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Production, Purification and Applications of Microbial L-Glutaminase

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ABSTRACT

Glutaminases are amidohydrolases that are produced by a wide range of microorganisms such as bacteria, yeast, and fungi. They catalyze the degradation of glutamine into glutamic acid. This unique property of glutaminases forms the basis for their applications in many industries, including pharmaceuticals and food fields. Microbial glutaminases are more significant than animal-derived glutaminases because they are more efficient, quick, stable, and compatible with downstream processes. Microbial glutaminases are expected to have high potential in the following areas: anti-cancer, antiviral and antioxidant therapy, oriental food flavor enhancers, biosensors and nutritional theanine production. This review focuses on glutaminase production and advances in their applications and how it can be optimized and purified.

Keywords: microbial glutaminase, glutamine, production, applications, anticancer.

1. Introduction

The most found amino acid in the human body is glutamine, which makes up 20% of all free amino acids in blood (Cruzat *et al.,* 2018). It is a non-essential amino acid for humans, but under catabolic stress circumstances brought on by trauma, burns, extreme exercise, and other diseases like cancer, it can become conditionally essential (Lacey & Wilmore, 1990).

Glutamine is involved in the metabolism of cells by providing the nitrogen necessary for the metabolism of different nitrogenous metabolic substrates (Sathish & Prakasham, 2010). Glutamine also plays a role in the maintenance of immune function, acid-base balance of bodily fluids, and metabolism of many metabolites (Altman *et al.,* 2016). Cells take up glutamine from the bloodstream or create it from de novo using glutamine synthetase (GS). GS is an enzyme that uses adenosine triphosphate (ATP) to convert glutamate and ammonia into glutamine (Kim *et al.,* 2021). GA is encoded by the Gls and Gls2 genes (Eagle, 1955), they are abundantly produced in the liver, kidney, skeletal muscle, and brain (Watford, 2000).

L-glutaminases are members of the amidohydrolase family, a phosphorylated enzyme that catalyzes glutamine hydrolysis to glutamic acid and glutamic ammonia by cleavage of the c-amide side chain, this enzyme has been isolated from all three life domains including prokaryotes and eukaryotes (Orabi *et al.,* 2020) (Figure 1).

In 1956 AD, research on the enzyme glutaminase was initiated by the accidental discovery of its relevance by Alexander B. Gutman and Tsai–Fan (Gutman & Yü, 1963). Subsequently, the interest in glutaminase began when its antitumor properties were discovered (Bauer *et al.,* 1971). In addition to its therapeutic role, L-glutaminase has also gained significance in the food industry as a flavoring agent (YOKOTSUKA, 1985). As a result, glutaminase has recently gained widespread recognition and its applications have broadened with the development of biotechnology (Figure2).

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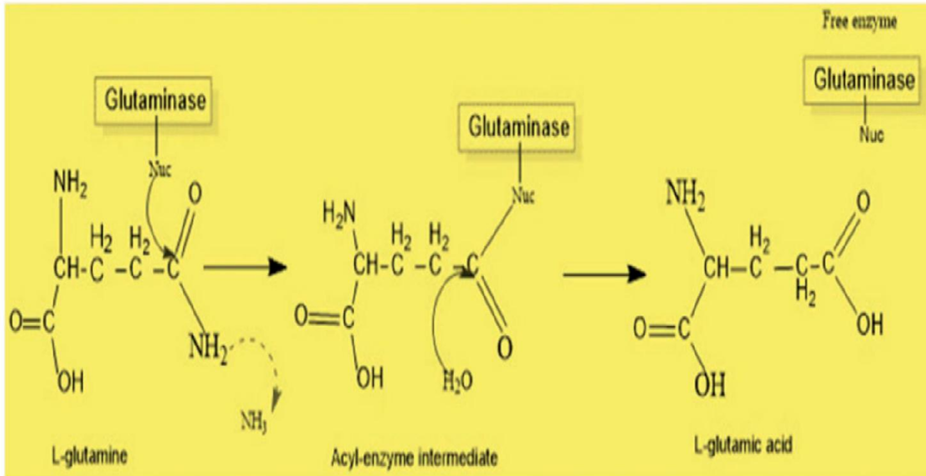

Fig. 1: Reaction mechanism of L-glutaminase (Amobonye *et al.,* 2019)

