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Research Article

MICROBIAL PRODUCTION OF L-METHIONINASE AND ITS BIOTECHNOLOGY APPLICATION

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ABSTRACT

L- Methioninase (methionine γ-lyase) has important biotechnological application because of exhibiting hydrolytic property to catalyze α-γ-elimination of L-methionine to α- ketobutyrate, methaneethiol and ammonia. The abnormality behavior of methionine based metabolic process result ageing, obesity, parkinsons, cardiovascular and cancer diseases among human being. The catalytic activity of L-methioninase could be used as enzyme supplementation therapy for these diseases. This enzyme is resent in wide range of organisms including, plant, bacteria, and fungi. Methioninase secreted from some bacterial species have high therapeutic value because of association with high immunogenicity and low substrate specificity. These enzymes are also used for cancer treatment by depleting supply of methionine from exogenous source to cancer cells. This review is an attempt to briefly describe the sources, mechanism of action, biochemical characterization and its therapeutic applications for treatment of ageing, obesity, parkinson's, cardiovascular disease and cancer.

INTRODUCTION

L-methioninase (EC 4.4.1.11) is a pyridoxal phosphate (PLP) dependent hydrolytic enzyme and also known as L-Methionine-γ-lyase, L-methionine-γ-demethiolase, methionase, and L-methionine-methanethiol-lyase. It is absent in mammalian system and intracellularly present in bacteria and extracellularly in fungi (1).

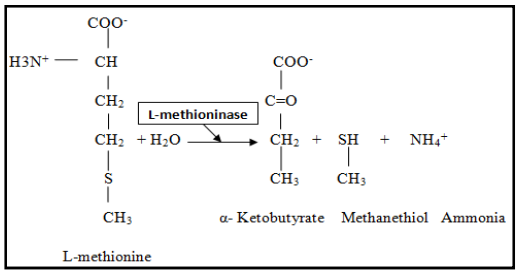

Fig 1 Catalytic pathway for catalysis of L-methioine by L-methioninase

This enzyme catalyzes the direct conversion of L-methionine ($C_5H_{11}NO_2S$) into α-ketobutyrate, ammonia, and methanethiol (2) by eliminating it's α γ-group as shown in Fig1. This enzyme was first isolated from the rumen bacteria (3, 4, and 5). Disruption and abnormalities in methionine metabolism and transmethyaltion are associated with the major disease in human, like ageing, obesity, parkinsons disease, heart disease and cancer. The therapeutic application of L-methioninase was studied from bacteria, yeast and fungi. L-methioninase secreted from bacterial source is mostly associated with high immunogenicity, low substrate specificity and hazardous effects to the kidney and liver (6). The therapeutic studies of this enzyme include studies of anti-homocysteine and anti-methionine chemotherapeutic treatment. This enzyme is also used for cancer treatment by depleting the growth of cancer cells to block the pathway for uptake of L-methionine from exogenous source (7). The incorporation of anti-mitotic and other cell-cycle specific, cytotoxic agent to L-methioninase improve its therapeutic effectiveness by cell cycle synchronization.

L-methioninase have high therapeutic value since it was reported as a potent anticancer agent against various types of tumor cell lines like glioblastoma, kidney, breast, colon and lung cancer (8,9). L-methionine as an essential amino acid which regulate cellular metabolism by synthesizing polyamines and glutathione which are further used for biosynthesis of protein, and expression of gene (10,11). The significant activity of this enzyme has been highly observed in patients of high-stage gastric cancer after applying chemotherapeutic drugs with methionine-free diet (12, 13).

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Thus, L-methioninase has received affordable attention as a therapeutic agent against various types of methionine-dependent tumors (14, 15 and 5). The injection of L-methioninase in nude mice intraperitoneally showed effective inhibition of the Yoshida Sarcoma and human lung tumor with minimal toxicity (16). Methioninase is chemically modified by coupling with Polyethylene glycol (PEG) polymer which has extended half-life, high methionine depletion activity, and low immunogenicity. L-methioninase has also lowered homocysteine levels in patients and hence applicable to treat cardiovascular disease (16).

