *et al*. 1984). In this study use have explored the biopotential of several filamentous fungi isolated from soil samples, for the production of some of the above referred enzymes.

Hemicellulose is the second most abundant polysaccharide in nature after cellulose. The major constituents of hemicelluloses are the hetero-1, 4-β-D-xylans and hetero-1, 4-β-D-mannans (galactoglucomannan, galactomannan, and glucomannan). The heteroxylans are found mainly in grasses, cereals, and hard wood (angiosperms). The mannans are more abundant in copra, plam, coffee and locust bean endosperms (Araujo and ward 1990). Mannanolytic microbes were found in soil compost and animal rumen (Zakaria *et al*. 1998). Biodegradation of β-mannans is caused by β-mannanose (1, 4-β-D-mannan manohidrolase [EC 3, 2.1.7.8]) produced from bacteria and fungi. The enzyme hydrolase the β-(1, 4)-Linkages in backbone of mannan polymer producing short chain mannoligosaccharides. Then these compounds can be further degraded by the action of β-mannansidase (β-D-mannosidase [EC3, 2.1.25]) and a α-galactosidase (EC3.2.1.22) (Duffaud, *et al*.1997). Mannan degradation from glucomannan and galactomannan produce mannooligosaccharide, mannobiose and mannose. Mannan-degrading enzymes can be used for numerous applications in food, feed, pulp, and paper industries. Proteases are most important group of industrial enzymes which account for about 60% of total enzymes in the market. Bacterial proteases are most significant compared with animal and fungal proteases and have wide range of industrials application. Among different proteases, alkaline, protease produced by microorganisms are of main interest from a biotechnological perspective and are investigated not only in scientific fields of protein chemistry and protein engineering but also in applied fields detergents, foods, tanney, pharmaceutical and leather industries. A proteases catalyses the hydrolysis of protein into peptides and amino acids and consists of one of the most useful enzyme groups. The possibility of using streptomyces for protease production has been investigated because of their capacity to secrete the proteins into extracellular media which is generally regarded as safe with food and drug administration (GRAS). Streptomyces sps that produce proteases include S. *clavuligerus*, S. *griseus*, S. *rimouses*, S. *thermoviolaceus*, S. *thermovulgaris* (James *et al*. 1991, Edward *et al*.1994). Streptomyces sps are heterotrophic feeders which can utilize both complex and simple molecules as nutrients. In addition to antibiotics streptomyces species liberate several extracellular enzymes (Gupta *et al*. 1995). They produce variety of extracellular proteases that have been related to aerial mycelium formation and sporulation (Kim and Lee 1995). The microorganisms of bacillus genus are known to be one of the most important source of enzymes and other biomolecules of industrial interest, being responsible for the supply of about 50% of the market for enzymes (Schallmey, *et al*. 2004). The worldwide market for enzymes is estimated at 1.6 billion dollars, 29% for the food industry, animal feed 15% and 56% in other application (Outtrup, *et al*. 2002).

Bacillus Subtilis produces a variety of extracellular enzyme including proteases amylases and lypolytic enzymes of great importance in industrial processes pharmaceutical, leather, laundry, food, and waste processing industries (Schallmey et al. 2004, Machium, *et al*. 1995). Amylases produced by Bacillus are heat resistant, which is commercially interesting because many processes require high temperatures, so the thermo sensibility to be a limiting factors (Konsoula, *et al*. 2006). Finally lipases catalyze the hydrolysis of triacylglycerols and are widely used in organic chemistry due to its high selectivity and specificity and therefore, receive much attention because of its potential use in industrial processes (Lesuisse, *et al*. 1993). Bacillus Subtilis secretes different types of lipases, which may vary according to different growth conditions, environmental factors pH and amino acid supply (Eggert, *et al*. 2003). Enzymes are potential biocatalysis for a large number of reactions. Microorganisms represent a viable alternative source of enzymes, as they may be cultured in large quantities within short time frames by fermentation, are amenable to genetic manipulation (Anbu *et al*. 2013). Fungal endophytes produce several extracellular enzymes pectinases, callulases, lipases, amylases, laccases, and proteases. Moreover fungal enzymes play a key role in biodegradation and hydrolysis, mechanisms of significant importance in protection against pathogens (Desire *et al*. 2014). Fungal enzymes are used in the beverage, food, confectionary, textile, and leather industries to simply the processing of raw materials. In additions enzymes are often more stable than those derived from the sources (Raju *et al*. 2015). The use of bioremediations technologies for removing these contaminants provides a safe and economic alternative to commonly used physical-chemical treatment. Bacterial extracellular enzymes mediated activity is the major process involved in the hydrolysis of organic pollutants. Bacterial communities contain abroad range of genetic information to build up specific enzyme for the biodegradation. Extracellular hydrolytic enzymatic enzymes thus produced can disrupt major chemical bonds in the toxic molecules and results in the reduction of their toxicity (Sharma and Shah, 2005, Lal and Sexena, 1982). To process the organic matter, microbes produced extracellular enzymes which degrade these large complex molecules into smaller ones, which are easily up taken by the microbes. These enzymes are divided into two major groups: oxidoreductases and hydrolases, which decompose complex molecules by breaking them down. These enzymes are very important in the ecosystem overall due to the large amount of necromass generated, maintaining an ecological balance several extracellular enzymes proteases, phenol oxidases, peroxidases and β-Nacetylglucosaminidases, are very important in this process as they contribute to the breakdown of organic compounds (Kang *et al*. 2005; Oyekola and Pletschke 2006; Acosta-Martinez *et al*. 2007). Life depends on the complex nature of chemical reactions carried out by specific enzymes may have for reaching consequences for the living organisms. Most of the industrial enzymes are of microbial oregion. Microbial enzyme is wide and microorganisms can be easily and rapidly cultivated thus forming an unlimited enzyme source. A wide range of microorganisms produced invertase and can thus utilize sucrose as a nutrient. Commercially, invertase is biosynthesized chiefly by yeast strains of *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*, invertase is extensively used in confectilonaries, food industries and in pharmaceuticals (Ashokkumar *et al*. 2001).