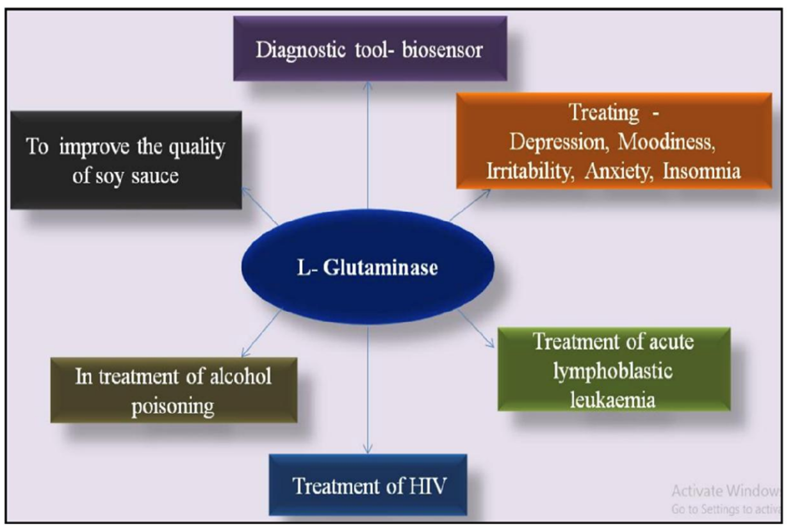

Fig. 2: L-glutaminase Applications (Prajapati & Supriya, 2017)

2. L-Glutaminase Sources:

Microorganisms like bacteria fungi and yeast, as well as macro-organisms, contain the glutaminase enzyme. Thus, glutaminases are ubiquitous in nature (Ardawi & Newsholme, 1983). Animals are not well-studied in the field of enzyme isolation from their tissues due to their complex organization (Yano *et al*., 1988). There is little evidence of plant glutaminase extraction due to less viable approaches (Yang *et al*., 2017). Microbes are the primary source of enzymes because they produce enzymes quickly, cheaply, gently, and are compatible with downstream extraction and purification steps (Saleem & Ahmed, 2021). (Table.1).

2.1. Bacterial Sources

Bacteria are widely considered to be the primary source of glutaminases, as the majority of commercially available glutaminases are derived from bacteria (Pandian *et al.,* 2014; Sathish *et al.,* 2018). Numerous bacterial genera play a role in the synthesis of glutaminases both extracellularly and intracellularly, including Bacillus sp., Pseudomonas, Actinobacterium and Escherichia coli (Klein *et al.,* 2002; Wakayama *et al.,* 2005).

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2.2. Actinomycetes Source

Actinomycetes are found in both terrestrial and marine environments. They are commercially important due to their ability to generate new metabolites. Actinomycetes, however, are relatively less studied as a source for L- glutaminase generation and are therefore candidates for the formation of this enzyme. Streptomyces spp. is particularly active as a substrate for L-glutaminase (Orabi *et al.,* 2019a).

2.3. Fungal Sources

The importance of fungi glutaminases as pharmaceutical agents is increased due to their stability, cost-effectiveness, and ease of production, as well as their high conceivability in the use of anticancer chemotherapy (Amobonye *et al.,* 2019). Numerous fungal species that belong to *Saccharomyces*, *Trichoderma*, *Acremonium*, *Penicillium*, and *Aspergillus* have been shown to be highly potent L-glutaminase producers with high productivity, high efficacy, and high antitumor activity against various cancer cell lines (Awad *et al.,* 2021).

Table 1: Some sources of L-glutaminase and their applications.

Source	Application	Reference
***Bacillus* sp. DV2-37**	Anticancer against human breast (MCF-7), hepatocellular (HepG-2), and colon (HCT-116) carcinoma	(Gomaa, 2022)
Halomonas meridiana	Anticancer against colorectal Cancer Cell Lines	Mostafa *et al.,* (2021)
***Tetragenococcus muriaticus* FF5302**	An aroma and flavor enhancer.	Dueramae *et al.,* (2023)
***Aspergillus versicolor* Faesay4**	Anticancer against human liver (HepG-2), colon (HCT 116), breast (MCF-7), lung (A-549), and cervical (Hela) cancer cell lines	Awad *et al.,* (2021)
***Pseudomonas* sp. RAS123**	Antimicrobial activity against Bacillus subtilis RCMB 015 (1) NRRL B-543 and Streptococcus mutants RCMB 017 (1) ATCC 25175	Elborai *et al.,* (2023)
Camel liver mitochondria	Anticancer against Hepatocellular carcinoma cell line (HepG-2)	Maharem *et al.,* (2020)

3. Enzymes Production

There are a variety of microbial systems and methods used to produce glutaminases. The two most widely used culture methods are submerged fermentation (SmF) and solid-state fermentation (SSF). The production of glutaminases under these conditions is characterized by high production volume and efficient process control (Mousumi, 2013). Commercial glutaminase production tends to be submerged fermentation, but solid-state fermentation has lots of benefits over submerged fermentation. These benefits include low water requirements, easy-to-find substrates, low production costs, and less risk of contamination (Astolfi *et al.,* 2019; Soccol *et al.,* 2017). In addition, some studies have shown that solid-state fermentation yields more product than submerged fermentation (Chahande *et al.,* 2018).

