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MICROBIAL L-METHIONASE: A PROMISING ANTI-CANCER ENZYME FOR TREATMENT OF VARIOUS CANCER

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ABSTRACT

On a worldwide scale, cancer has become a more prevalent cause related to mortality and morbidity. Enzyme therapies are becoming more widely used in medicine today, with numerous advantages in the treatment of diseases. Methionine amino acid plays a significant role in tumor cells. Methionine dependency of these cells ultimately results in apoptosis due to methionine limitation in tumor cells. Methionine amino acid has the potential to be used against several types of cancers. L-methionase is a microbial enzyme that has great therapeutic value as a potent anticancer agent against various types of tumor cell lines, including breast, lung, colon, kidney, glioblastoma, and neuroblastoma. The elimination of methionine is catalyzed by the L-Methionase enzyme, which results in the formation of α-ketobutyrate, methanethiol, and ammonia. L-methionine is absent in mammalian systems, but it is present intracellularly in bacteria and extracellularly in fungi. Methionine restriction can be beneficial in improving the effectiveness of chemotherapy by inhibiting the proliferation of tumor cells. Methionine is an essential amino acid that is involved in several significant functions in mammalian metabolism, such as the synthesis of proteins, the methylation of DNA, and polyamines. Methionine also plays an indispensable role in gene activation and inactivation due to methionine limitation in cancer cells.

Therapeutic interventions for various cancers have been developed using L-Methionase by introducing recombinant proteins to deplete methionine, which is essential for the growth of cancer cells. The current focus of clinical trials is on the application of L-methionase and methionine restriction in combination with chemotherapeutic regimens.

Keywords: L-Methionase, Anticancer Enzyme, Enzymes Therapy, Methionine Restriction

INTRODUCTION:

Cancer is a major health problem and continues to lead to death. Despite significant advances in cancer treatment, there is still a need for more effective and safer therapies **[1]**. The cancer cells proliferate abnormally, they require a high number of amino acids as nutrients because they are the building block for protein synthesis. So, without amino acids, tumor cells fail to function because proteins cannot synthesize. According to this concept, recent research has targeted amino acid metabolic enzymes that deregulated specific amino acid metabolism that is essential for cancer cell proliferation **[2]**.

Enzyme therapies are becoming more prevalent in medicine today, with many advantages in disease treatment. Enzymes have two significant features First; enzymes frequently bind and act on their targeted site with high affinity and specificity. Second, Enzymes are catalytic and convert numerous target molecules to the desired product. Enzymes are highly specialized and effective drugs that can change the body's biochemistry for the better than smaller molecules **[3]**. Enzymes have emerged as a promising therapeutic agent for a variety of illnesses, from metabolic deficiencies, such as fibrosis, joint problems, cancer, and cardiovascular diseases. There are 4 types of enzymes used in cancer treatment. 1] Arginase 2] Asparaginase 3] Glutaminase and 4] Methionase. Arginine-degrading enzymes consist of three main types of proteins: arginine. arginine deiminase, arginase, and arginine decarboxylase which exist in archaea, bacteria, and eukaryotes **[4]**. L-asparaginase is an important chemotherapeutic agent for acute lymphoblastic leukemia and other hematopoietic malignancies. L-asparaginase is an enzyme found in bacteria, fungi, yeast, actinomycetes, algae, and plants **[5]**. L-glutaminase is used as an effective agent in the cure of acute lymphocytic leukemia and HIV. L-glutaminase activity is found in abundance in the tissues of animals, plants, and microbes like bacteria, fungi, and actinomycetes. Microbial L-glutaminase has received greater attention for its

potential biotechnological applications in large-scale production **[6]**.

L- Methionase

L-Methionase is also known as methionine-γ-lyase, methionine lyase, methionine demethylase, and L-methionase. L-methionase belongs to the family of pyridoxal L-phosphate (PLP) dependent enzymes, and catalysis the α-γ elimination of L-methionine, converting into α-ketobutyrate, methanethiol, and ammonia. L-methionine is an essential Sulphur-containing amino acid for humans **[7]**. L-methionase is a microbial enzyme with high therapeutic value as a potent anticancer agent against various types of tumor cell lines like breast, lung, colon, kidney, glioblastoma, and neuroblastoma. Besides protein synthesis, methionine acts as a precursor for glutathione, polyamines, and S-adenosyl methionine (SAM). Glutathione plays a significant role in cellular antioxidant mechanisms, while polyamines are required for nucleotide biosynthesis and cell division. SAM is required for the methylation of DNA, RNA, and proteins. L-Methionase enzyme ranging from 149 to 173 kDa is composed of four subunits, each of which has a molecular weight of 41 to 45 kDa **[8]**.

L-methionine, an essential amino acid, is required for the survival and proliferation of human cancer cell lines and malignant tumors. Lack of methionine in the bloodstream arrests the carcinoma cells at the S-G2 phase of the cell cycle resulting in cell death **[9]**. L-Methionase is absent in mammalian systems and intracellularly present in bacteria and extracellularly in fungi **[10]**. L-methionase is recognized as an antibacterial, antiprotozoal, antifungal, and antioxidant agent **[11]**. L-methionase has a significant therapeutic value because it has shown effective results as an anticancer agent both *in vitro* and *in vivo*. *In vitro* studies have demonstrated that L-Methionase selectivity depletes L-methionine in cancer cells, leading to inhibition of protein synthesis and induction of apoptosis. *In vivo*, studies have also demonstrated the antitumor activity of L-Methionase inhibits the growth of breast cancer xenografts in mice **[12]**.

Now, recombinant Methionase (rMETase) from Pseudomonas putida was found to process favorable kinetic properties and was more stable **[13], [14]**. Recombinant Methionase has shown promising results in combinational therapies against cancer cells, where it is used with 5-fluorouracil and 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) have displayed significant activity in mouse models of colon cancer, lung cancer, and brain cancer **[15]**. It leads to the activation of prodrug selenomethionine, leading to its conversion from non-toxic to toxic

methylselenol, resulting in anti-tumor properties due to superoxide synthesis by thiol oxidation **[16]**. Further examination explored the pharmacokinetics properties of PEGylated L-Methionase by Lessening its antigenic properties, extending its half-life time, and reducing the hyperammonemia with maximum therapeutic efficacy **[17]**.

