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Proteinaceous Bacterial compounds isolated from different sources with multiple activities against cancer and microbial infection

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ABSTRACT : In recent years, global health authorities have had to deal with two significant problems : the alarming number of people suffering from cancer and the rise of antimicrobial resistance (AMR). Tremendous efforts and progress have been made towards finding a cure for cancer. Currently, bacterial proteins and peptides are important as antiproliferative agents. Some of these are already used in cancer treatment, others are in human clinical trials or studied in vitro. In this study glutaminase enzyme has been studied as anticancer and antimicrobial agent.

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INTRODUCTION

In recent years, global health authorities have had to deal with two significant problems: the alarming number of people suffering from cancer and the rise of antimicrobial resistance (AMR).

Cancer is one of the major reasons for morbidity and mortality in the world while becoming a major concern of human health in the 21st century. In the year 2018, there were about 17.0 million new cases of cancer reported along with 9.5 million deaths worldwide due to cancer (**A. Baerheim and H. Sandvik, 2018**).

Tremendous efforts and progress have been made towards finding a cure for cancer. However, numerous challenges have been faced due to adverse effects of chemotherapy, radiotherapy, and alternative cancer therapies, including toxicity to noncancerous cells, the inability of drugs to reach deep tumor tissue, and the persistent problem of increasing drug resistance in tumor cells. These challenges have increased the demand for the development of alternative approaches with greater selectivity and effectiveness against tumor cells. Uncontrolled proliferation and expeditious growth are the hallmark of cancer cells. They exhibit metabolic reprogramming to adapt to their enhanced nutrient requirements Furthermore, they become dependent on normal cells for the supply of some nutrients, mainly amino acids, and thus become auxotrophic in nature. On the contrary, normal cells manifest reduced requirements of amino acids. This amino acid requirement difference leads to metabolic susceptibility of cancer cells establishing a rationale for amino acid depletion therapy (AADT) **(Cantor et al., 2012; Tabe *et al.,* 2019; Vachher et al., 2020)**.

According to Hanahan and Weinberg, cancer cells exhibit six important changes in their own physiology: (1) self-sufficiency in signals of growth, (2) insensitivity to signals inhibiting growth, (3) resistance to apoptosis, (4) unlimited proliferative potential, (5) sustained angiogenesis and (6) metastasis. One of the available treatments for cancer is chemotherapy, which very often belongs to the main choice of treatment. Unfortunately, chemotherapy can lead to damage of healthy cells and tissues or development of drug resistance **(Raguz and Yagüe, 2008; Hanahan and Weinberg., 2011)**.

Another pandemic is hiding in plain sight. Antimicrobial resistance (AMR) is already a leading threat to global health and risks adversely affecting the environmental sustainability of the planet. The consequences of the continuing development and spread of AMR could be catastrophic (**Murray *et al.* 2022**).

Antimicrobials are agents intended to kill or inhibit the growth of microbes. They include antibiotics, fungicides, antiviral agents and parasiticides. Disinfectants, antiseptics, other pharmaceuticals and natural products may also have

antimicrobial properties (**Baquero *et al.*, 2019**).
AMR occurs when microbes such as bacteria, viruses, parasites and fungi are, or become, resistant to antimicrobial treatments to which they were previously susceptible. Antimicrobials are widely used in human and animal healthcare, and in crop and animal production (**Baquero *et al.*, 2019)**. Acquired resistance is an evolutionary response by microbes, which genetically change their DNA in such a way that they are no longer inhibited or killed by antimicrobials. AMR can be
intrinsic or acquired; the latter can occur through mutations, the acquisition of DNA from Environmental Dimensions of Antimicrobial Resistance Summary for Policymakers different microbes, or, in the case of bacteria, horizontal gene transfer (HGT) of mobile genetic elements (MGEs) (**Martínez *et al.* 2015**). Use and misuse of antimicrobials and other stressors (e.g. the presence of heavy metals and other pollutants) create favourable conditions for resistant microbes to develop). This can happen in the digestive tracts of humans and animals or in environmental media (e.g. water, sewage, soil and air). Resistant microbes can subsequently spread and be transmitted to humans, food animals, plants and wildlife because of complex interconnections across nature. There is strong evidence that antimicrobials are increasingly failing to cure infections, the pipeline of novel antimicrobials to take their place has faltered, and AMR therefore poses a significant threat to human, animal and plant health, and food security **(Wales and Davies 2015; Baquero *et al.*, 2019**; **Graham *et al.* 2019)**.

