

ENZYME BASED ASPARAGINE BIOSENSOR FOR THE DETECTION OF ASPARAGINE  
LEVELS IN LEUKEMIC SAMPLESPunia Sandeep<sup>1</sup>, Kumar Raman<sup>1</sup> and Kumar Kuldeep<sup>2\*</sup><sup>1</sup>Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Ambala, Haryana<sup>2</sup>Department of Biotechnology, Multani Mal Modi College, Patiala, Punjab\*E mail address: [kuldeepbio@gmail.com](mailto:kuldeepbio@gmail.com) Phone: +91-175-2214108 Fax numbers: +91-175-2212049

**ABSTRACT:** Plants have been used for the treatment of different diseases since 1100 BC. *Catharanthus roseus* is one of the medicinally important plants showing anti-tumor, anti-mutagenic and anti-microbial effects. L-asparaginase is extracted by *C. roseus* and is used for the development of biosensors. L-asparaginase breaks down asparagine into aspartic acid and ammonia, thus killing the tumor cells due to asparagine starvation. In the present study the L-asparaginase was extracted from *C. roseus* and immobilized in different matrixes such as agar, soil, clay and k-carrageenan. Out of the various immobilization techniques k-carrageenan gave the fastest response time, so it was chosen for the development of asparagine biosensor. In the leukemic blood samples the asparagine concentration was  $10^{-2}$  to  $10^{-3}$  M whereas in normal blood samples it was  $10^{-5}$  to  $10^{-6}$  M. The developed biosensor gave the fast response and were sensitive and reliable.

**Key words:** L-asparaginase, immobilization, leukemia, k-carrageenan, *C. roseus*.

**INTRODUCTION**

The use of plants for the treatment of various diseases dates back to 1000 BC (Kappor, 1990). The plants have been used for the treatment of various diseases in humans and animals. *Catharanthus roseus* is one of the such medicinal plant possessing anti-tumor, anti-microbial, anti-diabetic and anti-mutagenic effects. The plants produce primary and secondary metabolites for their growth and metabolism. The plant products which are needed for the growth of plants (organic acids and amino acids) are known as primary products and those which do not participate in growth processes are called secondary metabolites (Ghasemzadeh and Ghasemzadeh, 2011). The secondary metabolites serve various functions such as oils, flavoring agents, waxes, dyes, food additives and anti-tumor agents (Hussain et al., 2012). L-asparaginase is one of the secondary metabolites produced by various plants used for the treatment of leukemia. The normal cells can synthesize asparagine (amino acid needed for brain development) on their own but the cancer cells depend on asparagine in circulating blood (Henry et al., 2011). When L-asparaginase is injected into the blood it breaks down asparagine to aspartic acid and ammonia. Thus the cancer cells die as they get deprived of asparagine supply. L-asparaginase is used in baked foods to prevent the formation of acrylamide (a potent tumor causing agent) and in the development of biosensors for the detection of asparagine levels in different samples (Mottram et al., 2002; Ciesarova et al., 2006). For the development of biosensors L-asparaginase is immobilized into different matrixes. Immobilization serves a number of advantages such as repeated use, easy recovery, mild reaction conditions, efficient handling and fast response time. In the present study L-asparaginase was immobilized in agar, soil, clay and k-carrageenan for the development of biosensor to detect asparagine levels in leukemic blood samples.

**MATERIALS AND METHODS**

The chemicals and reagents used in the study were of analytical grade. These were purchased from HiMedia and Sigma. The *C. roseus* plants were collected from different regions of North India.

### Preparation of Crude Extract

The fresh leaves of *C. roseus* were used to extract the enzyme. The leaves were given multiple washing with distilled water and then homogenized with 0.15 M KCl buffer. The mixture was centrifuged at 8000 rpm at 4°C and the supernatant was taken as crude extract. This crude extract was stored at 4 °C until further use (Bano and Sivaramkrishnan, 1980).

### Immobilization of the Crude Extract

The crude extract was immobilized in agar, sand and k-carrageenan. For immobilization of crude L-asparaginase into the agar cakes 4% of agar was dissolved into water, heated and then cooled to 45-50 °C. To this 0.5 IU/ml of enzyme (20µl) was added alongwith 10 µl of phenol red indicator and poured into the petri plates. It was allowed to solidify and then cut into small pieces of 1.0 x 1.0 cm<sup>2</sup> (Mahajan et al., 2010).

