



A REVIEW ON SCREENING METHODS FOR EXTRACELULAR ENZYMES

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ABSTRACT

Soil contains a number of microorganisms with nutritional diversity. These bacteria produce many extra cellular and intracellular enzymes which are used for various purposes. Some of these extracellular enzymes are important for agro based industries that in turn opens scope for searching cost effective and easy to operate screening of such important extracellular enzymes. Present paper describes a review on screening methods for extracellular enzymes. It includes screening for extracellular amylase, lipase and proteases.

INTRODUCTION

Soil microorganisms are ability to excrete enzymes into the environment. Soil microbes very important in soil fertility and produce enzymes are very important in industrial commercial production such as food industries, beverage industries, textile industries, leather and taining industries etc. Organisms produced of extracellular enzymes. Soil microorganisms isolated were screened for extracellular enzymes production. Screening of proteases producing from bacillus sp. of different ecological environment can result in isolation of new alkaline protease with unique physiochemical characteristics (Singh *et al.*, 1999). One of the most important characteristics determines the industrial suitability of proteases is their requirement of high pH for optimum enzyme activity. Proteases are important industrial enzymes accounting for 60% of total global enzyme sales (Chun *et al.*, 2007, Ward, 1985; Chu, 2007). (Halpern, 1981). Proteases are studied in protein chemistry, protein engineering and for applications as cleaning agents, food additives and dehairing (depilating) agents. (Tsujiyo, Miyamoto *et al.* 1990. Lee *et al.*1992). *Actinomycetes* are good decomposers in soil via secretion of extracellular hydrolases meriting studies for potential applications as industrial enzymes. (McCarthy. 1987. Edwards *et al.* 1984.).

Actinomycetes in addition to antibiotic, elaborates extracellular enzymes, e.g. proteases, chitinases, amylases etc. Compared to bacillus spp., *Actinomycetes* have been less explored for proteases. (Ningthojam *et al.* 2009). Screen local *Actinomycetes* isolates for protease production, characterization of the best strain and optimization of enzyme production. Screening of enzymes isolates for enzymes activity and properties of the best producer such as incubation period, pH and temperature optima, best C and N sources, effects of protease inhibitors and metal ions for the extracellular protease activity and its potential as a detergent additive.

Screening

Screening provided by Sadler (1996) is that it is a process involving the determination of whether or not an individual proposal. Screening is therefore a decision-making process that is initiated during the early stage of the development of a proposal. Screening methods are very useful tools for examining simulations models that involved a large number of factors (Barton, 2011., Kleijnen, 2004., Sanchez and al., 2005., Welch *et al.* 1992). This screening stage should involve a minimum number of experiments and should not take much

computing time different screening methods that are useful in eliminating negligible factors so that efforts may concentrate upon just the important ones. Screening methods are used in specific research and industry have used simulation codes in order to forecast, to optimize and to make good decisions in the context of their studies (Azarian *et al.* 2011). Soil microorganisms were isolated to determine their potential to produce different different enzymes. Microbes were isolated and identified from soil sample. The microbes were screened for enzyme production.

Enzyme from microbial source generally meets industrial demand, due to their high yield and thermo stability. Microbial enzyme presents a wide spectrum of characteristics that make them useful for specific applications. Enzyme are natural origin and non-toxic and have great specificity of action hence and cambering about reactions not easily carried out, they work out best under mild condition of moderate temperature and near natural pH (Oyeleke *et al.* 2009). Soil microorganisms selected by initial screening were subjected to extracellular enzyme produced by different varieties of microorganisms such as *Fungi*, *Bacteria*, *Yeast*, and *Actinomycetes* (Devi *et al.* 2008). Organisms have evolved in saline environments and are able to overcome the dexterous effects and saturating concentration. Therefore, are isolated and screened for extremely halophilic bacteria that can produced high protease activity at high concentration in soil medium. The high protease activity obtained at 5% NaCl (Kanlayakrit and Bovornreunroj, 2002; 2003). The number of bacteria which are capable of producing antibiotics which include *Bacillus* (waites *et al.* 2008). *Actinomycetes* (Abdulkadir and Waliyu, 2012; Tiwari and Gupta, 2013, Cartwright *et al.* 1995) and most *Streptomyces* (Willey *et al.* 2008) and most isolates are soil bacteria.