Currently, bacterial proteins and peptides are important as antiproliferative agents. Some of these are already used in cancer treatment, others are in human clinical trials or studied in vitro. Suggested division of the described proteins and peptides is shown in **Figure 1**.
Microbial enzymes that degrade amino acids find a plethora of applications in AADT because of their candid accessibility, enhanced productivity and relative ease of manipulation. Being foreign in nature, these enzymes can be unstable and elicit an immune response. So, various strategies including genetic manipulations, encapsulation, and making fusion proteins to improve the pharmacokinetic properties of these anticancer enzymes need to be worked out before administration in cancer patients. Thus, understanding cancer cell metabolism may define novel therapeutic approaches to curb tumor growth **(Pokrovsky *et al.*, 2019)**.

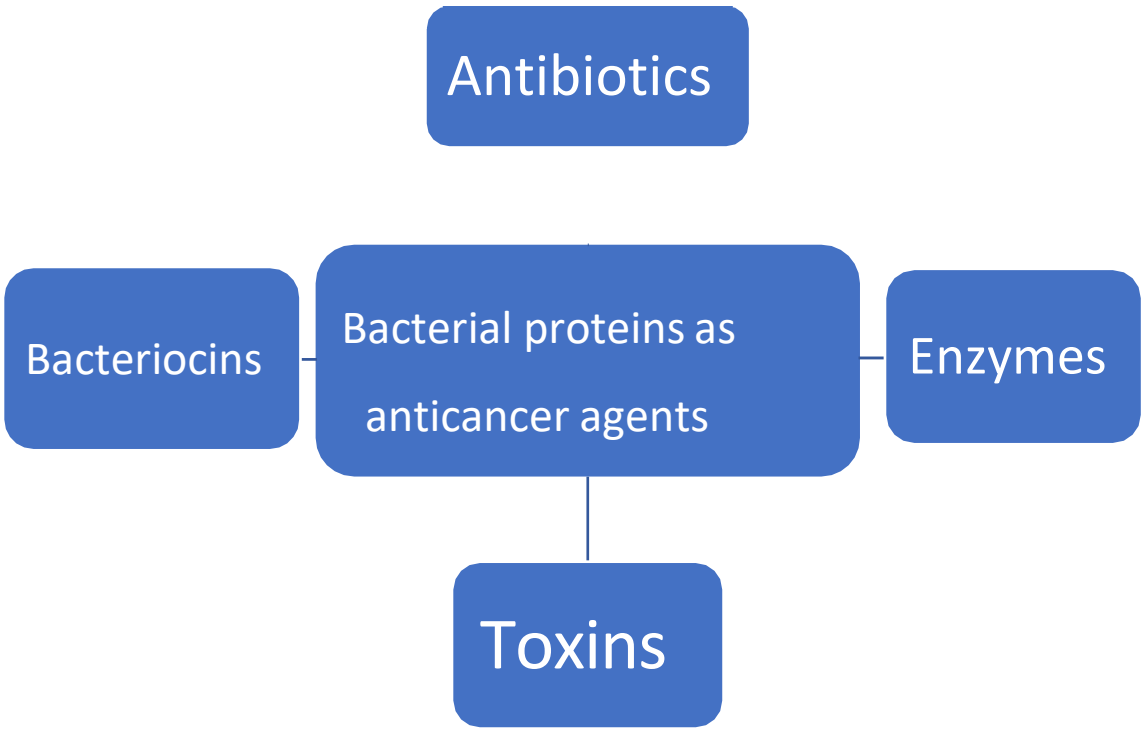


Fig (1): Anticancer proteins and peptides

1- Antibiotics

Antibiotics are the chemical compounds produced mostly by the microorganisms and injurious to other organisms from this group. It has been observed that some of the antibiotics also have anticancer activity and recently they have been used mainly as antitumor drugs **(Encyclopaedia Britannica, 2018)**.

1.1-Actinomycin D (Act D)

Actinomycin D (Act D) is a polypeptide antibiotic isolated from the genus Streptomyces. Act D intercalates into DNA, preventing the progression of RNA polymerases and causing inhibition of transcription. Nanomolar concentrations of Act D block transcription of RNA polymerase I and induce nucleolar stress by interfering with ribosome biogenesis. Act D is the first antibiotic used for treating cancer; these cancers include gestational trophoblastic neoplasia, Wilms tumor, Rhabdomyosarcoma, Ewing's sarcoma, and NPM1-mutated acute Myeloid leukemia **(Bensaude, 2011; Rao *et al.*, 2013; Falini *et al.*, 2015)**.

An FDA-approved NCI oncology drug that specifically targets and downregulates the stem cell transcription factor SRY (sex determining region Y)-Box 2 (SOX2), which leads to stem cell depletion within the tumor bulk. Furthermore, Act D has been shown to improve survival in preclinical models of recurrent glioblastoma **(Das *et al.*, 2017; Taylor *et al.*, 2020)**.