MICROBIAL SOURCES OF L-METHIONINE

The presence of L-Methionase has been reported in several organisms including plants, bacteria, fungi, actinomycetes, and protozoa except for humans. Even though L-Methionase exists in several plants and protozoa groups, due to tiresome extraction processes, other feasible sources such as bacteria and fungi were successfully explored by researchers **(Table 1)**.

Bacteria

L-methionase produces intracellular enzymes in most bacterial species. Both gram-positive and Gram-negative bacterial species have been reported to produce L-Methionase. Some of these are anaerobic *Porphyromonas gingivalis* **[16]** and *Treponema denticola* **[17]**, in eukaryotic pathogens such as *Entamoeba histolytica* **[18]**, furthermore, the bacteria *Pseudomonas putida* **[9]**, *Aeromonas sp* **[19]**, *Citrobacter freundii* **[15]** *and Lactococcus lactis; Clostridium sporogenes* **[19]** *Brevibacterium lines* **[20]**, *Triichomonas vaginalis* **[21]**, *Bacillus haynesii* **[22]** and *Hafnia alvei* **[23]**.

B. lines which are a normal flora present in a way of curd are a rich source of L-Methionase **[24]**. Since L-Methionase was discovered in *E. coli*, this enzyme has been found in various bacteria and it's considered a key enzyme in the bacterium metabolism of L-methionine **[25]**. Bacterium *hafnia alvei* bacteria from blue cheese to find bacterial strain secreting L-Methionase enzyme. Currently, low therapeutic efficiency and high levels of toxicity are caused by a few bacterial Methionase which increase the demand for new bacterial L-Methionase producers, with lesser side effects and more efficiency.

Fungi and yeast

Fungi are another potential source of L-Methionase along with bacteria. Several adverse side effects linked with bacterial L-Methionase often restrain their application. This obstruction demands a search for L-Methionase from novel sources. Fungi seem to be effective eukaryotes for enzyme production not only from the pharmacokinetics point of view but also from the cost of fermentation conditions. Moreover, the fungal enzyme possesses a lower immunogenic reaction during tumor therapy, which may be related to the structural competence of the

human immune system. L-methionase produce Extracellular an enzymes in fungal species.

Several studies were reported on the production and optimization of L-Methionase enzyme were investigated by using several agro-industrial residues by *Aspergillus flavipes* using solid-state fermentation **[26], [27]**. Fungi such as a *Trichoderma harzianum* **[28]** and *Penicillium notatum*. Some studies were reported on the partial characterization of L-Methionase from fungi including *Aspergillus sp*; *Penicillium sp* and *Humicola fuscoatra*. L-Methionase in the culture of yeast such as *Geotrichum candidum* **[29], [30]**, *Debaromyces hansenii*, and *Saccharomyces cerevisiae*. L-Methionase activity was quantitatively screened on a large number of isolated yeasts from various sources, including Egyptian soil, marine soil, marine water, and cheese products were quantitatively screened for L-Methionase activity.

Protozoa

In contrast to other organisms possessing L-Methionase, anaerobic parasitic protists, namely Entamoeba histolytica and trichomonas vaginalis a pair of L-Methionase isoenzymes such as MGL 1 and MGL 2 **[18]**.

L-METHIONASE MECHANISM OF ACTION

The mechanism of action of L-Methionase involves several steps. First, L-Methionase catalyzes the hydrolysis of L-methionine, producing L-homocysteine and methylthioadenosine (MTA) **[31]**.

L-homocysteine can be further metabolized into cysteine, which is another important amino acid required for cell growth. MTA is a potent inhibitor of S-adenosylmethionine (SAM), which is required for DNA methylation, a process that regulates gene expression. By inhibiting DNA methylation, MTA can lead to the activation of tumor suppressor genes and the inhibition of oncogenes, which can contribute to the anti-cancer effect of L-Methionase **[32]**. Furthermore, the depletion of L-methionine by L-methionase can also cause cancer cells to undergo programmed cell death or apoptosis **[33]**.

Overall, the mechanism of action of L-methionase involves the depletion of L-methionine, **(Figure 1)** the production of L-homocysteine and cysteine, and the inhibition of DNA methylation through the production of MTA these effects can lead to the inhibition of cancer cell growth and the induction of apoptosis, making L-Methionase a promising therapeutic agent for the treatment of cancer **[34]**.

METHIONINE RESTRICTION AND CANCER

Methionine

L-methionine is an essential amino acid that is important for various biochemical processes in the body. Cancer cells have a higher demand for L-methionine than normal cells and L-methionine supplementation could promote cancer cell growth. However, other studies have shown that L-methionine supplementation may have an anticancer effect by inhibiting cancer cell growth and inducing apoptosis **[35]**.

Methionine also plays a central role in the metabolism of all macromolecules control of gene expression, cytoprotection, and membrane integrity. Normal cells can grow on homocysteine, instead of methionine, due to their active methionine synthase. Hence, lowering plasma and cellular methionine levels with L-Methionase appears to be a promising therapeutic strategy for treating cancer. In terms of therapeutics, an effective enzyme should have little effect on normal cells and a low km value for methionine and activity toward homocysteine **[36]**.

Methionine Restriction (MR)

Methionine is an essential amino acid, and as such it must be included in the diet. Despite the consumption of methionine being essential for survival, studies have shown that limiting methionine in the diet of animal or cell culture media provides metabolic benefits such as decreasing adiposity, increasing insulin sensitivity, decreasing inflammation and oxidative stress, and extending lifespan **[37]**. Rats fed a diet with 80% less methionine lived 40% longer than rats fed a control diet. Dietary methionine restriction was also effective at extending lifespan in mice **[38]**. Methionine restriction has been shown to improve lifespan through a variety of processes, including a decrease in oxidative stress and inflammation, a change in autophagy, and an increase in hormones that protect against heart disease **[39]**. Another mechanism by which MR may extend lifespan is by providing a reduction in cancer incidence and an overall reduction in cancer mortality **[40]**. The ability of MR to improve insulin sensitivity and reduce adiposity may be directly related to its anticancer potential as several types of cancers are closely linked to obesity and insulin resistance and the anticancer effect on MR may be secondary to its ability to reduce adiposity and increase insulin sensitivity **[41]**.