The soil samples were taken from about 15 cm depth and were sieved through 2 mm mesh. The sample was allowed to separate into fine clay particles and coarse soil particles by sedimentation. After that the soil was treated with H<sub>2</sub>O<sub>2</sub> (30 %) for the removal of soil organic matter. 0.5 M CaCl<sub>2</sub> solution was added to the soil sample and then washed repeatedly with distilled water for the removal of Cl<sup>-</sup> ions. The soil and clay particles were separated out and 10.0 mg/ml of each were dispersed into double distilled water (ddH<sub>2</sub>O). They were subjected to ultrasonication at 100 W for 3 min. 0.5 ml of the soil and clay suspension was taken and 1.0 ml of 0.5 M Sodium Borate buffer (pH 8.6) was added with vigorous stirring. To them 10 µl phenol red alongwith 20 µl (0.5 IU/ml) of crude enzyme was added and final volume was made to 5 ml using ddH<sub>2</sub>O. The mixture was centrifuged at 19,000 g after shaking at 300 rpm at 25 °C for 3 hrs (Yan et al., 2010).

k-carrageenan solution (4 % w/v) was prepared and 18 ml of it was separated out. To this 0.5 IU/ml (20 µl) crude enzyme alongwith 10 µl phenol red was added and allowed to solidify in petri plates (Ellaiah et al., 2004). The k-carrageenan was cut into 1.0 x 1.0 cm<sup>2</sup> pieces. Asparagine concentrations were prepared from 10<sup>-10</sup> to 10<sup>-1</sup> M and the immobilized enzyme was added into each concentration. The response time for each technique was noted individually.

### Detection of Asparagine Levels in Normal and Leukemic Blood Samples

Out of the above techniques, the one which gave the fastest response was chosen for the development of asparagine biosensor. The k-carrageenan pieces were put into blood samples and their response time was noted.

## RESULTS AND DISCUSSIONS

### Immobilization of Crude Extract

The detection limit obtained using immobilized L-asparaginase was 10<sup>-10</sup> to 10<sup>-1</sup> M. The response time for L-asparaginase immobilized in agar was 14-7 sec and for k-carrageenan it was 10-3 sec. The L-asparaginase immobilized in soil and clay gave the response time of 12-4 and 11-3 sec respectively (Figure 1).

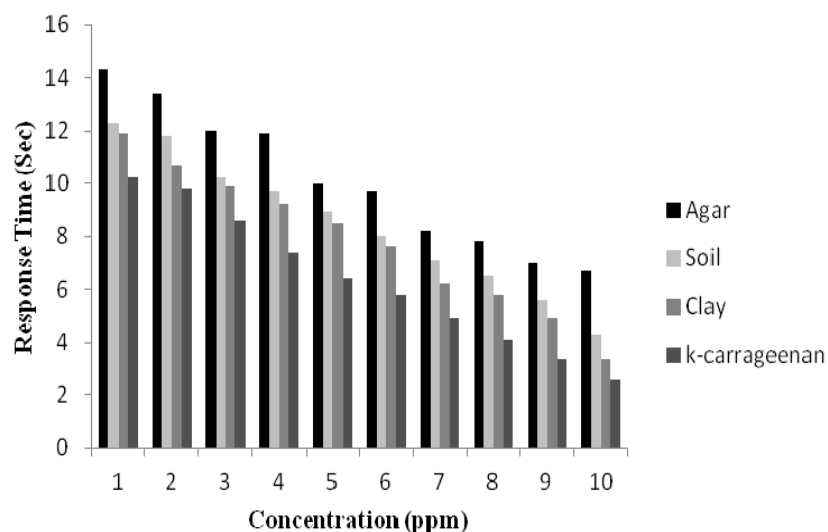


Figure 1: Response Time of Immobilized L-asparaginase



Figure 2: Immobilization of L-asparaginase in k-Carrageenan

### Detection of Asparagine Level in Normal and Leukemic Blood Samples

Out of the above applied techniques k-carrageenan showed the fastest response time so it was used for the development of asparagine biosensor. The normal and leukemic blood samples were taken and k-carrageenan pieces were put into the asparagine concentrations ( $10^{-10}$  to  $10^{-1}$  M). For the normal blood samples the asparagine concentration was  $10^{-5}$  to  $10^{-6}$  M and for leukemic samples it was  $10^{-2}$  to  $10^{-3}$  M (Figure 3). Asparagine biosensors were developed using L-asparaginase extracted from *Capsicum annum* (Kumar and Walia, 2012), *Withania somnifera* (Kumar et al., 2012), *Citrus lemon* (Kumar et al., 2013), *Cannabis sativa* (Pathak et al., 2014) and *Catharanthus roseus* (Punia et al., 2015) for the detection of asparagine levels in blood samples. The normal blood sample had  $10^{-4}$  to  $10^{-6}$  M asparagine concentration and in leukemic samples was  $10^{-2}$  to  $10^{-3}$  M.