Thus, the drug has been useful in the treatment of rare and highly aggressive cancers such as HB, embryonal sarcoma of the liver, Wilms tumor, rhabdomyosarcoma, Ewing's disease, and choriocarcinoma. Recent studies have revealed that Act D is cytotoxic to LCSCs without affecting immortalized normal hepatocytes. This process occurs via the activation of p53 in an AKT-mediated mechanism **(Zsíros *et al.*, 2010; Chen *et al.*, 2014; Sato *et al.*, 2019; Song *et al.*, 2019)**.

2- Bacteriocins

Bacteriocins constitute a heterogeneous group of ribosomal synthesized bacterial peptides or proteins with antimicrobial properties. Some of them also show anticancer activity **(Kaur *et al.*, 2015; Karpi´nski, *et al.*, 2016; Mandal *et al.*, 2016; Drider *et al.*, 2016)**. There are four classes of bacteriocins secreted by Gram-positive bacteria. Group I includes antibiotics or thermostable peptides with a molecular mass below 10 kDa. Class II contains thermostable bacteriocins without lanthionine. The molecular weight of these bacteriocins is below 10 kDa. In turn, group III includes thermolabile bacteriocins with a molecular mass above 10 kDa. Class IV consists of bacteriocins

requiring the presence of lipid or carbohydrate moieties for full activity **(Karpi´nski, *et al.*, 2016; Alvarez-Sieiro *et al.*, 2016; Gomes *et al.*, 2017).**

Bacteriocins isolated from Gram-negative bacteria are microcins secreted by Enterobacteriaceae with a molecular weight below 10 kDa and plasmid- encoded colicins with a molecular weight above 20 kDa **(Karpi´nski, *et al.*, 2016)**. The origin and biological activity of anticancer bacteriocins are shown in Table 1.

Table (1): Types, sources and biological targets of bacteriocins

	in/Peptide	**ce**	**gical Target: an Cancer Cell Lines**	**ences**
	in HC5	*tococcusbovis HC5*	t adenocarcinoma (MCF-7), liver hepatocellular carcinoma (HepG2)	**r *et al.*,2015)**
	ins A andE1	*richia coli*	t carcinoma , osteosarcoma (HOS), leiomyosarcoma (SKUT-1), sarcoma (HS913T)	**r *et al.*,2015)**
	osporulin10	*bacillus sp.* SKDU10	cal cancer (HeLa), sarcoma (HT1080), lung carcinoma (H1299) t cancer (MCF-7)	**lara *et al.*, 2017)**

3-Toxins

TO produce by the bacteria damage host tissues directly at the site of bacterial infection or may spread throughout the body. Some toxins are tried to be used for therapeutic purposes **(Henkel et al., 2010).** The source and biological target of bacterial toxins with anticancer activity are presented in Table 2.

Table (2): Types, sources and biological target of toxins

	ein/Peptide	**ce**	**gical Target: an Cancer CellLines**	**rences**
	inumneurotoxintype A	*ridiumbotulinum*	ate cancer (PC-3, LNCaP) breast cancer (T47D), neuroblastom (SH-SY5Y)	**dala *etal.*, 2013)**
	theriaToxin	*nebacterium diphtheriae*	nocortical carcinoma, lastomas cutaneous T-cell lymphomas (CTCL), breast carcinom (MCF7),cervical adenocarcinoma (HeLa)	**ee *et al.*,2016; s *et al.*,2017)**
	oxin A	*domonasaeruginosa*	eatic cancer (PaCa- 2), melanomas Melmet-44, MelRM, MM200), head an necksquamous carcinomas	**pi´nski *et al.*, 2013)**
	riolysin O	*ria monocytogenes*	t carcinomas F7, SKBR-3), emia	**howiak *et al.*, 2012)**

4- ANTI-CANCER ENZYMES

Microbial enzymes find diverse applications as anti-cancerous agents. Microbial enzymes that degrade amino acids find a plethora of applications in AADT because of their candid accessibility, enhanced productivity and relative ease of manipulation. Being foreign in nature, these enzymes can be unstable and elicit an immune response. So, various strategies including genetic manipulations, encapsulation, and making fusion proteins to improve the pharmacokinetic properties of these anticancer enzymes need to be worked out before administration in cancer patients. Thus, understanding cancer cell metabolism may define novel therapeutic approaches to curb tumor growth **(Pokrovsky *et al.*, 2019)**.

GLUTAMINASE

L-Glutaminase (EC.3.5.1.2) is an amidohydrolase that catalyzes the deamidation of L-glutamine, resulting in the production of L-glutamic acid and ammonia. L-Glutaminases are ubiquitous in the biological world, and organisms ranging from bacteria to human cells produce this important enzyme. L- Glutaminase has pulled in much attention as of late for its wide application in pharmaceuticals as well as being a hostile agent toward leukemia **(Pal and Maity,1992; Iyer and Singhal, 2010)**.

