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Microbial screening: Insights into production of enzymes with emphasis on proteases

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Abstract:

Microorganisms can be isolated at random from various natural sources and tested individually for their fermentation potential, but the chances of finding one of value by this means are not very great. Only a very few members of a natural microbial population will possess the characteristics being sought. Thus, to find a microorganism that can do what we want it to do is not a simple matter.The most successful approach to finding such a microorganism is to utilize some technique that will allow the obtaining and testing of large numbers of microorganisms, but without requiring that extensive studies be carried out on each individual organism. Such a technique is available and is known as screening. To make effective use of the screening approach, one must first have access to natural microbial source that contains many different types of microorganisms, whether most of these organisms are not known to possess the biosynthetic abilities in which we are interested.Proteases are most important enzymes in industrial set up and are amongst the most studied protein. In the present scenario, the proteases are of more commercial interest than peptidases. The present article is an overview on the various aspects of microbial screening with emphasis on Proteases.

Key words: Screening, Protease, Isolation, Enzymes, Fermentation

1. Introduction

The screening technique is used in various forms, depending on the type of microorganism that is desired, on the particular product of interest and on the source from which the microorganism are

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to be obtained (Casida L. E, 1968).Soil is the best source by which one couldacquirevastset of microbes. This is because much of debris of the world finds its way into the soil. Another source, which has not yet been explored to any extent, is sea water or marine mud. Examples of other natural microbial sources are compost, rumen content, domestic sewage undergoing treatment, manure, and spoiled foodstuffs or feedstuffs.

Various workers have estimated that plate counting and isolation procedures for total numbers and types of soil microorganisms, even though utilizing the best know media and incubation conditions, probably allow less than one percent of the soil microorganisms to be grown in laboratory. If this statement is reversed, we find that 99% or greater of the soil microorganisms may not yet have been cultivated in the laboratory. Apparently, these microorganisms are waiting for someone to devise a medium and cultural condition that will allow their growth in the laboratory. Obviously, this is of interest to one who wishes to isolate organisms with new biosynthetic capabilities, because it means that at least with soil, there are many microorganisms not previously described which are just waiting to be isolated and evaluated.

Screening is associated withuse of extremely selective procedure that allows detection as well as isolation of microbes which are ofinterestamidenormous population of microbes. This process to be more effective, must be able to help in discardingseveral valueless microbes with in a step or two, while on the other hand it should allow the easy discovery of small percentage of beneficial microbes present in the population. Microorganisms have been studied extensively, but the degree of detection and unearthing of novel metabolite from terrestrial microorganisms is declining. The biological vastness of marine environment in particular alsoprovidesvastpossibilities for unearthing novel natural products.

Incorporation of pH indicating dyeviz., bromothymol blue or neutral red into agar nutrient mediumwhich is not buffered enough aids in revealing microbes that produce organic acids or amines from numerous carbon substrates.Variations in the color of indicating dye aroundvicinityof colony to a color demonstrating an acidic or basicreaction forms basis of these compounds being produced (Bergey D, 1919). For the identification of microbes adept at producing useful antibiotics, screening methodology has been extensively used using well known

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crowded plate technique. Antibiotic activity is analyzed following zone of inhibitionaround the colonies capable of producingantibiotic (Donadio S, 2002).

Screening approach can also be utilized to identify microbeswhich are able to synthesize extracellular vitamins, amino acids or other metabolites. In this case too, the microbial source is diluted and plated followed by incubation. The selection of specific test organism to be made use plays a vital role. It is imperative tohave a definite growth requirement for particular metabolite and not to other metabolites, so that formation of the particular compound is indicated by zone of growth, or at least an increase in growth pattern, of the test organism around colonies which have formed the metabolite.

Primary screening helps in the detection as well as isolation of microbeswhich possess potential industrial applications. Secondary screening follows next, to further test the abilities of as well as gatherdataonsuch organisms that may have potential. Primary screening can yield only a few microbes or many microorganisms. However, very small number of these organisms will have real commercial value as this type of screening defines which microorganisms are capable to produce a compound without providing much information on the production or yield potential of the organisms. This limitation is rather taken care during secondary screening which allows further categorizing out of those microbeswhich have potential value for industrial processes and discarding of those lacking this prospect.

2.Enzymes and microorganisms

Microbial cells contain or produce a range of enzymes which are the biological catalysts for various biochemical reactions that leads to microbial growth and respirationfollowed by formation of fermentation products. Enzymes are either adaptive or constitutive. A constitutive enzyme is always produced in amounts usable by the cell regardless of whether the particular substrate of the enzyme is present in the growth medium. In contrast, microbial cells produce adaptive enzymes in usable amounts only in response to the presence of the particular enzyme substrate in the fermentation medium; the microorganism produces an adaptive enzyme only if required to do so to bring about degradation or change in a particular nutrient substrate otherwise

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not available to the cell. Microorganisms, however, differvastly from one another in their ability to synthesize adaptive enzymes (Estell D A, 1993).

Enzymes also may be either exocellular or endocellular. An endocellular enzyme is synthesized in the cell or at the cytoplasmic membrane and normally does not find its way into the fermentation medium surrounding the cell. Exocellular enzymes, which include most of the enzymes produced by microbial fermentation for commercial usage, are also produced by the microbial cell, but in addition are liberated to the fermentation medium so that the enzyme can hydrolytically attack and degrade polymeric substances too large or insoluble to pass through the microbial cell wall, or in some other manner inaccessible to the cell.Examples of exocellular enzymes are amylases, attacking starch, and protease, attacking protein.

