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Microbial L- Asparaginase an Enzyme with Encrypted Potential

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Many enzymes have been used as drugs like wise L-asparaginase attracted much attention because of its use as effective therapeutic agent against lymphocytic leukemia and other kinds of cancer in Human beings. Apart from medicine L-asparaginase also find applications in food industry. Though much has been unraveled still there may be much more encrypted in this enzyme as it's potential.

Key Words: Biocatalyst, Actinomycetes, Anticancer agent, Lymphoblastic, Leukemia.

Microorganisms are considered as mini factories' for the conversion of the raw materials into fine products like antibiotics, enzymes, vitamins, fatty acids etc. Commonly enzymes have been isolated and purified from microorganisms because of their broad biochemical diversity, feasibility of mass culture and easy genetic manipulation. Enzymes are biocatalyst which brings about specific biochemical reactions. It forms the part of metabolic processes of cells. L-asparaginase (L asparaginase amino hydrolase, E.C. 3.5, 11, LA) catalyses the hydrolysis of L asparagine into L-aspartic acid and ammonia.

L-asparaginase is the essential amino acid for growth of tumor cells. L-asparaginase was discovered way back in 1904 by Lang *et al*. The development of L-asparaginase as therapeutic agent began in 1953 and today it is considered as potent antitumor agent. Till 1970-80s this enzyme was looked with limited applications in cancer cure only. Later on when it was obtained from variety of sources its diversity and potential was understood. Though the discovery is very old new applications of L-asparaginase are still being discovered in many different fields.

L-asparaginase is broadly distributed among the plants, animals and microorganisms. Out of all microbes are considered as better source of this enzyme, because they can be cultured easily and its extraction and purification is also easy on large scale production.

Sources of L-asparaginase

Wide range of microorganisms like bacteria, fungi, algae and actinomycetes produce efficient amount of L-asparaginase. Gram positive bacteria received little attention so far for L-asparaginase production; hence researchers are prompted to screen large numbers of bacteria for activity.

Production of Purification of Microbial L-asparaginase

The optimization of nutritional requirements and operating conditions is an important step in any bioprocess development. There have been many reports about the production of L-asparaginase under different conditions by various microbes. L-asparaginase is generally produced by either submerged fermentation (Within Fermentor) or by Solid state fermentation (On surface of Trays). Ideally glucose

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as C source, yeast extract as N source, pH 6-8, Temperature 35-37°C, Incubation time varies between24-36 hrs for different organisms exhibits maximum L-asparaginase production. L-asparaginase finds its major applications in Cancer treatments hence it demands high degree of purity of the enzyme. Purification can be carried out by Gel filtration, Ione exchange chromatography, Ammonium sulfate precipitation.

Modification and Immobilization of L asparaginase

The very less half life and high immunogenicity of native enzyme forced researchers to modify the enzyme, resulting in its increased half life and better storage stability. Modification and immobilization increases thermostability of the enzyme. Reuse of the enzyme is also possible. Immobilization is carried out by encapsulation, entrapment and covalent attachment.

Table 1. List of major L-asparaginase producing microorganisms.

Microorganisms	Discovered by & Year
Achromobacteriaceae	Roberts J & *et al*,1972
Citrobacter	Bascomb S & *et al*,1975
Seratia marcescena (Nima)	Sukumaran C & *et al*, 1979
Vibrio succinogens	Radcliffe C & *et al*, 1979
Aeromonas sp	Benny KP , 1994
Alcaligens fecalis	Sakato H & *et al.*, 1970
Marinobacter hydrocarbanoclasticus	S Anil R & *et al.*, 2010
Pectobacterium caratovorum	Sanjay K & *et al.*, 2009
Thermus thermophilus	Pritsa AA & *et al.*, 2001
Erwinia sp.	Borkotaky B & *et al*, 2002
E.coli sp.	Warangkar S & *et al*., 2009
Yeast	
Candida utilis	Jo K & *et al* , 1995
Rhodosporidium toruloids CBS	Dunlop P & *et al*., 1975
Actinomycetes	
Nocardia sp.	Gunasekaran S & *et al* , 1995
Streptomyces sp.	Dhevendaran K & *et al*., 2002
Algae	
Chlamydomonas sp	Paul J , 1982
Fungi	
Aspergillus terrus	Siddalingeshwara KG & *et al*., 2011
Emericell nidulans	M Jayaramu & *et al*., 2010
Streptomyces gulbergensis	Amena S & *et al*., 2010
Medicinal Plants	
Withania somnifera L.(Ashwagandha)	Oza V & *et al*, 2011
Osimum sanctum (Tulsi)	S. M. Pradeep & *et al*., 2010a