L- Glutaminase exhibits its anticancer effect by depleting L-glutamine from the tumor cells, prompting their death as they are dependent on this amino acid. L-Glutaminase is actually a vital ordinary antioxidant that helps in avoiding human infections. Moreover, it is not related to lethal and cancer-causing effects like those of artificial antioxidants **(Mousumi and Dayanand, 2013; Unissa *et al.*, 2014)**.

L- Glutaminase is also used as an efficient antiretroviral agent along with its use in food industry as a flavor and

aroma-enhancing agent. Another important application of glutaminase is that it also plays an important role in biosensor as a monitoring agent for glutamine level measurement **(Roberts and McGregor, 1991; Pallem *et al.*, 2010)**.

Glutamine, one of the most abundant intracellular amino acids, plays an important role in satisfying the biosynthetic needs of proliferating cancer cells by providing carbons to produce tricarboxylic acid (TCA) cycle intermediates, glutathione, fatty acids, and nucleotides. As a result of glutamine being a major source of carbon molecules in tumor growth-facilitating metabolic pathways, many cancer cells often become "addicted" to Glutaminolysis (a rate limiting step in the TCA cycle) (**Moure *et al.*, 2001; Mousumi and Dayanand, 2013; Sajitha *et al.*, 2014)**.

Glutamine is an amino acid that plays a prominent role in cellular metabolic processes, engages in ammonia formation and glycosylation reaction, and in addition, provides the nitrogen necessary for the synthesis of various nitrogenous metabolic intermediates as nucleotides, glutathione, and hexosamine **(Binod *et al.*, 2017)**.

The rapid proliferation of colorectal cancer cells shows more nutritional requirements. The tumor cells are auxotrophic to some nutrients such as amino acids and hence it depends mainly upon the supply of these nutrients from normal cells. The glutamine-dependent colorectal tumor cells cannot survive without exogenous glutamine. The glutamine-deprivation therapy by L-glutaminase that hydrolyzes L-glutamine to L-glutamic acid and ammonia, selectively inhibits tumor growth by the blocking of de novo protein synthesis and increase of the superoxide level by oxidative stress that promotes the death of the cancer cells **(cantor *et al.*, 2012; Mustafa *et al.*, 2020; Singh *et al.*, 2013)**.

Glutaminolysis occurs via two steps, first step is catalyzed by glutaminase (GLS) and converts glutamine to glutamate. The second step converts glutamate to α-ketoglutarate (α-KG) and is catalyzed by glutamate dehydrogenase (GDH). Cancer cells addicted to glutaminolysis often rely on glutamine as the carbon source for the TCA cycle **(Roberts and McGregor, 1991; Pallem *et al.*, 2010)**.

In humans, GLS exists in two forms: kidney-type glutaminase (GLS1) and liver-type glutaminase (GLS2). While GLS1 is expressed ubiquitously, GLS2 is expressed primarily in the liver. Recent efforts have focused on targeting Glutaminolysis by inhibiting the GLS activity in cancer cells. Drugs targeting GLS activity such as BPTES (bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide 3) or CB-839 have gained attention owing to their potent inhibition of GLS1 activity and antiproliferative effect in multiple tumor subtypes including leukemia and triple negative breast cancer. Although glutamine metabolism has been shown to play a crucial role in tumorigenesis both in vitro and in vivo **(Chen and Cui, 2015; Xiang al., 2015; Altman *et al.*, 2016; Jin *et al.*,2016)**.

OCCURRENCE AND DISTRIBUTION

Glutaminase enzyme is ubiquitous in nature and reported in animals, plants, bacteria, actinomycetes, yeast, and fungi. Attempts are being made to replace enzymes, which traditionally have been isolated from animal tissues and plants to enzymes from microorganisms because microbial enzymes are cheaper to produce, more predictable, controlled, and reliable. Many bacteria synthesize extracellular and intracellular glutaminases such as *Bacillus sp., Pseudomonas, Actinobacterium sp, and Escherichia. coli*. The focal sources of fungal glutaminases are *Aspergillus sp*. and *Trichoderma sp.* **(Singh and Banik, 2013; Binod *et al.* 2017; Amobonye *et al.*, 2019; Aishwariyaa *et al.* 2020)**.

Reports showed that the majority of microbes producing L-glutaminase have been isolated from soil and aquatic (marine) environment**)**. L-glutaminases produced by terrestrial microorganisms have been reported to have some disadvantages such as unstable in extreme conditions, incompatible with human blood, and may induce a lot of side effects to patients. Thus, there is a great urgency to investigate other enzymatic sources **(Iyer and Singhal 2010; Yulianti *et al.*, 2012)**.

