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OVER VIEW

Microbial production of glutaminase enzyme

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ABSTRACT:

Enzymes are proteins highly specific in their actions on substrates and serve as biocatalysts. They are produced by cells in order to accelerate both the rate and specificity of metabolic reactions. Microbial enzymes are known for their unique characteristics over other sources due to their easy production on a commercial scale and stability. Different microorganisms are known to produce various enzymes such as bacteria, fungi and actinomycetes which produce a variety of extra-cellular and endo-cellular enzymes. Some of these actinomycetes enzymes have been isolated from the culture filtrates or the mycelium, concentrated and purified. Others have only been demonstrated in the mycelium of the organism. However, the ability to produce a variety of enzymes may be an attractive phenomenon in these microorganisms since they are nutritionally quite versatile. Microbial L-glutaminase has recently gained more attention due to its anticancer properties, in addition to its use as a flavor enhancer in food industry by increasing the amount of glutamic acid content in the fermented food .

Keywords:
Actinomycetes, Anticancer properties, Enzymes, Glutamic acid and L-Glutaminase.

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INTRODUCTION

Enzymes are highly selective catalytic proteins produced by living cells which may or may not contain a non-protein prosthetic group (Underkofler *et al.,* 1958).

Actinomycetes are considered to be preferred enzymes sources due to their production of extracellular enzymes. They are highly diverse group with numerous members representing important source of microbial enzymes. Actinomycetes genera are differentiated from each other based on morphological, biochemical, and physiological criteria. They act as decomposers of complex animal and plant materials resulting in release of simple substances, especially carbon and nitrogen which is easily utilized by other organisms, thus performing a vital role in life cycle. Due to their significant biochemical activities, Actinomycetes are used in commercial production of various substances such as antibiotics and enzymes (Waksman, 1950).

Because of its industrial and pharmaceutical applications, intensive research was conducted on L-glutaminase recently. L-glutaminase is produced by various terrestrial microorganisms such as *Pseudomonas* sp., *Acinetobacter* sp., *Escherichia coli*, *Bacillus* sp., *Hansenula* sp., *Candida* sp., *Aspergillus oryzae* and *Beauveria bassiana* (Sabu, 2003). Also few marine microorganisms such as *Micrococcus luteus, Vibrio cholera* and *Pseudomonas fluorescens* were reported to produce the enzyme (Chandrasekaran, 1997).

Definition

L-glutaminase is classified as an amidohydrolase enzyme which acts upon amide bonds of L-glutamine generating L-glutamic acid and ammonia. It is present in both microorganisms and mammalian tissues (Ohshima *et al.,* 1976b). Microbial sources of glutaminase showed a great role in various applications such as its use in fermented foods precisely in soy sauce and other related types, in addition to its use as anticancer agent which act by inhibition of glutamine utilization by the cancerous cells resulting in selective starvation of cancerous cells and their possible death (Santana *et al.,* 1968).

Glutaminase Producing microorganisms

Different types of organisms were reported to produce glutaminase enzyme. However, The selection of the right organism is very critical to obtain high yield of the required enzyme (Akujobi *et al.,* 2012). L-glutaminases from *E. coli, Pseudomonas* sp., *Bacillus* sp., and *Clostridium welchii* have been isolated and well studied (Wade *et al.,* 1971). In addition to these bacterial sources, the fungus Aspergillus oryzae showed a great ability to produce this enzyme. Among microorganisms, actinomycetes are widely recognized as preferable L-glutaminase sources because they generally produce extracellular enzymes, which facilitate the enzyme recovery from the fermentation broth such as glutaminase from *Streptomyces rimosus* (Sivakumar *et al.,* 2006).

Microbial Glutaminase Characteristics

Temperature is considered to be an important factor affecting the enzyme stability, The optimum temperature recorded by many glutaminases ranged from 40-50ºC. However, the temperature stability of glutaminase I (Micrococcus glutaminase) of *M. luteus* could be increased by the addition of 10% NaCl (Moriguchi *et al.,* 1994). The optimum temperature for *A. oryzae* glutaminase was around 37-45ºC and remained stable at up to 45ºC and the enzyme was completely inactive at 55ºC (Nakadai and Nasuno, 1989).

It is interesting that the exposure of *E. coli* glutaminase B to cold resulted in a reversible inactivation of enzymatic activity, while subsequent warming to 24ºC restored the activity. There was no difference in the molecular weight of the cold inactivated enzyme and the warm activated enzyme. The conformational changes which probably occur upon exposure to cold resulted from a weakening of the interaction among hydrophobic groups in the protein (Chou *et al.,* 1993).