L-asparaginase as an anticancer agent

In 1954, for the first time L-asparaginase's role as antitumor agent was deciphered and still today it is intensively studied as anticancer agent. L-asparaginase is useful for acute lymphoblastic leukemia in children and considered as therapeutic agent for many malignant tumors. L-asparaginase is required in larger quantities by tumor cells for proliferation as compared to normal cells. In the presence of L-asparaginase tumor cells are unable to absorb other important growth factors hence they fail to survive. This leads to the development of L-asparaginase as a potent antitumor and antilukemic drug. ELSPAR, ONCASPAR, ERWINASE and KIDROLASE are some of the brands of L-asparaginase, approved by FDA for

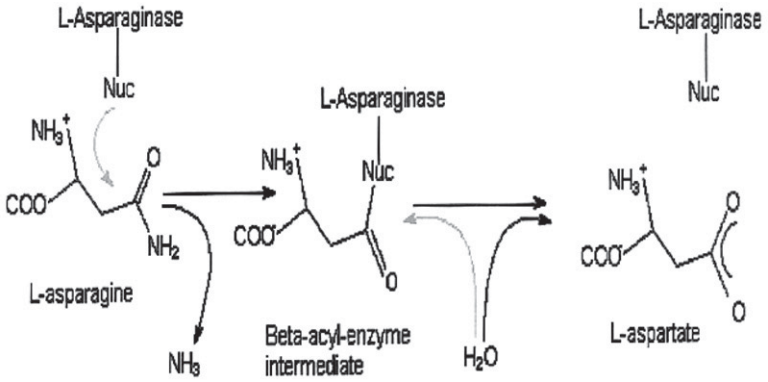

Fig. 1. Chemical reaction of L-asparaginase with structure

the treatment of acute lymphoblastic leukemia and lymphosarcoma.

Applications of L-asparaginase in food industry

Along with medicinal applications L-asparaginase also finds applications in food industry. The Acrylamide is formed from L-asparaginase and reducing sugars in carbohydrate-containing foods that are heated above 120°C. Examples of such foods include bread and other baked goods, fried or baked potato products, and flavoring agents. L-asparaginase is added to food prior the heating step. During heating, the enzyme gets denatured and thereby inactivated. It has been reported that Cancer causing acrylamide can be eliminated using gene technology to degrade L-asparaginase, the free amino acid which is precursor to acrylamide prior to baking during bread preparation. It has been reported that 57-68% reduction of acrylamide can be achieved with addition of 5% Pea flour in wheat bran and whole grain breads without any negative impact on color and sensory properties. The positive effect in reduction of acrylamide formation in French fries and other potato products has been reported in 2008.

CONCLUSION

Several groups of microorganisms have potential of L-asparaginase production and they have many practical and clinical uses. L-asparaginase constitutes one of the most biotechnologically and biomedically important group of therapeutic enzymes accounting for about 40% of the total worldwide enzyme sales. This enzyme has been successfully used for the medical treatment of leukoses and certain other malignant neoplasms. The discovery of the antitumorigenic property of L-asparaginase has contributed to the rapid development of the production of the enzyme. The largest pharmaceutical firms in the U.S.A., England, Germany and Japan are manufacturing highly purified L-asparaginase at the present time. The demand for L-asparaginase will increase several fold in coming years due to its potential industrial application as food processing aid in addition to its clinical applications.

L-asparaginase production using microbial systems has attracted considerable attention, owing to the cost-effective and eco-friendly nature. A wide range of microorganisms such as filamentous fungi, yeasts, and bacteria have proved to be beneficial sources of this enzyme. Still there is tremendous scope in screening of novel L-asparaginase sources studying their properties and applications.