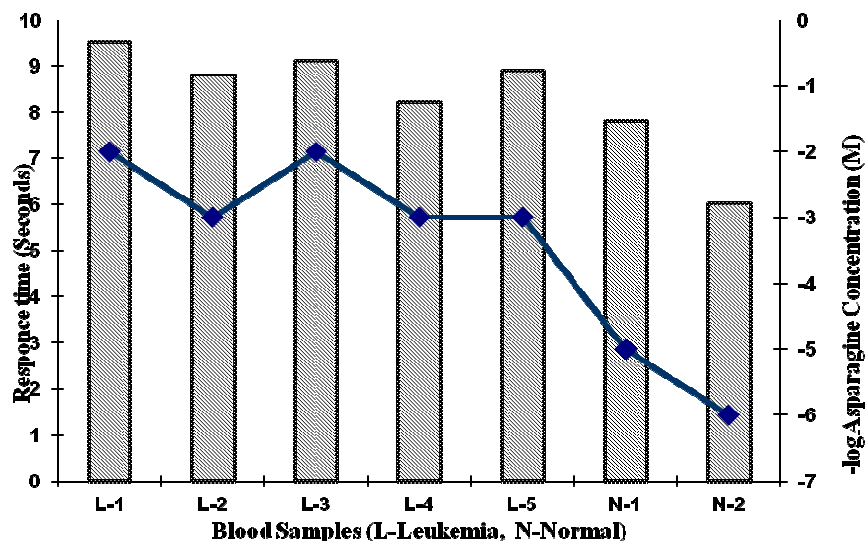


Figure 3: Asparagine Concentration in Normal and Leukemic Blood Samples

### CONCLUSION

L-asparaginase was extracted from fresh *C. roseus* and used for the development of asparagine biosensor to detect asparagine levels in normal and leukemic samples. The normal blood samples contained  $10^{-5}$  to  $10^{-6}$  M asparagine and leukemic samples had  $10^{-2}$  to  $10^{-3}$  M. The developed biosensor was reliable, sensitive and gave fast response time.

### ACKNOWLEDGEMENT

The authors wish to thank Modi Education Society and Dr. Khushvinder Kumar, Principal, M.M. Modi College, Patiala for encouragements.

## REFERENCES

- Bano M. and Sivaramakrishnan V.M. (1980). Preparation and properties of L-asparaginase from green chillies (*Capsicum annum* L.). *Journal of Biosciences*: Vol.2 (4), 291-297.
- Ciesarova Z, Kiss E. and Boegl P. (2006). Impact of L-asparaginase on acrylamide content in potato products. *Journal of Food and Nutrition Research*: Vol.45 (4), 141-146.
- Ellaiah P, Prabhakar T, Ramakrishna B, Taleb A.T. and Adinarayana K. (2004). Production of lipase by immobilized cells of *Aspergillus niger*. *Process Biochemistry*: Vol.39 (5), 525-528.
- Ghasemzadeh A. and Ghasemzadeh N. (2011). Flavonoids and phenolic acids: role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*: Vol.5 (31), 6697-6703.
- Henry L.K, Iwamoto H, Field J.R, Kaufmann K, Dawson E.S, Jacobs M.T, Adams C, Felts B, Zdravkovic I, Armstrong V, Combs S, Solis E, Rudnick G, Noskov S.Y, DeFelice L.J, Meiler J. and Blakely R.D. (2011). A conserved asparagine residue in transmembrane segment 1 (TM1) of serotonin transporter dictates chloride-coupled neurotransmitter transport. *Journal of Biological Chemistry*: Vol.286, 30823-30836.
- Hussain M.S, Fareed S, Ansari S, Rahman M.A, Ahmad I.Z. and Saeed M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences*: Vol.4 (1), 10-20.
- Kappor, L.D. (1990). *CRC handbook of ayurvedic medicinal plants*. Boca Raton, Florida, CRC Press, pp. 416-417.
- Kumar K, Kataria M. and Verma N. (2012). Plant asparaginase-based asparagine biosensor for leukemia. *Artificial Cells Blood Substitute and Biotechnology*: Vol.41 (3), 184-188.
- Kumar K, Punia S, Kaur J. and Pathak T. (2013). Development of plant asparagine biosensor for detection of leukemia. *Journal of Pharmaceutical and Biomedical Sciences*: Vol.35 (35), 1796-1801.
- Kumar K. and Walia S. (2012). L-asparaginase Extracted From *Capsicum annum* L and Development of Asparagine Biosensor for Leukemia. *Sensors & Transducers Journal*: Vol.144 (9), 192-200.
- Mahajan R, Gupta V.K. and Sharma J. (2010). Comparison and suitability of gel matrix for entrapping higher content of enzyme for commercial applications. *Indian Journal of Pharmaceutical Sciences*: Vol.72(2), 223-228.
- Mottram D.S, Wedzicha B.L. and Dodson A.T. (2002). Acrylamide is formed in the Maillard reaction. *Nature*: Vol.419, 448-449.
- Pathak T, Kumar R, Kaur J. and Kumar K. (2014). Isolation of L-Asparaginase from *Cannabis sativa* and Development of Biosensor for Detection of Asparagine in Leukemic Serum Samples. *Research Journal of Pharmacy and Technology*: Vol.7 (8), 850-855.
- Punia S, Kaur J, Kumar R. and Kumar K. (2015). Potentiometric biosensor for asparagine detection. *International Journal of Research in Ayurveda and Pharmacy*: Vol.6, 282-284.
- Yan J, Pan G, Li L, Quan G, Ding C. and Luo A. (2010). Adsorption, immobilization and activity of  $\beta$ -glucosidase on different soil colloids. *Journal of Colloid and Interface Science*: Vol.348, 565-570.



ISSN : 0976-4550

# INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY



Email : [ijabpt@gmail.com](mailto:ijabpt@gmail.com)

Website: [www.ijabpt.com](http://www.ijabpt.com)