This review will highlight the production, characterization of L-methioninase from different microbial strains and their effective therapeutic studies to treat various diseases.

Mechanism of Action of L-Methioninase

The hydrolytic activity of L-methioninase was started by attacking internal aldimine structure with the amine group of the methionine. The external aldimine would be formed by the transformation of Schiff's base and enzyme would be released from the lysine group. This released enzyme would further attack on tyrosine moiety and removes α- position of methionine from the hydrogen group resulting formation of ketimine. Quinonoid would be further formed by releasing thiol group after donating hydrogen group to the β- positions from the tyrosine moiety of hydroxyl group. The imine bond would be further attacked the water moiety and released the α-keto acid. The Internal aldimine formation would be further occurred by attacking lysine moiety of amine group from L-methioninase on amine bonds with release of ammonia (17, 2 and 18).

Sources of L-Methioninase

L-methioninase reported in different microorganism which includes bacteria, fungi, protozoa, yeast*, Arabidopsis thaliana,* and potato (19, 20). L-methioninase is produced as intracellular enzymes in many bacterial species. Both gram positive and gram negative bacteria are being reported to produce L-methioninase. Gram negative bacteria have gained more consideration as compared to gram positive bacteria.

Among fungal strains, some are reported to produce L-methioninase intracellular and some are producing extracellular (21). The production of L-methioninase from bacterial and fungal strains is being listed on table 1**.**

Table 1 Bacterial and fungal sources of L-methioninase

Bacterial Species	**References**
Achromobacter sp	Ruiz-Herrera and Starkey 1970 (22)
Aeromonas sp	Nakayama *et al*., 1984 (23)
Arthobacter sp	Bonnarme *et al*., 2000 (24)
Bacillus subtilis	Hullo *et al*. 2007 (25)
Brevibacterium linens	Amarita *et al*., 2004 (26)
Citrobacter sps.	Manukhov *et al*., 2005, Faleev *et al*., 1996 (27,17)
Clostridium sp.	Weisendanger and Nisman 1953; Kreis and Hession 1973a, b (5,27,28)
Corynebacterium glutamicm	Bonnarme *et al*., 2000 (24)
Escherichia coli	Ohigashi *et al*., 1951 (29)
Micrococcus luteus	Ohigashi *et al*., 1951 (29)
Pseudomonas sps.	Tanaka *et al*., 1976 (30)
Proteus rottgeri	Chen *et al*., 1971 (31)
Staphylococcus equorum	Bonnarme *et al*., 2000 (24)
Yarrwia lipolytica	Bondar *et al*., 2005 (32)
Fungal species	**References**
Aspergillus sps.	Khalaf and El-Sayed 2009, Ruiz- Herrera and Starkey 1969 (21,22)
Cladosporium sps.	Abu- Seidah and Youssef 2000, Khalaf and El-Sayed 2009 (33,21)
Debaromyces hansenii	Bonnarme *et al*., 2001 (24)
Fusarium sps.	Khalaf and El-Sayed 2009, (21)
Geotrichum candidum	Ruiz- Herrera and Starkey 1969 (22)
Humicola fuscoatra	Faleev *et al*., 1996 (17)
Kkuveromyces lactis	Bondar *et al*., 2005 (32)
Microsporum gypseum	Stahl *et al*., 1949 (34)
Mucor racemosus	Khalaf and El-Sayed 2009 (21)
Oidium lactis	Akobe 1936 (35)
Penicillium sps.	Khalaf and El-Sayed 2009 (21)
Trichoderma koningii	Khalaf and El-Sayed 2009 (21)
Schizophyllum commune	Challenger 1959 (36)
Scopulariopsis brevicaulis	Challenger 1959 (36)

Other microbial sources

Yeast like *Sacchromyces cerivisiae, Geotrichum candidum, Candida tropicalis, Debaromyces hasenii* are being reported to produce extracellular L-methioninase (1, 37). Some protozoans are also producing L-methininase extracellularly which includes *Entamoeba histolytica* (38), *Porphyromonas gingivalis* (39), *Treponema denticola* (40), *Trichomonas vaginalis* (41).