The level of enzyme production from microbial is affected by a range of physicochemical parameters, including pH and temperature, as well as media components such as inoculum size and incubation period, carbon and nitrogen source, peptone source, and NaCl source. The highest rate of glutaminase from bacteria production was observed in a medium that was supplemented with1% (w/v) glucose as carbon source, 1% (w/v) peptone as nitrogen source, 5% (w/v) NaCl, the initial pH of 7.0, at 37 °C, using 20% (v/v) inoculum size after 96 h of incubation (Gomaa, 2022). The maximum glutaminase activity of fungi is achieved when incubated at a temperature of 40 °C, with an initial pH of 8.0, a carbon source of 2% sucrose, a nitrogen source of 1.5% sucrose, and an incubation period of 6 days (Awad *et al.,* 2021).

L- glutaminase activities are measured by estimating the number of ammonia or acids that are liberated by the reaction due to glutamine hydrolysis (Orabi *et al.,* 2019b). Several methods have been proposed and published for the determination of L-glutaminase activity, the most widely used being the plat assay and the Nesslerization methods. Ammonia is estimated in the quantitative test of L-Glutaminase using the Nesslerization test. To perform the test, either the cell lysis sample (for intracellular enzymes) or the supernatant of the crude enzyme (for extracellular enzymes) is maintained at an appropriate temperature with L glutamine for 10 minutes. Subsequently, the reaction is terminated by the addition of trichloroacetic acid (TCA). Nessler's reagent is employed to evaluate the liberated ammonia, which results in a yellow color (El-Sayed, 2009; Moorthy *et al.,* 2010). The extracellular protein content in the crude enzyme is determined by using the Lowery method (Lowry *et al.,* 1951).

4. Application of L-Glutaminase

4.1. Therapeutic Application

4.1.1. Glutaminase as an antitumor agent

Enzymes have been proposed as a viable option for cancer treatment due to their biological catalytic nature. It is anticipated that the potential side effects associated with the use of enzymes for cancer therapy will be less pronounced than those associated with chemotherapy (Amobonye *et al.,* 2019).

The initial investigation into the anti-tumor properties of glutaminases was based on the finding that they were unable to synthesize glutamine, which is a metabolite essential to the survival of neoplasms but not necessary for the growth of host cells (Fernandes *et al.,* 2016).

The first study on the anti-cancer properties of glutaminases produced by the Pseudomonas spp, revealed that they inhibit the growth of the Gardner lymphosarcoma (6C3HED) and L-1210 leukemia cells (Greenberg *et al.,* 1964).

4.1.2. Antiviral activity of glutaminase

In vitro studies have demonstrated that glutaminases have antiviral properties; however, few studies have specifically highlighted their biological activity. Pseudomonas 7A glutaminase (PGA) has been demonstrated to inhibit the replication of mice retroviruses by reducing glutamine levels and inhibiting their mRNA translation, resulting in the cessation of viral replication (Roberts & McGregor, 1991).

In 2001, a patent was filed for the use of PGA in antiviral therapy as an antiretroviral agent for the treatment of certain HIV/AIDS infections. In a culture medium with a glutaminase concentration of 0.016 Lg/ ml, the virus was 50% inhibited by PGA and 100% inhibited by a higher concentration of 0.4 Lg/ ml (Roberts *et al.,* 2001).

4.1.3. Antibacterial activity of glutaminase

The enzyme is present in the protective blood cells and is associated with bactericidal activity through a mechanism dependent on glutamine to produce superoxide (Castell *et al*., 2004; Márquez *et al*., 2006). Limited but promising studies have been performed to investigate the antibacterial potential of glutaminase enzymes. The glutaminase enzyme from Penicillium citrinum has been tested against a variety of human and fish pathogens, with the highest activity being observed against the pathogens Vibrio parasahaemolysis and Edwardsiaella tarda (Sajitha *et al*., 2014).