The salt-tolerance of glutaminase is an important parameter in industrial processes that include high-salinity. It was reported that the high-salt concentration (nearly 3 M NaCl) used in the process of soy sauce fermentation resulted in remarkable inhibition of the koji mold (*A. oryzae*) Glutaminase (Koibuchi *et al.,* 2000).

Methods Used for Microbial Glutaminase Production

Two methods are known for the production of microbial glutaminase.

Submerged (Liquid) Production Method

In this method, the sterile media together with the enzyme producing organism were introduced into large fermentors (Tanks) followed by constant mixing and supply of sterile air (Schuegerl *et al.,* 1991). Actinomycetes glutaminases showed a high salt tolerance in this production method. Reports showed that *Streptomyces rimosus* isolated from estuarine fish recorded high salt tolerance and the highest enzyme production obtained at temperature 27ºC, pH 9.0 and both glucose and malt extract proved to be the best carbon and nitrogen sources for maximum enzyme production (Imada *et al.,* 1973).

Surface Production Method

This method includes the use of solid support on which microorganisms are grown. Surface production method (solid state fermentation) showed 25 to 30 fold increase in enzyme production when compared with submerged production (Sabu *et al.,* 2000b).

Wheat bran was found to be a favorable support for microorganisms in the process of glutaminase production (Kashyap *et al.,* 2002). In addition to wheat bran many other solid supports showed high efficiency in the enzyme production such as ground nut cake powder, copra cake powder and sesamum oil cake (Prabhu and Chandrasekaran, 1995). Polystyrene beads, supported by mineral salts and glutamine are another form of solid supports used for the enzyme production.

By using this method it was found that L-glutaminase producing marine *alkalophilic Streptomyces* sp. SBU1 which was isolated from Cape Comorin coast, India gave highest enzyme production after 4 days of incubation and at 14% Corn steep liquor (Krishnakumar *et al.,* 2011).

Applications

L-glutaminase has received great attention due to its valuable applications in several fields especially in medicine and its use as an anticancer agent either alone or together with any other agents is known as enzyme therapy, In addition to its role as flavor enhancer by increasing the glutamic acid content of food. Also glutaminase applications extend to the enzyme utilization as biosensor in analytical purposes by measuring the levels of L-glutamine and finally in the manufacture of fine chemicals such as theanine when used with baker's yeast.

Glutaminase as Enzyme Therapy

Glutaminase can be used as alternative for cancer treatment as enzyme therapy. The mechanism for glutaminase therapy includes that L-Glutaminase act on its substrate (L-glutamine) and breaks it down leading to the selective destruction of the tumor cells accompanied by inhibition of both protein and nucleic acid biosynthesis due to glutamine starvation and this is attributed to the inability of cancerous cells to synthesis glutamine (Tanaka *et al.,* 1988). This is due to the fact that some types of cancerous cells utilize glutamine greatly (Lazarus and Panasci, 1986). Concerning this finding various enzymatic therapies developed to deprive L-glutamine to cancerous cells (Roberts *et al.,* 1970).

Glutaminase as Flavor Enhancer

Glutamate is a famous amino acid and considered as a natural constituent of many fermented or aged foods, such as soy sauce, fermented bean paste and cheese (O`Mahony and Ishi, 1987). It gives these types of food their desired taste (Chou and Hwan, 1994). Glutamate (Glutamic acid) accumulated in these food types as a result of protein hydrolysis by proteolytic enzymes such as glutaminase and protease have a vital role in food industry (Tambekar and Tambekar, 2011).

Glutaminase as biosensor

L-glutaminase is used as biosensor to monitor the L-glutamine levels in body fluids. This technique is more applicable than previously used methods and characterized by its high specificity compared with cell based sensors in addition to its fast response. This has led to intensive use of glutaminase in clinical purposes especially that is derived from mammalian tissues.

Glutaminase and Manufacture of Various Chemicals

Theanine (γ-l-glutamyl ethylamide) is synthesized by theanine synthetase (EC 6.3.1.6) in plants and known for its capability to inhibit stimulation by caffeine, in order to enhance the effects of the anticancer agents. Bacterial glutaminases together with baker's yeast are used to produce theanine (Tachiki *et al.,* 1998). Also L-glutaminase is used in the manufacture of γ –glutamyl alkamides by the transfer of γ-glutamyl from a donor molecule such as glutamine or glutathione to a glutamyl acceptor like ethylamine or glycyl glycine by catalysis.

Conclusion

Due to their important applications, Microbial glutaminases gained much attention among the commercially important enzymes. Their role in the biotechnological industries, in addition to their medical applications as anticancer agents created the need for searching of high potential microorganisms strains. The advantages of the microbial glutaminases - such as their stability and large scale production - over other sources made microorganisms represent a desirable source for the enzyme production. This brief review revealed the microbial sources of the enzyme and its characteristics, in addition to the production methods and extended to its various applications.

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