Methionine Restriction in Cancer

Cancer cells have a higher demand for and are often starved of nutrients and oxygen which are needed in abundance for rapid growth. L-methionine plays an essential role in the methylation of DNA, polyamine synthesis, and mammalian protein synthesis **[42]**. Methionine also plays a central role in the metabolism of all macromolecules and controls gene

expression, cytoprotection, and membrane integrity. Methionine restriction is a principal approach in the metabolic control of cancers and exhibits methionine dependence for survival and proliferation. Methionine restriction has a significant positive impact on fruit fly lifespan extension. Therefore, methionine disintegration with L-Methionase is found to be an important strategy for the control of certain ailments. So, in the presence of L-Methionase malignant cells are deprived of vital growth factors that result in the exhaustion of methionine, and eventually, the tumor cell dies off **[43-46]**.

Even though Methionine is an essential amino acid, it can be substituted with homocysteine, which is transformed into methionine in a reaction catalyzed by methionine synthase. That utilizes cobalamine and 5-methyl tetrahydro-folic acid as cofactors **[47]**. This enzyme supports normal cells to grow on nutrients supplemented with homocysteine, folic acid, and cobalamin (B12) with the lack of methionine. Supplementation of homocysteine in the place of methionine or disintegration of methionine with Methionase in methionine-dependent tumor cells growing exponentially in culture results in cell cycle arrest, strict inhibition of mitosis, and eventual death (Mazzucchelli *et al*., 2013; Meyskens *et al*., 2014; Sinha *et al*., 2014). Methionine deprivation affects tumor cells with the propensity to divide and cause them to arrest predominantly in the last S/G2 phase and are susceptible to spontaneous death. A variety of tumors including Lewis lung carcinoma, several human colon cancer lines, and glioblastoma have shown controlled growth by L-Methionase **[51], [52], [53], [54] (Table 2 and 3)**.

L-METHIONASE USE IN VARIOUS CANCER

Methionine Restriction and Breast Cancer

The second most prevalent type of cancer diagnosed in women is breast cancer. Breast cancer cells are hormone receptor-positive if they express either of the estrogen and progesterone receptors and are considered HER-2-positive if the breast cancer cells overexpress the protein, HER-2 9 human epidermal growth factor receptor 2) TNBC makes up about 16% of all breast cancer diagnoses. Few studies have examined the efficacy of MR in breast cancer models **[55-57]**.

TNBC has fewer treatment options than forms of breast cancer due to the lack of response to hormone therapy and drugs that target HER-2. MR may provide an easy to enhance the efficacy of potential treatment options for TNBC. Tumor necrosis factor (TNF) related apoptosis-inducing ligand (TRAIL) receptors are an exciting possibility for cancer treatment

due to their ability to induce apoptosis in cancer cells while having little on normal cells **[58], [59]**. Despite their efficacy in preclinical studies, TRAIL receptors have not been successful in human clinical trials. Methionine deficiency increases TRAIL-R2 expression in TNBC cells but not in healthy breast epithelial cells. Further, a methionine-free diet suppresses breast cancer growth and enhances the efficacy of the TRAIL-receptor-2 monoclonal antibody in inhibiting breast cancer growth in mice. Recent studies have been undertaken to determine if blocking the ability of MR to activate the integrated stress response will enhance its efficacy in treating TNBC.MR has been shown to activate two kinases nonderepressible 2 (GCN2) and protein kinase R like endoplasmic reticulum kinase blocking and enhancing the efficacy of methionine restriction nutrients in TNBC **[60- 63]**.

Methionine Restriction and Colorectal Cancer

The fourth most common cause of cancer-related death in the United States is colorectal cancer. The relationship between methionine restriction and colorectal cancer has been established in tumor prevention and treatment in animal and cell culture models **[64]**. A recent study evaluated the effect of MR on two patient-derived xenograft models of colorectal cancer. Methionine restriction (86% methionine-restricted diet) was initiated either two weeks before inoculation to determine the effects of MR on cancer prevention, or when the tumor became capable to test the treatment effect of MR. Importantly, MR also enhances the efficacy of 5-fluorouracil, a chemotherapeutic drug with great efficacy against colorectal cancer in the PDX model **[65-66]**.

Methionine Restriction and Prostate Cancer

Prostate cancer is the second leading cause of cancer death among adult men in the US and current treatment options include hormonal therapy to decrease testosterone levels, radiation therapy, or surgical procedures. While there are options available for prostate cancer, there are no known interventions to prevent the development of cancer **[5], [67-69]**. Using a well-characterized mouse model for prostate cancer, it was shown that dietary MR inhibits prostate cancer development especially in the anterior and dorsal lobes of the prostate, where are most severe lesions are found. While the mechanism by which MR inhibits prostate cancer cell proliferation, inhibiting the insulin/GF-1 axis, or reducing polyamine synthesis. The cells of the prostate produce high levels of polyamines and inhibition of polyamine

synthesis is effective at suppressing tumor growth in prostate cancer **[70]**.

Thymidylate synthase is another enzyme that MR can target in prostate cancer cells. Thymidylate synthase is the enzyme that catalyzes the methylation of deoxy uridylic acid during nucleotide biosynthesis and thus an important target for cancer treatment. The chemotherapy drug 5-fluorouracil inhibits TS activity by disrupting the action of TS, causing DNA and RNA damage, making 5-fluorouracil an effective and commonly used cancer treatment **[71]**. However, 5-fluorouracil has been reported to increase TS protein expression, resulting in 5-fluorouracil drug resistance. Several studies have shown that MR and 5-fluorouracil have synergistic anti-cancer effects. MR selectively reduces TS activity in prostate cancer cells by 80% within 48 h but does not affect TS activity in normal epithelial cells. Importantly, MR also reduces TS protein expression, potentially explaining the synergy between MR and 5-fluorouracil **[72]**.