L-glutaminase from various microbial sources has attracted high attention in various biological activities. The anti-tumor activity of *Alcaligenes faecal*is L-glutaminase against HeLa cell line, and from *Bacillus*

cereus MTCC 1305 toward hepatocellular carcinoma (Hep- G2) cell line, were reported. L-glutaminase from *Pseudomonas 7A* has antiviral activity against retroviral disease by disruption in mRNA translation and repression of the viral replication **(Roberts *et al.*, 2001; Singh *et al.*, 2013; Pandian *et al.*, 2014)**

Also, L-glutaminase from *Bacillus amyloliquefaciens* has been used as a flavor enhancer in foods, while the enzyme from *Bacillus cereus* LC13 showed antioxidant activity with ascorbic acid. A biosensor for monitoring the L-glutamine in pharmaceutical powders was progressed by immobilizing L-glutaminase from *Hypocria jecorina* onto nanorods of zinc oxide and chitosan **(Ye *et al.*, 2013; Albayrak *et al.*, 2016; Amobonye *et al.*, 2019)**.

which include actinomycetes**,** bacteria, fungi, and yeasts are important sources of glutaminases. As expected, the glutaminases sourced from these microbes vary in their physicochemical and biochemical properties. Bacteria are considered important sources of glutaminase as most commercial glutaminases are of bacterial

origin. Bacterial glutaminases are generally produced extracellularly **(Ito *et al.*, 2011; Revanth and Raju, 2013; Pandian *et al.*, 2014; Rajan *et al.*, 2015; Kumar *et al.*, 2019)**.

MODE OF ACTION

Glutaminases have received much attention in the last decade due to their catalytic ability to deaminate glutamine to glutamic acid and ammonia. This property has made them valuable in different industrial applications. Although the precise mechanism of L-glutaminase action is still unknown, we propose a simple two-step reaction which has abeta-acyl-enzyme intermediate similar to other enzymatic hydrolytic reactions. In the first step, the nucleophilic residue of the enzyme is activated by a strong base and attacks the amide carbon atom of L- glutamine generating a beta acyl-enzyme intermediate.

This is followed by a nucleophilic attack on the ester carbon, the nucleophile is activated by a water molecule at this step (Figure 2). The same mechanism has been proposed for asparaginase, an enzyme classified in the same group of serine- prostrate and product with glutaminase. Glutaminase has shown its ability to significantly inhibit the proliferation of some cancer cell lines thus raising the possibility of its application as an alternative to chemotherapy **(Cachumba *et al.*, 2016; Irajie *et al.*, 2016; El-Gendy *et al.*, 2017)**.

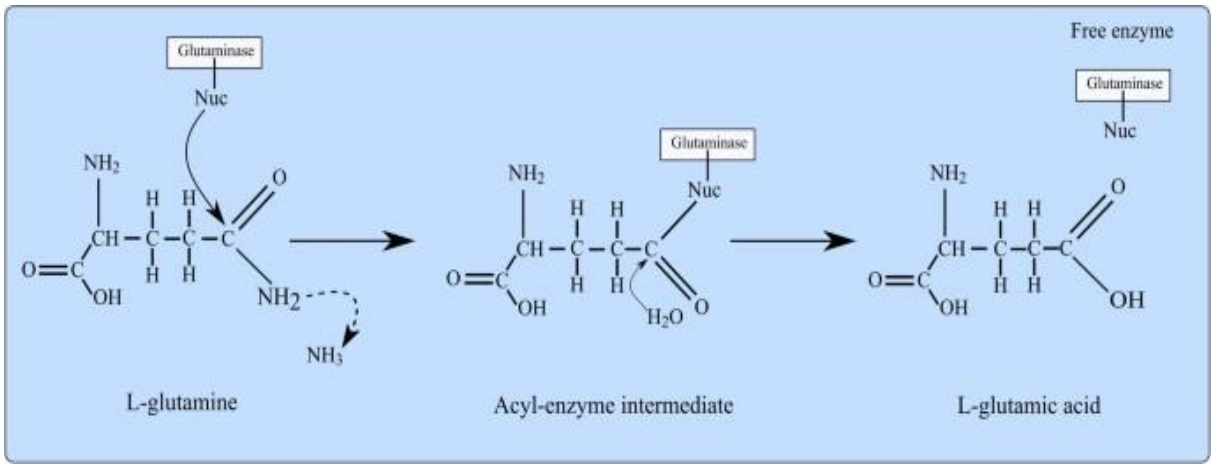


Fig (2): mode of action of glutaminase enzyme adapted from (A. Amobonye *et al.*,2019)

STRUCTURE OF GLUTAMINASES

Most structural elucidations have been conducted on mammalian glutaminases; however, some progress has also been carried out on microbial glutaminases. Glutaminases possess similar structural motifs, catalytic mechanisms, and a common evolutionary ancestor with members of serine-b-lactamases and penicillin-binding proteins (**Irajie *et al.*, 2016)**.