A recent study found that the glutaminase enzyme RAS123 has high antibacterial activity against Bacillus subtilis RCMB 015 (1) NRRL B-543 followed by Streptococcus mutants RCMB 017 (1) ATCC 25175 (Elborai *et al*., 2023).

4.1.4. Antioxidant activities

In aerobic metabolism, free radicals are released and are essential for many biochemical processes (Tiwari, 2001). However, they can also play a role in the development of life-threatening diseases such as cancer, Alzheimer's disease and cardiovascular diseases (Alam *et al.,* 2013).

Antioxidant compounds are used to protect against these adverse effects by scavenging and detoxifying the free radicals (Mousumi, 2013). Various marine enzymes have been studied for their ability to scavenge free radicals, such as L-glutaminase from marine yeast Rhodotorula sp. DAMB1(Sarkar *et al.,* 2020), marine Bacillus subtilis strain JK-79 (Kiruthika & Swathi, 2019) and marine actinobacteria strain BSAIP5 (Sarkar *et al.,* 2014).

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Although the mechanism of action of the enzyme is not fully understood, it is thought that it can either donate its hydrogen atoms to free radicals or release an acidic product, glutamic acid (Amobonye *et al.,* 2019).

5. Food Applications: Chinese/Japanese soy sauce fermentation

The amino acid L-glutamate, which is released through the catalytic activity of glutaminase, is widely used in the food industry as a flavoring amino acid, such as in the production of soy sauce (Kijima & Suzuki, 2007).

Glutaminases are known to be salt tolerant, which is a desirable property in food applications because fermentation processes are conducted under high concentrations of salt (Amobonye *et al.,* 2019). These properties are commonly found in enzymes derived from marine sources (O'toole, 1997; Yulianti *et al.,* 2012).

For example, the efficacy of the enzyme L glutaminase from Bacillus amylobacillus Y-9 examined in the production of glutamic acid with applications in the Chinese soy sauce fermentation and the results of the study were positive (Ye *et al.,* 2013).

6. Manufacture of Fine-Chemicals

Theanine (N-ethyl-L-glutamine), an amino acid found in tea that is water-soluble and non-protein, is a nutraceutical that has been extensively studied. It has been shown to be beneficial for the immune system (Zhang *et al*., 2019), hepatoprotection (Gong *et al*., 2018; Williams *et al*., 2019), fat accumulation and nerve cell protection (Dubey *et al*., 2018).
Theanine can be synthesized using glutaminase, which is relatively cost-effective and has the advantage of being produced in its naturally occurring form, L-form. Theanine is produced by glutaminase, a process in which glutamine is hydrolyzed to glutamic acid and ethylamine is reacted with to form the amino acid theanine (Mu *et al*., 2015). The synthesis of Theanine is one of the most important submissions in the industry, and the most notable theanine synthesizing capability is attributed to glutaminase from Pseudomonas nitroreducens IFO 12694 (Takashi *et al*., 1996).

7. Glutaminase as biosensors

Biosensors are widely employed in the agricultural and food sectors to detect environmental contaminants. The enzyme glutaminase is commonly employed in the fabrication of biosensor systems for the quantification of glutamine levels in biological fluids or fermentation broths (Unissa *et al*., 2014). The enzyme contacts the analyte, and the biological reaction is converted into electrical signals via the sensor.

The key features of biomass sensors are stability, cost-effectiveness, sensitivity, and repeatability. A biosensor based on the glutaminase from Hypocria jecorina has been demonstrated to be a reliable biomass sensor for the detection of L glutamine levels in pharmaceutically derived glutamine powder (Albayrak & Karakuş, 2016).

8. Conclusion and Future Prospects

In recent years, glutaminase has been extensively studied in a variety of industries, including pharmaceuticals, and food, but at a much slower rate than other major industrial enzymes. One of the major obstacles to glutamine industrial applications is its relatively high cost. The current glutaminase production levels are not sufficient to support the clinical trials required to facilitate glutaminase for medical applications and other applications. One of the most hopeful future alternatives is to explore high-yield strains using conventional and metagenomic approaches. More emphasis should be put on exploring technologies to produce glutamine with enhanced properties that meet diverse industrial needs. Recent advances in biotechnology offer a fertile ground to develop glutamine enzymes, and their applications in improving human life quality will continue to grow. With the high potential glutaminase in industrial applications, glutamine is expected to be extensively researched in its various uses in the future.

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