FUTURE PROSPECTS

The unique catalytic reaction of L-Methionase and its limited distribution in pathogens but not in humans make this enzyme a promising target for designing novel chemotherapeutics agents. Tumor cells show enhanced methionine dependence in comparison to normal cells. The greater requirement of methionine by rapidly growing tumor cells supports high protein synthesis and regulation of DNA expression yet it can be exploited by the use of Methionase-based therapy to rapidly deplete the cancerous cells. Thus, the restriction of methionine is an important strategy in cancer growth control particularly in malignant cells that exhibit dependence on methionine for their survival and proliferation. Currently, selenomethionine is also used as a prodrug in cancer therapy along with the enzyme L-Methionase that converts prodrug into active toxic chemicals that cause the death of cancerous cells. More recently, a fusion protein consisting of L-Methionase linked to annexin-V has been used in cancer therapy. The fusion proteins have advantages in that they have specificity only for cancer cells and do not harm normal cells.

CONCLUSION

In conclusion, Microbial L-Methionase is a promising enzyme for the treatment of various cancers. This enzyme has been shown to have potent anti-cancer properties both *in vivo* and *in vitro*. Additionally, microbial L-Methionase has been found to have minimal toxicity and few side effects making it an attractive treatment option for cancer patients. While future research is needed to fully understand the potential of this enzyme in cancer therapy, the available evidence

suggests that microbial L-Methionase could be a valuable addition to a weapon of anti-cancer treatment.

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REFERENCES

[1] J. Liu, J. Huang, P. Xin, G. Liu, and J. Wu, "Biomedical applications of methionine-based systems," *Biomater Sci*, vol. 9, no. 6, pp. 1961–1973, 2021, doi: 10.1039/d0bm02180f.

[2] M. H. Hendy, A. H. Hashem, W. B. Sulieman, M. H. Sultan, and M. Abdelraof, "Purification, Characterization and anticancer activity of L-methionine γ-lyase from thermo-tolerant Aspergillus fumigatus," *Microb Cell Fact*, vol. 22, no. 1, pp. 1–11, 2023, doi: 10.1186/s12934-023-02019-z.

[3] N. Salim, A. Santhiagu, and K. Joji, "Purification, characterization and anticancer evaluation of l-methioninase from Trichoderma harzianum," *3 Biotech*, vol. 10, no. 11, 2020, doi: 10.1007/s13205-020-02494-w.

[4] B. Borek, T. Gajda, A. Golebiowski, and R. Blaszczyk, "Boronic acid-based arginase inhibitors in cancer immunotherapy," *Bioorg Med Chem*, vol. 28, no. 18, p. 115658, 2020, doi: 10.1016/j.bmc.2020.115658.

[5] S. A. Alzaeemi *et al.*, "Improvement of L-asparaginase, an Anticancer Agent of Aspergillus arenarioides EAN603 in Submerged Fermentation Using a Radial Basis Function Neural Network with a Specific Genetic Algorithm (RBFNN-GA)," *Fermentation*, vol. 9, no. 3, p. 200, 2023, doi: 10.3390/fermentation9030200.

[6] L. Kothapalli and C. L. M. V V, "Physicochemical characterization, cytotoxicity and anticancer activity of L-glutaminase from marine Streptomyces luteogriseus," vol. 10, pp. 1154–1171, 2023.

[7] A. abozeid, "Short Review on Microbial L- methioninase and its Applications," *Bulletin of Faculty of Science, Zagazig University*, vol.

2022, no. 4, pp. 107–139, 2023, doi: 10.21608/bfszu.2022.148461.1155.

[8] H. S. Fernandes, C. S. Silva Teixeira, P. A. Fernandes, M. J. Ramos, and N. M. F. S. A. Cerqueira, "Amino acid deprivation using enzymes as a targeted therapy for cancer and viral infections," *Expert Opin Ther Pat*, vol. 27, no. 3, pp. 283–297, 2017, doi: 10.1080/13543776.2017.1254194.

[9] M. Zolfaghar, M. A. Amoozegar, K. Khajeh, H. Babavalian, and H. Tebyanian, "Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran," *Mol Biol Rep*, vol. 46, no. 3, pp. 3275–3286, 2019, doi: 10.1007/s11033-019-04787-7.

[10] B. Mh, "Microbial source of L-methioninase as anticancer agent," *J Am Sci*, vol. 16, no. 4, pp. 73–80, 2020, doi: 10.7537/marsjas160420.06.Keywords.

[11] M. Hendy, A. Hashem, M. Sultan, A. El-Ghamery, and M. Abdelraof, "L-methionine γ-lyase from Thermo-tolerant fungi: Isolation, Identification of the potent producers, and Statistical Optimization of Production via Response surface methodology," *Egypt J Chem*, vol. 0, no. 0, pp. 0–0, 2021, doi: 10.21608/ejchem.2021.79178.4555.

[12] H. I. LIM, Y. SUN, Q. HAN, J. YAMAMOTO, and R. M. HOFFMAN, "Efficacy of oral recombinant methioninase and eribulin on a pdox model of triple-negative breast cancer (tnbc) liver metastasis," *In Vivo (Brooklyn)*, vol. 35, no. 5, pp. 2531–2534, 2021, doi: 10.21873/INVIVO.12534.

[13] A. A. Prihanto, "Bacillus subtilis UBTn7, a potential producer of L - Methioninase isolated from mangrove, Rhizophora mucronata," *IOP Conf Ser Earth Environ Sci*, vol. 137, no. 1, 2018, doi: 10.1088/1755-1315/137/1/012077.

[14] B. Kharayat and P. Singh, "Study of media optimization and kinetic modeling of l-methioninase from Pseudomonas stutzeri," *Vegetos*, vol. 32, no. 3, pp. 370–380, 2019, doi: 10.1007/s42535-019-00032-5.

[15] V. V. Kulikova *et al.*, "Gene cloning, characterization, and cytotoxic activity of methionine γ-lyase from Clostridium novyi,"

IUBMB Life, vol. 69, no. 9, pp. 668–676, 2017, doi: 10.1002/iub.1649.

[16] M. Yoshimura, Y. Nakano, Y. Yamashita, T. Oho, T. Saito, and T. Koga, "Formation of methyl mercaptan from L-methionine by Porphyromonas gingivalis," *Infect Immun*, vol. 68, no. 12, pp. 6912–6916, 2000, doi: 10.1128/IAI.68.12.6912-6916.2000.