Hence, glutaminases are classified into this group of proteins together with asparaginases, transpeptidases, DD-peptidases, the A, C, and D serine b-lactamases. As expected, the family tree of glutaminases covered in this section highlights the divergence between two main groups. The fungal glutaminases are all grouped together in one clade 1 while clade 2 comprises glutaminases from bacteria and actinomycetes. The *Micrococcus luteus* K3 glutaminase (mglu) is the most investigated of all glutaminases owing to its distinct salt tolerance (**Yano *et al.*, 2006)**.

The peptide sequence of mglu is organized into the N-terminal, C- terminal, and the peptide linkage. The peptide sequences of other studied glutaminases like *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Corynebacterium glutamicum* , *Geobacillus kaustophilus* (308 AA), and PGA (337 AA) are all shorter compared to mglu. The N-terminal domain has been shown to be conserved among *Bacillus subtilis*, *Escherichia coli*, *Geobacillus kaustophilus*, and *Micrococcus luteus*, thus suggesting its key role in the catalytic action of the enzymes and in their close homology. The structure of four glutaminases from *Bacillus subtilis* (YbgJ and YlaM) and *Escherichia coli* (YbaS and YneH) shows significant similarity **(Weingand-Ziad et al.,2003; Brow et al., 2008; Yoshimune et al., 2010)**.

APPLICATIONS OF GLUTAMINASE

FOOD APPLICATIONS

Glutaminase has found applications in the food industry for its flavor- enhancing abilities. The content of glutamic acid in food formed on account of glutamine hydrolysis is related to the desirable tastes of oriental fermented foods like miso, sufu, and soy sauce **(Rastogi and Bhatia, 2019**; **Cho *et al.*, 2018)**.

Production of theanine

Nutraceuticals are products isolated from foods that have therapeutic benefits or provide protection against chronic diseases. Theanine (N- ethyl-L-glutamine), a water-soluble non proteinous amino acid commonly found in tea, is a well-studied nutraceutical. Theanine has been shown by different authors to improve the immune system, to be hepatoprotective, to reduce fat accumulation and protect nerve cells. Many of the positive health benefits of tea consumption have been ascribed to its theanine content **(Dubey *et al.*, 2018; Gong *et al.*, 2018; Sharma *et al.*, 2018; Williams *et al.*, 2019; Zhang *et al.*, 2019)**.

Enzymatic synthesis of theanine using glutaminase is relatively economic and has the additional advantage of synthesizing the amino acid in its naturally occurring L- form. Glutaminase hydrolyzes glutamine to glutamic acid, which then reacts with ethylamine to produce theanine **(Mu *et al.*, 2015)**.

Therapeutic applications

(i)-Antibacterial activity of glutaminase

The discovery of glutaminase in human neutrophils raised speculation about their antibacterial activity. Subcellular fractionation of the enzyme on the neutrophil cell surface showed that glutaminase was enriched in the secondary granules and could be released into the cell culture medium upon stimulation with phorbol-12-myristate- 13-acetate.

The presence of the enzyme in the defense blood cells was linked to their bactericidal action through a glutamine-dependent mechanism for superoxide production. Few, but promising investigations have been made into the antibacterial activity of glutaminase enzymes **(Castell *et al.*, 2004; Marquez *et al.*, 2006)**.

Glutaminase from *Penicillium citrinum* was tested against different human and fish bacterial pathogens and the maximum activity was observed against *Vibrio parahaemolyticus* and *Edwardsiella tarda*. It was shown that a purified glutaminase from a recombinant AYA 20–1 strain, which was constructed by intergeneric protoplast fusion from *Trichoderma sp*. Gen 9 and *Cladosporium sp*. Gen 20, had significant antibacterial activity against some strains of *Pseudomonas aeruginosa* and *Corynebacterium* xerosis NRRL B-1397 **(Sajitha *et al.*, 2014; El-Gendy *et al.*, 2017)**.