Soil is rich in microorganisms which are capable of producing antibiotics (Brun and Skimkets, *et al.* 2000). The traditional approach is 'random screening' in which bacteria are isolated grown and their activity spectrum was assessed. Even this has been done for more than 50 years still we are getting results in favor to us and thus we are sticking with this approach (Wawrik *et al.* 2007). Soil microorganisms produces enzymes are involved in various Biological processes, eg. In cell cycle, differentiation and other. These enzymes classified as: 1) alkaline phosphatases; 2) high molecular mass acid phosphatases; 3) low molecular mass acid phosphatases; 4) purple acid phosphatases; 5) protein phosphatases. (Vincent *et al.* 1992). Alkaline phosphatases are used in enzyme linked immune absorbent assays (ELISA), Non isotopic probing blotting and sequencing system (Dong and Zeikus 1997).

Screening for extracellular amylase producing microbes

Microorganisms are preferred sources of these enzyme because of their rapid growth, the limited space required for their cultivation and they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various application (Lalitha Kumari, *et al.*, 2012). Screening method to determine the soil microorganisms. The optimization for screening method used for the identification of microbial enzyme from other natural source of great importance fungus and bacteria isolated were screen for amylases carboxy methylcellulases and proteases are widely used in the industry for the manufacture of pharmaceuticals etc. Screening and isolation of lipase

producing microorganisms have the ability to catalysed a Wide variety of reaction in aqueous and non aqueous phases (Sexena *et al.*, 2003). Microbial lipase have also received more attention due to their selectivity, stability and substrate, specificity (Treichet *et al.* 2010). Extracellular lipase production by bacteria are influenced by the composition of the growth medium, cultivation conditions and many physico-chemical (pH and temperature) and nutrition facto (carbon, nitrogen, and lipid sources) (Jaegar *et al.* 1994). A variety of extracellular lipases of bacterial origin with different properties and specificities have been described and characterized. Extracellular lipase was isolated from many different bacterial species including *Bacillus* (Ertugrul, *et al.*, 2007) and *Pseudomonas* (Kiran, *et al.* 2008. Wang *et al.* 2009). Lipase production by *Acinetobacter* radio resistance under alkaline conditions in the presence of n-hexadecane was evaluated (Chen *et al.* 1998). *Acinetobacter* strain has been isolated from a variety of sources, including, soil (Bompensieri *et al.* 1986) and water (Blaise and Armstrong, 1973).

Soil microorganisms have been the most important source that has found many applications in the fields of medicine, Pharmacy and agriculture etc. Most of the antibiotics use for the treatment of various infectious diseases is microbial products (Tawiah *et al.* 2012). Studies of soil microorganisms are potentially rich source of unique bioactive substance (Fenical, 1993). Since the discovery of penicillin and other antimicrobial agents by Alexander Fleming in 1928 (Fleming 1929). Some of these are morphological, physical, chemical and mineralogical, characteristics (Bhatt *et al.* 1990). The soil contains about 10% of organic matter, this have a tremendous effect on soil chemical and physical properties. It consist of organic matter such as carbon, oxygen, hydrogen, nitrogen and smaller quantities of sulphur and other elements. The impacts of soil on agricultural productivity and sustainability can be determined by analyzing the physical, chemical and biological parameters of the soil (Mohan *et al.* 2007). The microbial production of proteases is preferred more than other source because microbes can be grown and genetically modified easily. (Sharma *et al.* 2015). Protease enzyme can produce eco-friendly products and so they play a vital role in morden biotechnology industries (Abebe *et al.* 2014). The microbial proteases was laks pathogenicity. Therefore they can be grow easily in culture medium and they have wide industrial application. (Daniel *et al.* 1984). Microbial protease directly excreted production medium due to their extracellular nature, thus simplifying purification of enzyme as compared to enzyme extracted from animal and plant. (palsaniya *et al.* 2012).

Extracellular proteases catalyse hydrolysis of polypeptides into free amino acid or Smaller polypeptide chains in the outer cell environment and allow the cell to take up and utilize product hydrolysis. (Joo and chang 2005). Insoluble macromolecules such as collagen, keratin, chitin, cellulose, lignin and casein are biodegraded by microbial extracellular enzymes with the ability to act on dense substrates (Abbas *et al.* 1989, Bockle *et al.* 1995).

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