[17] H. Fukamachi, Y. Nakano, S. Okano, Y. Shibata, Y. Abiko, and Y. Yamashita, "High production of methyl mercaptan by L-methionine-α-deamino- γ-mercaptomethane lyase from Treponema denticola," *Biochem Biophys Res Commun*, vol. 331, no. 1, pp. 127–131, 2005, doi: 10.1016/j.bbrc.2005.03.139.

[18] M. Tokoro, T. Asai, S. Kobayashi, T. Takeuchi, and T. Nozaki, "Identification and Characterization of Two Isoenzymes of Methionine γ-Lyase from Entamoeba histolytica: A key enzyme of sulfur-amino acid degradation in an anaerobic parasitic protist that lacks forward and reverse trans-sulfuration pathways," *Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42717–42727, 2003, doi: 10.1074/jbc.M212414200.

[19] B. Lin, G. Tian, and Y. Liu, "Mechanistic insights into the γ-elimination reaction of l-methionine catalyzed by methionine γ-lyase (MGL)," *Theor Chem Acc*, vol. 136, no. 9, pp. 1–11, 2017, doi: 10.1007/s00214-017-2140-9.

[20] K. Pavani and S. V. Saradhi, "Original Research Article Cloning and expression of Methionine- -lyase (MGL) of Brevibacterium linens," vol. 3, no. 3, pp. 615–631, 2014.

[21] B. C. Lockwood and G. H. Coombs, "Purification and characterization of methionine y-lyase from," *Biochem.J.*, vol. 279, pp. 675–682, 1991.

[22] B. B. Kotramada Bopaiah *et al.*, "Purification, characterization, and antiproliferative activity of L-methioninase from a new isolate of Bacillus haynesii JUB2," *J Appl Pharm Sci*, vol. 10, no. 10, pp. 54–61, 2020, doi: 10.7324/JAPS.2020.10106.

[23] W. A. Alshehri, "Bacterium Hafnia alvei secretes L-methioninase enzyme: Optimization of the enzyme secretion conditions," *Saudi*

J Biol Sci, vol. 27, no. 5, pp. 1222–1227, 2020, doi: 10.1016/j.sjbs.2020.02.008.

[24] F. Amarita, M. Yvon, M. Nardi, E. Chambellon, J. Delettre, and P. Bonnarme, "Identification and functional analysis of the gene encoding methionine-γ-lyase in Brevibacterium linens," *Appl Environ Microbiol*, vol. 70, no. 12, pp. 7348–7354, 2004, doi: 10.1128/AEM.70.12.7348-7354.2004.

[25] R. de la Salud Bea, L. J. North, S. Horiuchi, E. R. Frawley, and Q. Shen, "Antimicrobial activity and toxicity of analogs of wasp venom emp peptides. Potential influence of oxidized methionine," *Antibiotics*, vol. 10, no. 10, pp. 1–11, 2021, doi: 10.3390/antibiotics10101208.

[26] A. S. A. El-Sayed, "L-methioninase production by Aspergillus flavipes under solid-state fermentation," *J Basic Microbiol*, vol. 49, no. 4, pp. 331–341, 2009, doi: 10.1002/jobm.200800318.

[27] A. S. A. El-Sayed, L. E. Ruff, S. E. A. Ghany, G. S. Ali, and S. Esener, "Molecular and Spectroscopic Characterization of Aspergillus flavipes and Pseudomonas putida L-Methionine γ-Lyase in Vitro," *Appl Biochem Biotechnol*, vol. 181, no. 4, pp. 1513–1532, 2017, doi: 10.1007/s12010-016-2299-x.

[28] N. Salim, A. Santhiagu, and K. Joji, "Process modeling and optimization of high yielding L-methioninase from a newly isolated Trichoderma harzianum using response surface methodology and artificial neural network coupled genetic algorithm," *Biocatal Agric Biotechnol*, vol. 17, pp. 299–308, 2019, doi: 10.1016/j.bcab.2018.11.032.

[29] P. Bonnarme, K. Arfi, C. Dury, S. Helinck, M. Yvon, and H. E. Spinnler, "Sulfur compound production by Geotrichum candidum from L-methionine: Importance of the transamination step," *FEMS Microbiol Lett*, vol. 205, no. 2, pp. 247–252, 2001, doi: 10.1016/S0378-1097(01)00485-2.

[30] P. Bonnarme, K. Arfi, C. Dury, S. Helinck, M. Yvon, and H. E. Spinnler, "Sulfur compound production by Geotrichum candidum from L-methionine: Importance of the transamination step," *FEMS Microbiol Lett*, vol.

205, no. 2, pp. 247–252, 2001, doi: 10.1016/S0378-1097(01)00485-2.

[31] D. Wanders, L. A. Forney, K. P. Stone, B. E. Hasek, W. D. Johnson, and T. W. Gettys, "The Components of Age-Dependent Effects of Dietary Methionine Restriction on Energy Balance in Rats," *Obesity*, vol. 26, no. 4, pp. 740–746, 2018, doi: 10.1002/oby.22146.

[32] D. Wanders, K. Hobson, and X. Ji, "Methionine restriction and cancer biology," *Nutrients*, vol. 12, no. 3, 2020, doi: 10.3390/nu12030684.

[33] B. C. Lee, A. Kaya, and V. N. Gladyshev, "Methionine restriction and life-span control," *Ann N Y Acad Sci*, vol. 1363, no. 1, pp. 116–124, 2016, doi: 10.1111/nyas.12973.

[34] X. Gao *et al.*, "Dietary methionine influences therapy in mouse cancer models and alters human metabolism," *Nature*, vol. 572, no. 7769, pp. 397–401, 2019, doi: 10.1038/s41586-019-1437-3.

[35] N. M. Iyengar, A. Gucalp, A. J. Dannenberg, and C. A. Hudis, "Obesity and cancer mechanisms: Tumor microenvironment and inflammation," *Journal of Clinical Oncology*, vol. 34, no. 35, pp. 4270–4276, 2016, doi: 10.1200/JCO.2016.67.4283.

[36] Y. Kubota *et al.*, "Long-term Stable Disease in a Rectal-cancer Patient Treated by Methionine Restriction With Oral Recombinant Methioninase and a Low-methionine Diet," *Anticancer Res*, vol. 42, no. 8, pp. 3857–3861, 2022, doi: 10.21873/anticanres.15877.