Microorganisms have two types of extracellular enzymatic system: The hydrolytic system, which produce hydrolyses and is responsible for cellulose and hemicelluloses degradation; and a unique oxidative and extracellular ligninolytic system, which depolymerises lignin (Perez *et al*. 2002). Two major families of enzymes are involved in ligninolysis are lignin proxidases and laccases, proxidases catalyze the oxidation of a number of substrates in the presence of hydrogen peroxide and use a wide range of substrate, have been found widely throughout plants, animals and microorganisms signifying their important role in biological system (Franssen, 1994; Rob *et al*. 1997). The finding that proxidases from fungi and bacteria have a wide range of potential biotechnological applications has caused an increased interest in finding new species that produce beneficial peroxidase activity (Orth *et al*. 1993; Mereer *et al*., 1996; Kang *et al*. 1996). Although peroxidases are ubiquitous, two main factors limit their exploitation (a) the levels of enzyme production and (b) the instability of peroxidase activity under conditions high pH and temperatures, conditions generally encountered in industrial processes. Environmental conditions on the production of high level of extracellular peroxidase activity and tnhe partial characterization of the crude enzymes produced by streptomyces sp. F6616 isolated from soil in Turkey, indicating a number of key properties of the enzyme and investigate its biotechnological potential in the biological treatment of wheat straw and Kraft pulp. *Serratia* is an opportunities gram-negative bacterium belonging to the tribe *Klebsiellae* and the large family *Enterobacteriaceae*. It occurs naturally in soil and water. It is a potent producer of industrially important enzymes and secretes several extracellular proteins, including chitinase, a lecithinase, a hemolysin, siderphores, lipase, proteases and nuclease (Hines, *et al*. 1988, Aucken, *et al*.1998). *Serratia* is also a potential insect pathogens and chitinase secreted by it plays an important role in its virulence, together with protease and lecithinases (Uchiyama, et al. 2003). The characterization of extracellular microbial enzymes is important for understanding their role in pathogenesis of infectious disease, as they play a, major role in causing cytotoxicity in mammalian cells, to improve their application in biotechnology (Brurberg, *et al*. 2000). Although, the production of extracellular enzymes has been reported by *Serratia spp*. From clinical isolates (Hines, *et al*. 1988, Aucken, *et al*.1998, Brurberg, *et al*. 2000). The production of extracellular metabolites is a wide spread feature of microbial biology, these compounds contributing to diverse functions, competition, and inter-and intra- specific interaction. Fungi are known to produce a range of extracellular enzymes in particular hydrolase, which will aid in their acquisition of nutrient from the surrounding environment. A small number of studies have addressed this aspect of their biology in Antarctia (Fenice *et al*. 1997; Bardner *et al*. 1999; Kasieczka-Burnecka *et al*.2007; Krishnan *et al*. 2011). Extracellular hydrolase enzymes generally function to degrads soil organic matter with the producing cells. In addition to soil organic matter deriving from primary production, it has been reported that soil micro fungi contribute significantly in the bioremediation of hydrocarbono contaminated soil (Ferrari *et al*. 2011).