Thus though much of L-asparaginase sources, properties and applications has been unraveled , still it appears that a long way to go for this amazing enzyme as currently explored L-asparaginase is making a molehill out of the mountain!!!!!!.....

REFERENCES

1. Agarwal A, Sanjay K, Verranki VD, Effect of chemical and physical parameters on the production of L-asparaginase from a newly

isolated *Serratia marcescens* SK-07, *Letters in Applied Microbiology*. 2011; **52**(4), 307–313.

2. Amena S, Vishalakshi N, Prabhakar M., Dayanand A, Lingappa K, Production, purification and characterization of l-asparaginase from *Streptomyces gulbargensis*, *Brazilian Journal of Microbiology*, 2010; **41**: 173-178.
3. Anese M, Quarta B, Frias J, Modeling the Effect of Asparaginase in Reducing Acrylamide formation in Biscuits, Dublin Institute of Technology, ARROW@DIT 2011.
4. Benny KP, Biological Properties of L-Asparaginase- School of Biosciences, Mahatma Gandhi University,Kottayam –Full thesis 1994. http://www.mgutheses.org/
5. Bascomb S, Banks GT, Skarstedt MT, Fleming A, Betthlem KA, The Properties and Large-scale Production of-Asparaginase from Citrobacter, *Journalof General Microbiology*, 1975; **91**: 1-16.
6. Ciesarová Z, Kiss E, Boegl P, Impact of L-asparaginase on acrylamide content in potato products, *Journal of Food and Nutrition Research* 2006; **45**(4), 141-146.
7. Capizzi, RL, Bertino JR, Handschumacher RE, L-asparaginase Review, Annual. *Review of. Medicine*, 1970; **21**: 433-444.
8. Dhevendaran, K, Anithakumari YK- 2002, L-asparaginase Activity in Growing Conditions of Streptomyces spp. associated with Therapon jarbua and Villorita cyprinoids of Veli Lake, South India-Fishery technology, society of fisheries technoloigists , vol 39; PART 2, pages 155-159.
9. Dhevagi – 2006, Isolation and characterization of L-asparaginase from marine actinomycetes-*Indian journal of Biotechnology*, pp 514-520.
10. Gulati R, Saxsena RK, Gupta R – 1997, Rapid plate assay for screening of L-Asparaginase producing microorganisms , *Letters in applied microbiology*,24,23-26
11. Necati B, Yilmaz N, Sener E – 2010, The effect of pea (*Pisum sativum* L.)-originated asparaginase on acrylamide formation in certain bread types, *International Journal of Food Science & Technology*-Volume 45, Issue 12, pages 2470–2476.
12. Oza V, Parmar PP , Patel DH , Subramanian RB – 2011, oning, expression and characterization of L-asparaginase from Withania somnifera L. for large scale production,Online –Spriger
13. Pedreschi F, Kaack K and Granby K -2008, The effect of asparaginase on acrylamide.
14. Roberts J, John S. Holcenberg, William C. Dolowy –1972, Isolation, Crystallization, and Properties of Achromobacteraceae Glutaminase-Asparaginase with Antitumor Activity, *The Journal opbiookal chemistry* - Vol.247, No. 1,Issue of January 10, pp. 81-90,
15. S. M. Pradeep, Mahmood R, Jagdeesh KS -*2010*, Screening and characterization of L-asparaginase producing microorganisms from tulsi (*Ocimum sanctum. L*) , *Karnataka J. Agric. Sci.*,23 (4) : 660-661.
16. S Anil R, R. Arunasri, Y. Jayachandra and M. B. Sulochana -2010, Screening of extracellular hydrolytic enzymes from marinobacter hydrocarbonoclasticus strain ak5-, *The Bioscan International Journal of Life Science*-5(1) : 97 – 99.
17. Savitri, Asthana N, Azmi W –2003, Microbial L-asparginase a potent antitumor enzyme , *Indian journal of Biotechnology*,Volume 2 :184-194
18. Taeymans D – 2005, Acrylamide update by European Food and Drink Industry,JAOAC Int,88,234-241,
19. Verma N, Kumar K, Kaur G, Anand S- 2007, L-asparaginase a promising chemotherapeutic agent, *Critical review in Biotechnology*, Volume 27: 45-62.