Developing L-Asparaginase with Low Glutaminase Activity for Therapeutic Applications

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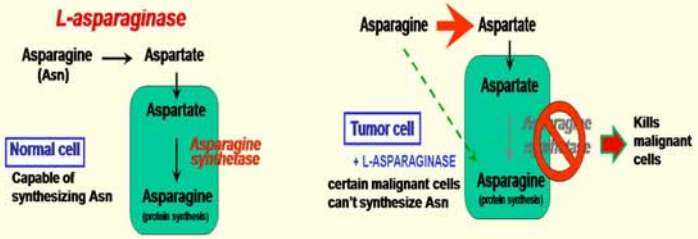
Why L-Asparaginase is important?

L-asparaginase (L-asparagines amidohydrolase EC 3.5.1.1) has the physiological function of hydrolyzing amide group of the side chain in L-asparagine to produce L-aspartate and ammonia. It (ASNase) inhibits protein synthesis in T-cells by catalyzing the conversion of L-asparagine to L-aspartate and ammonia, and this catalytic reaction is essentially irreversible under physiological conditions.

This spectacular property of the enzyme is its use in chemotherapeutic treatment of certain kinds of lymphoblastic malignancies, mainly in **acute lymphoblastic leukaemia** (ALL) and **lympho-sarcoma** (Lee *et al.*, 1989; Verma *et*



Mode of action of L-Asparaginase

At present, only 3 main preparation of asparaginase are used in treatment (*E. coli* asparaginase, its PEGylated form and *Erwinia* asparaginase).

However, the use of this enzyme involved huge expenses coupled with a high demand. Therefore, a great deal of interest has emerged in studying the possibilities of harnessing potential microorganisms that house this enzyme.

Limitation of commercially available L-Asparaginase

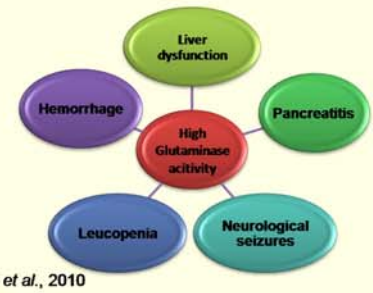
Erwinia chrysanthemi

E. coli

Source: http://www.uspharmacy1.com/anti-cancer-medicines.html

Drug from these sources, however, is strongly immunogenic and associated with side effects (Godfrin and Bertrand, 2003).

Problems: High doses of L-Asparaginase also reduces the level of glutamine in serum due to its **Glutaminase activity** (limiting factor for its use as a drug). Therefore, it is desirable to search for **robust/engineered** L-asparaginase with novel properties that can produce an enzyme with less adverse effects.



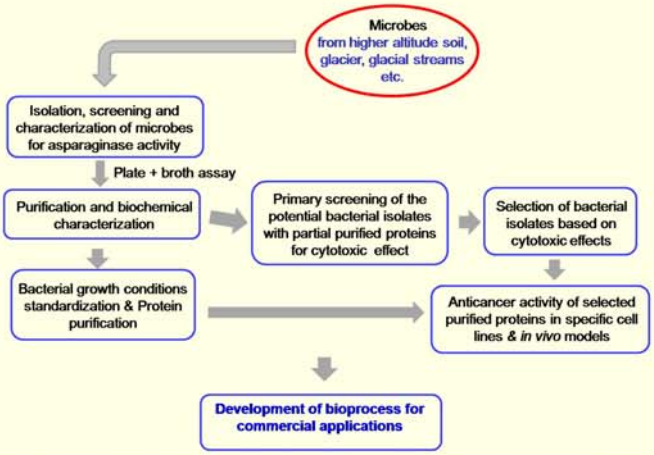
Duval *et al.*, 2010

CSIR-IHBT Positioning

An efficient enzyme from Himalayan microbial source which has new important therapeutic function with low toxicity has been discovered.

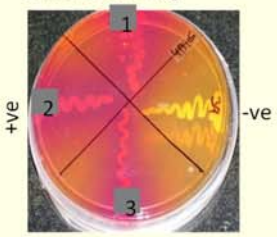
. More than 300 pure bacterial isolates from higher altitude regions of western Himalayas has been screened for extracellular L-asparaginase production.

. 24 isolates were potentially highly active.



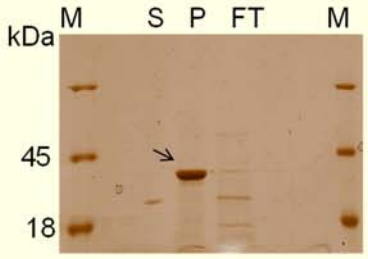
Flow diagram showing various activities to develop L-Asparaginase with Low Glutaminase Activity

Bacterial screening



Highly efficient bacterial positive (pink color) and negative (yellow color) for Asparaginase activity are shown.

Protein purification



Purified protein (~37 kDa) on SDS-PAGE is shown. M, protein mol weight marker; S, supernatant; P, purified protein; FT, flow through from column.

Companies currently marketing L-Asparaginase drug

1. ALPHARMA PHARMACEUTICAL, New England Ave, Piscataway Township, NJ 08854, United States.
2. MEDAC PHARMA Upper Wacker Dr #704, Chicago, IL 60606, United States.
3. FIRST PHARMACEUTICAL, Malaysia.
4. EUSA PHARMA, Breakspear Park, Breakspear Way, Hemel Hempstead HP2 4TZ, United Kingdom.
5. CELON, Plot No. 264, HUDA Techno Enclave, Patrika Nagar, Madhapur, Hyderabad, India

Business potential:

World enzyme market will grow $6.2 billion by 2020 and 40% will account for therapeutic enzymes. Asparaginase contribute to 1/3 (~$82 crores equivalent to 5576 crores INR) of total sales.

It is estimated to be $57,893/30 weeks (~40,00,000 INR) treatments and cost gets double in case of allergic response. EUSA's company improved Asparaginase drug Erwinaze cost about $150,000 to $175,000 (more than one crore) for a full course of treatment. Sigma company sell 1000 units of Asparaginase in the powder form with a price of more than 90,000 INR.

L-Asparaginase is a well known enzyme for its chemotherapeutic properties. This drug has been already approved by FDA, USA in the year 2011 made by EUSA Pharma (USA). The currently available Asparaginase drug derived from *E. coli* and *Erwinia* has hypersensitivity reactions along with other associated side effects.