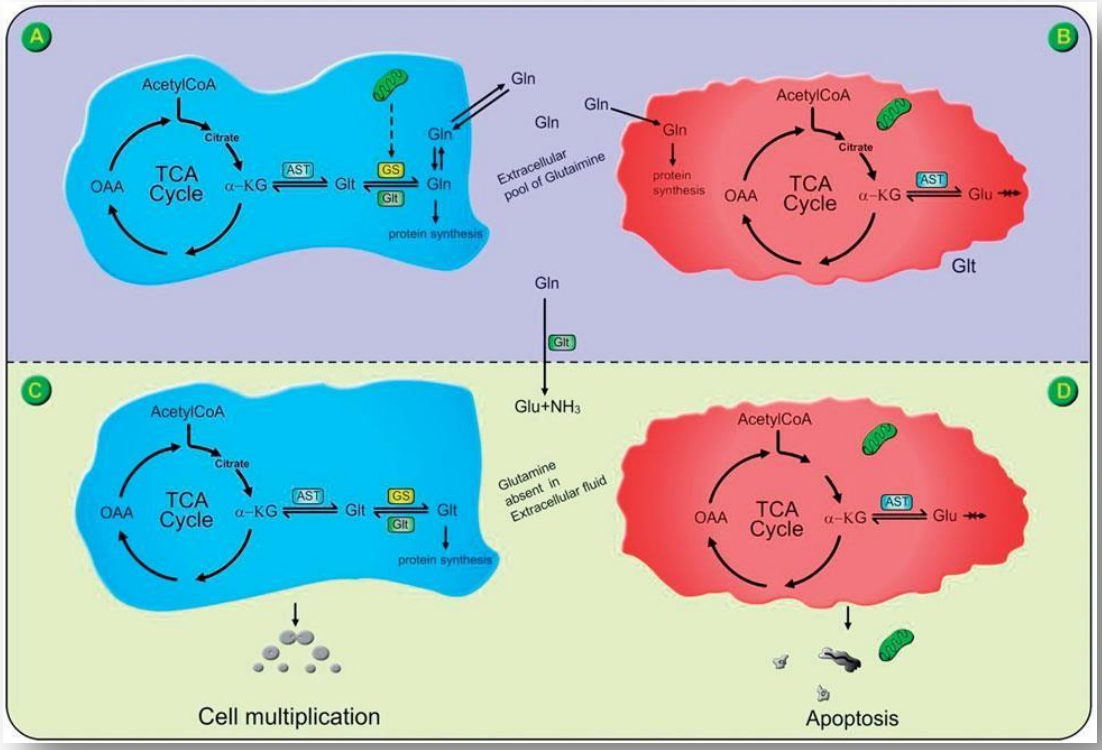
(ii)-Glutaminase as an antitumor agent

The search for better alternatives to cancer treatment has been a major area of scientific effort, and the use of enzymes has been considered one of the viable alternatives. Being a biological catalyst, it is opined that the side effects of using enzymes for cancer treatment will be lower when compared with chemotherapy. The substrate specificity of enzymes has also made them an attractive option in this regard. Glutaminase, arginase, asparaginase, tyrosinase, a- and b-glucosidase, and b-galactosidase are some of the enzymes that possess antitumor activities and have potential in the treatment of some types of cancer **(Godoy-Gallardo *et al.*, 2019; Liu *et al.*, 2019; Vimal and Kumar, 2019)**

The application of glutaminase was premised on initial findings that neoplastic cells are unable to synthesize glutamine and therefore have a high demand for external glutamine for their growth. Therefore, glutaminase as a result of its ability to deamidate glutamine is expected to eliminate a metabolite in the human system that is essential for survival of the neoplasm, but not nutritionally essential for the host cells **(Fernandes *et al.*, 2017)**.

A schematic illustration highlighting this mechanism is presented in Figure 3.

Fig (3): The fate of glutamine in normal (A, C) and tumor (B, D) cells in the presence of glutaminase. Gln: glutamine; Glt: glutaminase; GS: glutamine synthetase; Glu: glutamic acid; OAA: oxaloacetate; TCA:



tricarboxylic acid. **(A. Amobonye *et al.*,2019)**

The first investigation into the antitumor property of glutaminase revealed its inhibitory effect on the growth of the Gardner lymphosarcoma (6C3HED) and L-1210 leukemia cells. In the same study, it was shown that glutaminase, sourced from *Pseudomonas spp.*, in combination with azaserine enhanced the degree of tumor growth inhibition. Subsequently, glutaminase was administered intravenously in patients with acute lymphoblastic leukemia and acute myeloid leukemia **(Greenberg *et al.*,1964)**.

The administered enzyme exhibited antileukemic effects in all tested patients despite its short half-life (80 min). The synergistic activity of glutaminase with other compounds against the proliferation of tumor cells was shown by the administration of a complex formed from *Pseudomonas 7A* glutaminase and antitumor antibodies. The complex inhibited tumor cells to an extent far exceeding the inhibition by either glutaminase or antibody alone **(Spiersand Wade, 1976)**.

Various in vitro studies have also revealed the activity of glutaminase against the proliferation of tumor cell lines using the MTT (3-(4,5- dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) cell proliferation assay. *Alcaligenes faecalis* KLU102 glutaminase was able to reduce the viability of HeLa cells in a dose dependent manner, with an IC50 value of 12.5 mg/ml within a 24 h period **(Pandian *et al.*, 2014; Tallur *et al.*, 2014; Aly *et al.*, 2017)**.