Structure and Metabolic Pathway of L-Methioninase

In eukaryotic organisms, methionine metabolism and transmethyaltion are of great importance in modification and regulation of nucleic acids, lipids and proteins. The differential methylation process of gene regulates their expression in the myriad of cells in eukaryotic organism.

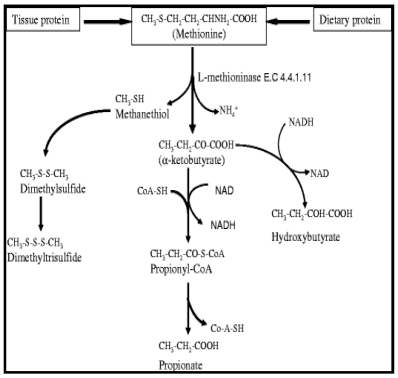

Fig 2 Deamination pathway of L- methionine by L-methioninase

Research has been carried out by many scientists to elucidate the structure of L-methioninase enzyme at the molecular level. Its structure has been predicted as tetramer similar to cystathionine- β-lyase from *Escherichia coli* (42,43). The molecular level structure of L-methioninase from *Citrobacter freundii, Clostridium sporogenes, Entamoeba histolytica, Micromonospora echinospora and Pseudomonas putida* have been thoroughly investigated (44,45). The analysis of strucutre of L- methioninase from *Pseudomonas putida* reveals the presence of f six conserved amino acids residues, Tyr59, Arg 61, Tyr114, Cys116, Lys240 and Asp241.These residues are present in the centre of the substrate, close to the pyridoxal phosphate in tetramer fashion (23, 46).

The mutational studies of *Entamoeba histolytica* and *Trepenoma vaginalis* from the L-methioninase isozymes demonstrated that the cysteine residues directly contribute to the specificity of substrate (47, 48). L- methioninase from the *Brevibacterium linens* neither degrades cystathionine nor

cysteines, whereas in the plant *Arabidopsis thaialana*, L-methioninase degrades cysteine, but hardly degrades cystathionine (49). The corresponding cysteine residues were substituted by glycine in both *Arabidopsis thaialana* and *Brevibacterium linens* (49, 50). The three dimensional structure of the external aldimine of *Citrobacter freundii* of L-methioninase with competitive inhibitor glycine has been determined at 2.45Å resolution (49).

Biochemical Characterization of L-methioninase

Different methods of L-methioninase for the purification and production from various organism have been reported which include submerged fermentation (SmF) and solid state fermentation (SSF) (51, 52). L-methioninase is used as an anticancer drug, which requires high level of purity. Some L-methioninase enzyme has reported intracellular in nature, which is difficult to handle as compared to extracellular enzymes. L-methioninase production from microbial sources is mostly methionine dependent. L-methionine present in aqueous medium and can be immediately oxidized via Maillard reaction in the presence of reducing sugars and ions and forming amadori compounds that subsequently reduce their bioavailability as carbon and nitrogen for the organism.

Many microorganisms including fungi degrade methionine but do not grow on it, because of their inability to metabolize the demethiolated product of α-ketobutyric acid and methanthiol residues of L-methionine. The inability of filamentous fungi to grow on L-methionine can be overcome by using co-dissimulator such as glucose and other carbohydrates. The uptake of L-methionine by fungi will be reduced after carrying fermentation process in carbon-free medium. The highest yield of L- methioninase was reported for production media supplemented with 1% glucose as a codissimilator because of the activation of plasma membrane H^+ adenosine triphosphatase (ATPase) (53). L-methionine is provided as inducer for its production from many microbial strains like *Pseudomonas ovalis*, *Achromobacter starkeyi, Aspergillus flavipes*, and *Yarrwia lipolytica* (22, 32). The optimum concentration of L-methionine was reported as 0.8% for producing higher amount of L-methioninase in these microbial strains. L-methioninase from *Geotrichum candidum* and *Pseudomonas putida* showed higher yield for production media without L-methionine (24). The optimum production of L-methioninase was reported in fungal strains growing in production media supplemented with 0.32% KH_2PO_4 and 0.24% K_2HPO_4.