[37] L. Zhou, Z. Chen, and C. Liu, "Identification and verification of the role of crucial genes through which methionine restriction inhibits the progression of colon cancer cells," *Oncol Lett*, vol. 24, no. 2, pp. 1–12, 2022, doi: 10.3892/ol.2022.13394.

[38] T. Ogawa *et al.*, "S-adenosyl-L-homocysteine extends lifespan through methionine restriction effects," *Aging Cell*, vol. 21, no. 5, pp. 1–6, 2022, doi: 10.1111/acel.13604.

[39] T. K. Kjaer *et al.*, "Long-term Somatic Disease Risk in Adult Danish Cancer Survivors," *JAMA Oncol*, vol. 5, no. 4, pp. 537–545, 2019, doi: 10.1001/jamaoncol.2018.7192.

[40] R. Dhankhar, V. Gupta, S. Kumar, R. K. Kapoor, and P. Gulati, "Microbial enzymes for deprivation of amino acid metabolism in malignant cells: biological strategy for cancer treatment," *Appl Microbiol Biotechnol*, vol. 104, no. 7, pp. 2857–2869, 2020, doi: 10.1007/s00253-020-10432-2.

[41] K. Suganya, K. Govindan, P. Prabha, and M. Murugan, "An extensive review on L-methioninase and its potential applications," *Biocatal Agric Biotechnol*, vol. 12, no. July, pp. 104–115, 2017, doi: 10.1016/j.bcab.2017.09.009.

[42] Y. Kubota *et al.*, "Elimination of Axillary-Lymph-Node Metastases in a Patient With Invasive Lobular Breast Cancer Treated by First-line Neo-adjuvant Chemotherapy Combined With Methionine Restriction," *Anticancer Res*, vol. 42, no. 12, pp. 5819–5823, 2022, doi: 10.21873/anticanres.16089.

[43] H. Jeon *et al.*, "Oncotarget 67223 www.impactjournals.com/oncotarget Methionine deprivation suppresses triple-negative breast cancer metastasis in vitro and in vivo," *Oncotarget*, vol. 7, no. 41, pp. 67223–67234, 2016, [Online]. Available: www.impactjournals.com/oncotarget/

[44] E. Strekalova, D. Malin, D. M. Good, and V. L. Cryns, "Methionine deprivation induces a targetable vulnerability in triple-negative breast cancer cells by enhancing TRAIL Receptor-2 Expression," *Clinical Cancer Research*, vol. 21, no. 12, pp. 2780–2791, 2015, doi: 10.1158/1078-0432.CCR-14-2792.

[45] S. H. Rajanala, R. Ringquist, and V. L. Cryns, "Methionine restriction activates the integrated stress response in triple-negative breast cancer cells by a GCN2- and PERK-independent mechanism.," *Am J Cancer Res*, vol. 9, no. 8, pp. 1766–1775, 2019, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/31497357%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6726988

[46] H. Zhang *et al.*, "Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer," *Mol Cancer*, vol. 20, no. 1, pp. 1–

22, 2021, doi: 10.1186/s12943-021-01431-6.

[47] D. Komninou, Y. Leutzinger, B. S. Reddy, and J. P. Richie, "Methionine restriction inhibits colon carcinogenesis," *Nutr Cancer*, vol. 54, no. 2, pp. 202–208, 2006, doi: 10.1207/s15327914nc5402_6.

[48] R. Mazzucchelli, M. Scarpelli, A. Lopez-Beltran, L. Cheng, R. Di Primio, and R. Montironi, "Treatment effects in prostate cancer following traditional and emerging therapies," *Int J Immunopathol Pharmacol*, vol. 26, no. 2, pp. 291–298, 2013, doi: 10.1177/039463201302600202.

[49] R. Sinha *et al.*, "Dietary methionine restriction inhibits prostatic intraepithelial neoplasia in TRAMP mice," *Prostate*, vol. 74, no. 16, pp. 1663–1673, 2014, doi: 10.1002/pros.22884.

[50] F. L. Meyskens, A. R. Simoneau, and E. W. Gerner, "Chemoprevention of prostate cancer with the polyamine synthesis inhibitor difluoromethylornithine," *Recent Results in Cancer Research*, vol. 202, pp. 115–120, 2014, doi: 10.1007/978-3-642-45195-9_14.

[51] A. Struck, M. L. Thompson, L. S. Wong, and J. Micklefield, "S -Adenosyl-Methionine-Dependent Methyltransferases : Highly Versatile Enzymes in Biocatalysis , Biosynthesis and Other Biotechnological Applications," pp. 2642–2655, 2012, doi: 10.1002/cbic.201200556.

[52] J. Y. Ahn, J. S. Lee, H. Y. Min, and H. Y. Lee, "Acquired resistance to 5-fluorouracil via HSP90/Src-mediated increase in thymidylate synthase expression in colon cancer," *Oncotarget*, vol. 6, no. 32, pp. 32622–32633, 2015, doi: 10.18632/oncotarget.5327.

[53] B. C. Halpern, B. R. Clark, D. N. Hardy, R. M. Halpern, and R. A. Smith, "The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture," *Proc Natl Acad Sci U S A*, vol. 71, no. 4, pp. 1133–1136, 1974, doi: 10.1073/pnas.71.4.1133.

[54] S. Lu and D. E. Epner, "Molecular mechanisms of cell cycle block by methionine restriction in human prostate cancer cells," *Nutr Cancer*, vol. 38, no. 1, pp. 123–130, 2000, doi: 10.1207/S15327914NC381_17.

[55] K. Wallis *et al.*, "Methionine stress induces ferroptosis in methionine dependent cancer cells," *bioRxiv*, 2020.

[56] S. Lu, S. M. Hoestje, E. M. Choo, and D. E. Epner, "Methionine restriction induces apoptosis of prostate cancer cells via the c-Jun N-terminal kinase-mediated signaling pathway," *Cancer Lett*, vol. 179, no. 1, pp. 51–58, 2002, doi: 10.1016/S0304-3835(01)00852-7.