Conclusion

The production of extracellular enzymes including amylases, protease, cellulose, lipase etc and extracellular metabolites is a wide spread feature of microbial biology and other industries. Produce of extracellular enzymes from microorganisms, screen of potential biotechnological enzyme producers the optimization condition of the fermentation media can be implemented in large scale for production of extracellular enzymes great potential as an alternative substrate to the production of enzymes. These enzymes could be found in different organisms. The production of extracellular enzymes by endophytes may be involved in the host plant and phytopathogenic microorganisms. Extracellular hydrolytic enzymes produced by fungal endophytic, particularly cellulase and pectinase. On the basis of above discussion it can be concluded that there is ample scope of research in the field of industrial enzymes.

References

Alves, S.B, (1998). Fungus entomopatogenicos, In: Alves, S.B. control microbiano deinsetos, Piracicaba, Feal, and P. 289-381.

Ashokkumar *et al*. (2001). Optimization of media for a-fructofuranosidase production by *Aspergillus Niger* in submerged and solid state fermentation process biochemistry. 37: 331-338.

Acosta- Martinez V. *et al*. (2007).Enzyme activities as effected by soil properties and land use in tropical water shed. Applied soil Ecology. A section of Agriculture, Ecosystems and environment, 35, 36-45.

Aucken H. M. *et al*. (1998). Microbes Drug Resist. 6, 111-117.

Araujo, A. and O.P. Ward, (1990). Extracellular mannanase and galactanases from selected fungi J. industrial microbiology. 6: 171-178.

Bradner JR. *et al*. (1999). Qualitative assessment of hydrolytic activities in Antarctic micro fungi grown at different temperatures on solid media. World J. microbes' biot 15: 131-132.

Brurberg, M.B. *et al*. (2000). Recent Res. Develop microbiology, 5, 187-204.

Bennett, J.W. (1998). Mycotechnology: the role of fungi in biotechnology J. Biotechnology., 66, 101-107.