Enzymes are proteins and hence, they possess the physical and chemical characteristics of proteins. Thus, some enzymes are inherently unstable, their protein being easily denatured so that enzymatic activity is lost. Each enzyme has its own pH and temperature optimum for activity, although there may be more than one enzyme capable of attacking a specific substrate, but varying in these conditions of pH and temperature optima. Enzymes demonstrate specificity for the particular substrates that they attack and this property is of competitive economic advantage for the commercial usage of enzymes since, because of this specificity, the side reactions often accompanying chemical reactions usually are not associated with enzymes. Enzymes act at relatively low temperatures, that is, temperatures below those which denature the enzyme protein and not under the high temperature, catalyst-associated conditions often required for chemical reactions. Since enzymes are easily denatured, an enzyme reaction can be stopped at an opportune moment simply by denaturing the enzyme, as for instance by the application of acid, heat, etc. The protein nature of enzymes also provides an additional bonus as regards their relative ease of recovery from fermentation culture broths.

Amylase and proteases find use in food industry and trypsin in baby food. Brewing industry utilizes amylase, glucanases, proteases, β-glucanases and arabinoxylanases are used to clarify fruit juices. Cellulase finds application in the bio-fuel industry (Kumar C G, 1999).In comparison to the industrial use of enzymes, therapeutically useful enzymes are needed in relatively minute

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amounts but withhigh purity and specificity. Cost of these enzymes is usually on the higher side but is comparable to the competing agents having such therapeutic value or treatment regimen.

Enzyme production from microbial origin for various commercial purposes utilizes various fungi, bacteria and yeasts.Bacteria and fungi usually yield similar enzymes, although the particular enzymes from these sources could vary in pH and temperature optima among several other characteristics. Also, within any one broad group of organisms, such as the bacteria or fungi, there is vast variation amongdifferent genera as to their capability to produce a particular enzyme. This variability also extends to the species within a genus and even to strains within a species. In addition, the production of a particular enzyme varies with particular medium employed and with various physical and chemical factors of the fermentation. Genetic stability of the microbial strains also plays an important role and as a result, cultures must be preserved in such a manner as to maintain their enzyme-producing ability. Thus, microbial strains are carefully selected so that they possess high enzyme-yield capacity and genetic stability under the particular conditions employed in the fermentation. Microbial enzymes obtainedthrough fermentation include invertase, amylase, protease, pectinase, catalaseamongothers.

3.Proteases:

Proteolytic enzymes are produced by various bacteria, such as species of *Bacillus, Psuedomonas, Clostridium, Protues*to name a few and by fungi such as *Aspergillus niger, Aspergillus oryzae, Aspergillus flavus*and *Penicillumroquefortii.* However, the enzymes obtained from these are mixtures of proteases and peptidases, with the formertypically being excreted to the fermentation medium during growth, while the latter often are liberated on autolysis of the cells.

Proteolytic enzymes are employed in industries such as textile (Alagarasamy S, 2002), detergents, food processing, animal nutrition, pharmaceuticals, paper industry etc. The range of protease application has expanded to many streamsincluding clinical, medicinal and analytical chemistry (Negi S, 2006), Fungal proteases present a wider pH activity range than do animal or bacterial proteases, and to a certain extent this results in a wider range of uses for the fungal proteases.

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3a.Fungal proteases

Various fungi produce protease enzyme in good yield, and the commercial production of fungal protease has utilized *Aspergillus wentii, Aspergillus oryzae, Mucordelemar*and *Amylomycesrouxii*. The fungus is usually grown on wheat bran. At sporulation, the various fungal proteolytic enzymes are present in the medium and the proteases are recovered by procedures for fungal protease production, have been studied and the indications are such fermentations may become commercially feasible. Even though many commercial proteases have their origin from microbes belonging to genus *Bacillus,* fungi too display a wide variety of proteases compared to bacteria. Furthermore most fungi are usually regarded as safe (GRAS) strains which can be utilized to produce extracellular enzymesthat can be easily recovered from fermentation broth (Wu T Y, 2006). The yield of extracellular enzymes is considerablyaffected by physicochemical parameters and thus these must be optimized for maximum production of enzymes through effective optimization techniques.

3b. Bacterial proteases

Bacterial proteases offer improved potential compared with animal and fungal proteases, accounting for 20% of the world market (Debananda S, 2006). Bacterial protease production utilizes strains of *Bacillus subtilis.* The *Bacillus subtilis* strains are specially selected for high protease activity and not for amylase activity. High carbohydrate content medium is made use to stimulate protease activity as well as to suppress amylase production, although the final product does contain some amylase activity. The fermentation is incubated 3 to 5 days at 37^{o}C in pans containing a shallow layer of fermentation medium, and the harvest procedure is similar to that for bacterial amylase, except that concentration of broth is carried out at reduced pressure and temperatures of less than 40^{o}C in order to protect the enzyme from denaturation.

4. Conclusion

While it is known that many different types of microorganisms occur in the soil, it is not so evident just what portion of these organisms have, to date been isolated to pure laboratory culture.Fungi, bacteria and yeasts have been vastly used for enzyme production

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for commercial purpose. Protease class of enzymes are employed widely in pharmaceutical, detergent, food processing and animal nutrition to name a few.The value addition from such enzymes is vast and explores the possibility of its further research for its wider applications.

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