[57] W. O. Jonsson, N. S. Margolies, and T. G. Anthony, "Dietary sulfur amino acid restriction and the integrated stress response: Mechanistic insights," *Nutrients*, vol. 11, no. 6, 2019, doi: 10.3390/nu11061349.

[58] T. Takakura *et al.*, "Physicochemical and pharmacokinetic characterization of highly potent recombinant L-methionine γ-lyase conjugated with polyethylene glycol as an antitumor agent," *Cancer Res*, vol. 66, no. 5, pp. 2807–2814, 2006, doi: 10.1158/0008-5472.CAN-05-3910.

[59] N. Goseki, M. Endo, T. Onodera, and G. Kosaki, "Anti-tumor effect of L-methionine-deprived total parenteral nutrition with 5-fluorouracil administration on Yoshida Sarcoma-bearing rats," *Ann Surg*, vol. 214, no. 1, pp. 83–88, 1991, doi: 10.1097/00000658-199107000-00013.

[60] J. R. Hens *et al.*, "Methionine-restricted diet inhibits growth of MCF10AT1-derived mammary tumors by increasing cell cycle inhibitors in athymic nude mice," *BMC Cancer*, vol. 16, no. 1, pp. 1–13, 2016, doi: 10.1186/s12885-016-2367-1.

[61] P. Bonnarme, L. Psoni, and H. E. Spinnler, "Diversity of L-Methionine catabolism pathways in cheese-ripening bacteria," *Appl Environ Microbiol*, vol. 66, no. 12, pp. 5514–5517, 2000, doi: 10.1128/AEM.66.12.5514-5517.2000.

[62] L. Ronda *et al.*, "Exploring methionine γ-lyase structure-function relationship via microspectrophotometry and X-ray crystallography," *Biochim Biophys Acta Proteins Proteom*, vol. 1814, no. 6, pp. 834–842, 2011, doi: 10.1016/j.bbapap.2010.06.017.

[63] K. S. Dike and I. A. Ekwealor, "Production of L-methionine by

Bacillus cereus isolated from different soil ecovars in Owerri , South East Nigeria.," *Eur J Exp Biol*, vol. 2, no. 2, pp. 311–314, 2012.

[64] T. A. Seiflein and J. G. Lawrence, "Methionine-to-cysteine recycling in Klebsiella aerogenes," *J Bacteriol*, vol. 183, no. 1, pp. 336–346, 2001, doi: 10.1128/JB.183.1.336-346.2001.

[65] Y. Tan, M. Xu, and R. M. Hoffman, "Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells in vitro," *Anticancer Res*, vol. 30, no. 4, pp. 1041–1046, 2010.

[66] R. M. Hoffman and S. Yano, "Tumor-specific S/G2-Phase cell cycle arrest of cancer cells by methionine restriction," *Methods in Molecular Biology*, vol. 1866, pp. 49–60, 2019, doi: 10.1007/978-1-4939-8796-2_5.

[67] S. Chaturvedi, R. M. Hoffman, and J. R. Bertino, "Exploiting methionine restriction for cancer treatment," *Biochem Pharmacol*, vol. 154, no. May, pp. 170–173, 2018, doi: 10.1016/j.bcp.2018.05.003.

[68] D. Sato and T. Nozaki, "Methionine gamma-lyase: The unique reaction mechanism, physiological roles, and therapeutic applications against infectious diseases and cancers," *IUBMB Life*, vol. 61, no. 11, pp. 1019–1028, 2009, doi: 10.1002/iub.255.

[69] K. A. Carr and M. J. Arduino, "Isolation and Characterization of Bacterial L-Methioninase as an Effective Anti-Tumor Agent," *Bios*, vol. 52, no. 1, pp. 13–22, 1981, [Online]. Available: http://www.jstor.org/stable/4607648

[70] S. A. Khalaf and A. S. A. El-Sayed, "L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions," *Curr Microbiol*, vol. 58, no. 3, pp. 219–226, 2009, doi: 10.1007/s00284-008-9311-9.

[71] T. Nagahama, N. Goseki, and M. Endo, "Doxorubicin and vincristine with methionine depletion contributed to survival in the Yoshida sarcoma bearing rats," *Anticancer Research*, vol. 18, no. 1 A. pp. 25–31, 1998.

[72] M. A. Abu-Tahon and G. S. Isaac, "Comparative study of a new alkaline L-methioninase production by Aspergillus ustus AUMC 10151 in submerged and solid-state fermentation," *Brazilian Archives of Biology and Technology*, vol. 59, no. December, pp. 1–10, 2016, doi: 10.1590/1678-4324-2016150484.