Cheng, HR and Jiang, N., (2006). Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnology letters, 28(1): 55-9. Doi: 10. 1007/s10529-005-4688-2.
Dias, B.A., *et al.* (2008).Cuticle degrading proteases produced by the entomo pathogenic fungus *Beauveria Bassiana* in the presence of coffee bery borer cuticle. Braz, J. Microbiology 39,301-306.
Desire, MH. *et al.* (2014). Enzymes and qualitative phyto chemical screening of endophytic fungi isolated from Lantana Camara Linn. Leaves, J. Applied biology, biotechnology 2: 16 10.7324/JABB. 2601.
Duffaud, G.D. *et al.* (1997). Purification and characterization of extremely thermo stable β-mannosidase, and α-galactosidase from the *Hyperthermophilic eubacterium Thermotoga neopalitana* 5068. Applied environment microbiology 63: 169-177.
Dekker, M. (2003). Handbook of fungal biotechnology. Dilip, K. Arora, ed., New York, 600p.
Dervilly, G.; *et al.* (2002). Isolation and characterization of high molecular mass water-soluble rabinoxylans from barley malt. Carbohydr. Polym. 47,143.
Eggert, T. *et al.* (2003). Extracellular lipases from *Bacillus Subtilis*: regulation of gene expression and enzyme activity by amino acid supply and external pH FEMS microbiology Lett. 225, 319-324.
Franssen M.C.R. (1994). Halogenation and oxidation reactions with haloperoxidases. Biocatalysis, 10: 87-111.
Fenice M. *et al.* (1997). Production of extracellular ernzymes by Antarctic fungal strains. Polar boil. 17: 275-280.
Ferrari B.C. *et al.* (2011). Recovering greater fungal diversity from pristine and diesel fuel contaminated sub-Antarctic soil through cultivation using both a high and a low nutrient media approach front microbiology 2: 1-14.
Gupta, R., *et al.* (1995). Chitinase production by *Streptomyces viridificans*: it's potential in cell wall lysis J. Applied Bacteriology. 78: 378-383.
Harbak, L.; Thygesen, H.V. (2002). Safety evolution of a Xylanases expressed in bacillus Subtilis. Food chem. Toxicology 40, 1.
Hines, D.A, *et al.* (1988). J. Bacteriology 170, 4141-4146.
James, P.D.A., M. Iqbal, C. Edwards and P.G.G. Miller, (1991). Extracellular protease activity in antibiotic producing *Streptomyces thermovioleceus*. Current microbial. 22: 377-382.
James, J.A; Lee, B.H., (1997). Glucoamylases. Microbial source, industrial applications and molecular biology a review J. Food Biochem. 21, 1-52.
Jiang, C. and XuL, (1996). Diversity of aquatic Actinomycetes in lakes of the middle plateau, Yunnan, China. Applied and Environmental microbiology, 62 (1), 249-253.
Krishna, A. *et al.* (2011).Extracellular hydrolases enzyme production by soil fungi from king George Island, Antarctic. Polar Biology 4, 1535-1542.
Kasieczka-Burnecka M. *et al.* (2007). Purification and characterization of two cold adapted extracellular tannin acyl hydrolases from an Antarctic strain verticillium sp. p9. Applied microbiology, biotechnology. 77: 77-89.
Kang, M. *et al.* (1990). Isolation and characterization of soil *streptomyces* involved in 2,4-dichiorophenol oxidation J. microbiology, biotechnology, 9: 877-880.
Konsoula, Z. Liakopoulou-Kyriakides M. (2006). Starch hydrolysis by the action of an entrapped in alginate capsules α-amylase from *Bacillus Subtilis* process Biochem. 41, 343-349.
Kim, I. and Lee. (1995). Physiological roles of leupeptin and extracellular protease in mycelium development of *Streptomyces exfoliates* SMF 13 microbiology, 141: 1017-1025.
Kashyap, D.R. *et al.* (2001). Applications of pectinases in the commercial sector. A review bioresource technology, 77, 215-227.
Lal, R., Sexena, D.M. (1982). Accumulation, Metabolism and effects of organo chlorine insecticides on microorganisms, microbiology Rev, 46. 95-127.
Lesuisse, E.*et al.* (1993). Purification and preliminary characterization of the extracellular lipase of *Bacillus Subtilis* 168, an extremely basic pH-tolerant enzyme. Eur. J. Biochemistry, 216, 1550-160.
Mercer, D.K. *et al.* (1996). Screening actinomycetes for extracellular peroxidase activity. Applied environment, microbiology. 62: 2186-2190.
Machius, M., and, G., Huber, R. (1995). Crystal structure of Calcium-depleted Bacillus *licheniformis* α-amylase at 2.2A` resolution, J. Mol. Biol., 246, pp. 545-559.
Marks. V. (1996). An improved glucose oxidase method for determining blood, CSF and urine glucose levels. Clin. Chim. Acta, 251, 19-24.
Mustafa, U., Kaur, G. (2009).Extracellular enzyme production in *Metarhizium anisoplia* isolates. Folia microbiology 54(6), 499-504.
Outtrup, H., Jorgensen, S.T. (2002). The importance of bacillus species in the production of industrial enzymes, In: R. Berkeley, M. Heyndrickx, N. Logen, D. De Vos (Eds), Application and system of bacillus and relatives, Wiley-Black well science Inc. Malden, MA, United States, 206-218.
Oyekola, O. and Pletschke B. (2006). ATP sulphurylase an enzymatic marker for sulphate reduction soil biology and biochemistry, 38, 3511-3515.
Orth, A.B., Royse, D.J., Tien, M. (1993).Ubiquity of lignin degrading peroxidases among various wood degrading fungi, applied environment microbiology, 59: 4011-4023.
Perez J.*et al.* (2002). Biodegradation and biological treatments of cellulose, hemicelluloses and lignin an over view, Int. microbiology 5: 53-63.

Porter, D.D. Porter, H.G.,(1984). A glucose oxidase immune enzyme stain for the detection of viral antigen or antibody on nitrocellulose transfer blot. J. immunology method 72, 1-9.

Polizeli, M.L.T.M. *et al*. (2005). Xylanases from fungi: properties and industrial applications. Review applied microbiology, Biotechnology. 67, 577-591.

Pandey, A. *et al*. (2000). Advances in microbial amylases review, Biotechnology applied biochem. 31,135-152.

Twomey, L.M. *et al*. (2003). The effect of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Animal feed sci technology, 108 (1-4), 71-82

Uchiyama, T. *et al*. (2003).J. Bacteriology 185, 1776-1780.

Wyckoff, HW. (1987). Structure of Escherichia coli alkaline phosphatase determined by X-ray diffraction. In: Torriani-GoriniA, FG Rothman, S. Silver, A. wright and E. Yagil (eds), phosphatase metabolism and cellular regulation in microorganisms. America Society for Microbiology, Washington, DC, P. 118-126.

Zakaria, M.M., *et al*. (1998). Optimization for β-mannanase production of a *Psychrophilic bacterium*, *Flavobacterium sp*. biotechnology, biochemistry 62: 655-6s60.