Glutaminase from *Penicillium brevicompactum* NRC 829 also suppressed the growth of human cell line hepatocellular carcinoma (Hep-G2), with an IC50 value of 63.3 lg/ml. Recently, a purified glutaminase from *Streptomyces sp*. D214 was shown to be the most effective with an IC50 value of 10 mg/ml against MCF-7 tumor cell line. Recombinant glutaminase may be more useful in cancer treatments as recombinant technology allows for tailor making enzymes with improve properties suitable for clinical trials. The enzyme obtained by intergeneric protoplast fusion of strains of *Cladosporium* with *Trichoderma* was shown to cause gradual inhibition of cancer cell viability **(Elshafei *et al.*, 2014; Aly *et al.*, 2017; El-Gendy *et al.*, 2017;)**.

The concept of targeting glutamine metabolism in cancer cells, originally justified by the number of glutamine metabolic pathways, has been strengthened by studies demonstrating that glutamine metabolism is regulated by oncogenes. However, lymphocytes are also dependent on glutamine metabolism which suggests that immunosuppression may be a side effect of drugs that target glutamine metabolism for cancer therapy. This

could be overcome by understanding the biochemistry of glutaminase transport in both normal and cancer cells and exploiting the differences to target cancer cells **(Vander Heiden, 2011; Kim and Kim, 2013)**

Another limitation of glutaminase is its short half-life in vivo which reduces the duration of its action and requires an intermittent supply by intravenous injection, which is expensive. Therefore, efforts should be directed to identify novel glutaminases with higher stability, and also to engineer enzymes to increase their stability. Existing glutaminases can also be integrated into modified-release drug products that have the property to alter the rate and timing of the release of the active pharmaceutical agent.

Cancer cells, especially, cannot synthesize L-glutamine as they lack the proper functioning glutamine biosynthetic machinery (L-glutamine synthetase) and therefore require large amount of L-glutamine for their rapid growth. These cells depend on the exogenous supply of L-glutamine for their survival and rapid cell division, as it is a primary tool for donation of its nitrogen, which aid in protein, nucleic acid, lipid formation and participate in oxidative metabolism.

Furthermore, glutaminase is already present in mitochondria, but it must be at the level that allows sequential and fast degradation of glutamine **(Lukey *et al.* 2013)**. Hence, the use of L-glutaminase deprives the tumor cells of L- glutamine and causes selective death of L-glutamine dependent tumor cells. The glutamine-deprivation therapy with L-glutaminase that hydrolyzes L-glutamine to L- glutamic acid and ammonia, not only selectively inhibits tumor growth by the blocking of the de novo protein synthesis, but also increase in the superoxide level of oxidative stress that promotes the death of the cancer cells. Thus, it can act as a possible candidate for enzyme therapy. For example, L-glutaminase has been receiving more attention as an antileukemic agent for treatment of acute lymphoblastic leukemia (ALL) and other types of cancer **(Elshafei *et al.*, 2014; Orabi *et al.* 2019; Mustafa *et al.*, 2020)**.

L-glutaminase enzyme produced by halotolerant isolates can be used for increasing the level of glutamine catabolism and stopping cancer development. In addition, the chemical nature of seawater could provide microbial sources producing enzymes that could have fewer side effects when used in therapeutic applications. Thus, marine bacteria have recently attracted attention for the l- glutaminase production**.** *Halomonas meridiana* was first reported as an l-glutaminase producer that is used as an anti- colon cancer agent by (**Kiruthika and Swathi 2019; Zolfaghar *et al.* 2019; Mostafa *et al.*,2021).**

To accomplish the biosynthetic need, the cancerous cell causes modification and reprogramming of metabolic pathways the type of change observed in glycolytic pathway that is generally referred to as "Warburg Effect". However, there is a constant upsurge of a better alternative for cancer treatment. By using marine biocatalysts and by lowering down its ominous side effects, the marine biocatalysts uphold significant consideration and can be used as an alternative to chemotherapeutic treatment. In this respect, the wide range of substrate specificities of marine-originated enzymes could be an appealing option. There are biocatalysts reported for anticancer property and used widely for the treatment of some kind of cancer, i.e glutaminase, asparaginase, arginase, tyrosinase, methionase and galactosidase **(Vimal and Kumar 2019; Liu *et al.* 2019; Godoy-Gallardo *et al.* 2019)**.

However, the applicability of cancerous cells to synthesize glutamine demand increases as the cancer cell is unable to synthesize glutamine on its own for growth and survival. Because of the capability of glutaminase to deaminate the glutamine, it can be predicted to eliminate a key metabolite from the system that is necessary for the survival of cancer cell; on the contrary, this metabolite is not nutritionally required for a host **(Fernandes *et al.* 2017).**

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