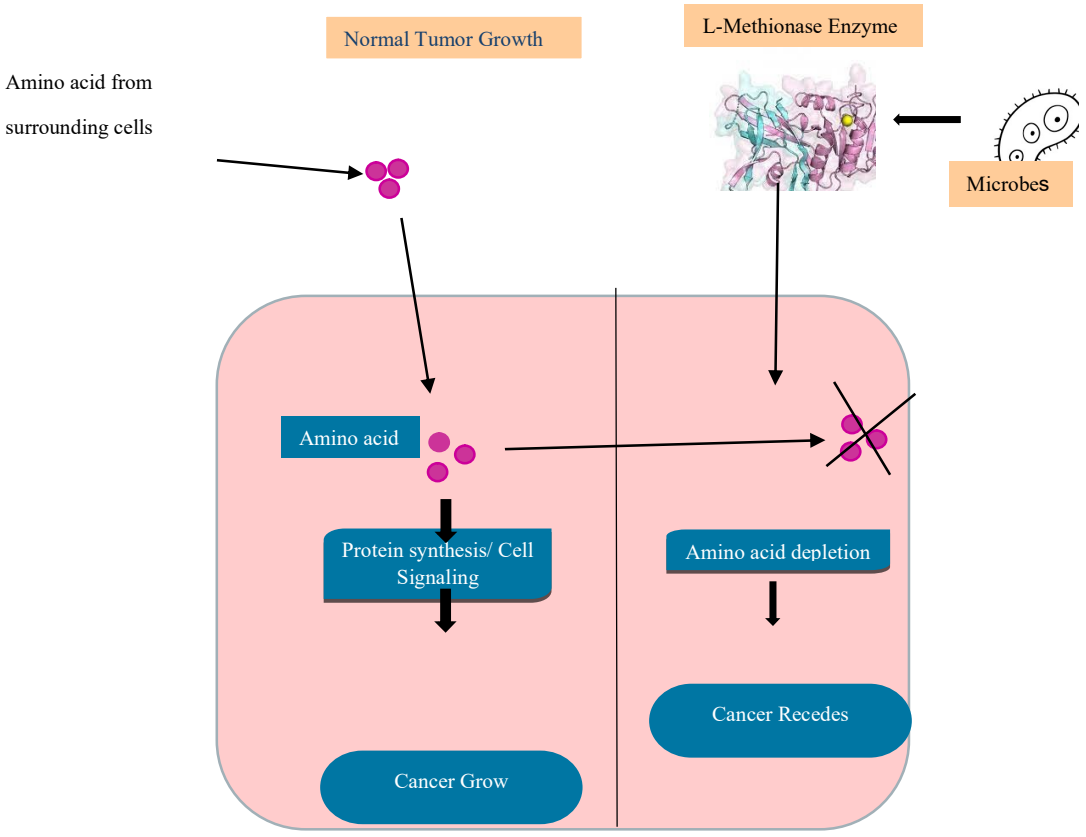

Figure 1: Mechanism of Action of L-Methionase Enzyme

Table 1: Microbial Sources of L-Methionase

Sources	Microbial Species	References
Bacteria	***Aeromonas sp***	**(Strekalova *et al*., 2019)**
	Arthrobacter sp	**(Jonsson *et al*., 2019)**
	Bacillus subtilis	**(Jambulingam & Sudhakar, 2019; Saengkerdsub, 2012)**
	Brevibacterium linens	**(Amarita *et al*., 2004)**
	Citrobacter freundii	**(Kahraman *et al*., 2011)**
	C.intuemedius	**(Faleev *et al*., 1996)**
	Clostridium sp	**(Kulikova *et al*., 2017; Pokrovsky *et al*., 2018)**
	Bacillus hynesii	**(Kotramada Bopaiah *et al*., 2020)**
	Bacillus cereus	**(Dike & Ekwealor, 2012)**
	Klebsiella aerogenes	**(Seiflein & Lawrence, 2001)**
	Pseudomonas putida	**(A. S. A. El-Sayed *et al*., 2017; Inagaki, n.d.)**
	Pseudomonas suturzi	**(abozeid, 2023)**
	Yarrwia lipolytica	**(Bondar *et al*., 2005)**
Fungi	***Aspergillus flavipes***	**(El-Sayed, 2009)**
	Aspergillus fumigatus	**(Hendy *et al*., 2023)**
	Aspergillus carneus	**(Kebeish & El- Sayed, 2012)**
	Trichoderma harzinum	**(Salim *et al*., 2019, 2020)**
	Candida tropicalis	**(Selim *et al*., 2015)**
	Aspergillus ustus	**(Abu-Tahon & Isaac, 2016)**
	F.solani	**(Kubota *et al*., 2022)**
	Geotricum candidum	**(Bonnarme *et al*., 2001)**
Protozoa	***Entamoeba histolytica***	**(Tokoro *et al*., 2003)**
	Treponema denticola	**(Fukamachi *et al*., 2005)**

Table 2: Methionine Restriction and Cancer in Cell Culture		
Cancer Model	**Effect of Methionine Restriction**	**References**
Lymphatic leukemia, human monocytic leukemia, rat liver epithelial cell, rat liver fibroblasts, human breast fibroblasts, human prostate fibroblasts	**Normal cells can grow methionine-depleted homocysteine-supplemented media, while cancer cells can't survive.**	**(Halpern *et al*., 1974)**
Human prostate cancer cell line, primary prostate epithelial cell	**MR synergistically enhances the anti-tumor effect of 5-FU by depletion of reduced folates, selective inhibition of thymidylate synthase, and creation of an imbalanced nucleotide pool.**	**(Lu *et al*., 2003)**
Human prostate cancer cell line	**MR in PC3 cells reduces mitochondrial membrane potential and induces caspase-dependent and independent apoptosis.**	**(Mazzucchelli *et al*., 2013)**
Human prostate cancer cell line	**MR led to an accumulation of the cyclin-dependent kinase inhibitors p21 and p27.**	**(Lu & Epner, 2000)**
Human prostate cancer cell line, human cervical carcinoma cell line	**MR induces apoptosis of prostate cancer cells via the N terminal kinase-mediated signaling pathway.**	**(Wallis *et al*., 2020)**
Human TNBC cell line	**Methionine deprivation increases the sensitivity to potential cancer drugs in TNBC cancer cells by enhancing TRAIL receptor-2 expression.**	**(Lu *et al*., 2002)**
Human TNBC cell line and a mouse model of TNBC	**Methionine deprivation inhibited the migration and invasion of cancer cells. In addition, methionine deprivation reduced the activation of FAK and expression of MMP-2 and MMP-9.**	**(Jonsson *et al*., 2019)**
Human TNBC cell line and mouse fibroblasts	**MR inhibited growth and induced apoptosis in TNBC cells in a GCN2 and PERK-independent mechanism.**	**(Strekalova *et al*., 2015b)**

Table 3: Methionine Restriction and Cancer in Animal Model		
Cancel model	**Effect of Methionine Restriction**	**References**
Human gastric cancer xenograft in nude mice	**Methionine depletion increased the 5-FU antitumor activity by modulating intra-tumoral folate metabolism.**	**(Takakura *et al*., 2006)**
Rat with Yoshida sarcoma	**Methionine deprivation inhibits tumor growth and metastasis with administration of 5-FU.**	**(Goseki *et al*., 1991)**
Mice injected with human pre-malignant breast epithelial cell line	**Methionine restriction inhibits the growth of breast tumors by increasing cell cycle inhibitors in nude mice.**	
F344 rat treated with azoxymethane to induce colon cancer	**Methionine restriction inhibits colonic tumor development during the post-initiation phase of carcinogenesis partially due to proliferation inhibition.**	**(Komninou *et al*., 2006)**
Transgenic Adenocarcinoma of the mouse prostate	**Methionine restriction inhibits prostatic intraepithelial neoplasia in TRAMP mice.**	**(Hens *et al*., 2016)**