Table 2 Purification methods and properties of L-methioninase from different microbes

Microbes	Molecular weight (kDa)	K_m(mM)	Catlytic efficiency	Optimum pH	Optimum temp(°C)	References
Aeromonas sps	41	-	-	8.0	25-30	Nakayama *et al.*, 1984 (23)
Arabidopsis thaliana	48	72	9.5	8.0	30	Goyer *et al.*, 2007 (49)
Aspergillus flavipes	47	6.5	39.6	7	37	El-Sayed 2011 (53)
Brevibacterium linens	43	6.12	-	25	6.8-8	Suwabe *et al.*, 2011 (54)
Candida tropicalis	46	0.5	-	45-55	6-8	Dias and Weimer 1998a,1998b (36,55)
Clostridium sporogenes	37.5	90	-	-		Kreis and Hession 1973a, 1973b (7,28)
Citrobactor freundii	43	0.7	6.5	-	-	Ruiz-Herrera and Starkey 1969 (22)
Fusobacterium nucleatum	43	0.32	-	25	-	Nakayama *et al.*, 1984 (23)
Pseudomonas ovalis	45	1.33	-	37	7.2	Tanaka *et al.*, 1976 (30)
Pseudomonas putida	42	0.8	-	37	8	Manukhov *et al.*, 2006 (27)
Trichomonas vaginalis	44	4.3	-	37	7-8	Lockwood and Coombs 1991 (41)
Treponema denticola	43	0.55	-	-	-	Fukamachi *et al.*, 2005 (40)
Streptomyces sp.	47	0.7	441	-	-	Huang *et al.*, 2014 (19)
Streptomyces variabilis 3MA2016		0.43	-	30-40	7-8	El-Awady *et al.*, 2017 (56)

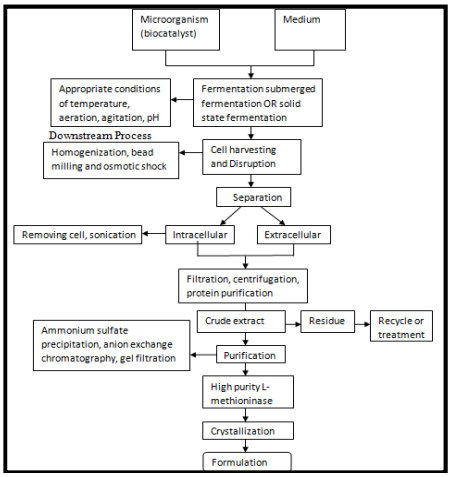

Fig 3 Schematic representation for an industrial process for L-methioninase production

Different Assay Method for Estimation of Activity of L-Methioninase

One international unit of L-methioninase has been defined as the amount of enzyme required to liberate 1micromolar of methanethiol product under standard condition. Methanethiol is an organosulfure compound which is specrophotometrically estimated by using Ellman's reagent (DTNB) (5, 5′-dithio-bis (2-nitrobenzoic acid) (57). TNB (2-nitro-5-thiobenzoic acid) as coloured species was released after reaction of DTNB with a sulfhydryl group of the methanethiol which was spectrophotometrically measured in visible range. Other product of α-ketobutyrate was measured spectrophotometrically with "Oxidation followed by coupling reaction using MBTH (3-Methyl-2-Benzothiazoline Hydrazone) (58,59). The released product of α-Keto acids after αɣ-elimination of methioninase was estimated as 2,4-dinitrophenylhydrazone derivatives by thin-layer chromatography (TLC) in terms of specific R_f values (60,61). The deamination rate was calculated with the help of nessler's reagent by forming a precipitate of mercury (II) amido-iodide with end product ammonia (62). The colour of the precipitate varies from yellow to brown, depending on the quantity of ammonium ion, which could be measured at 450 nm (63,64).

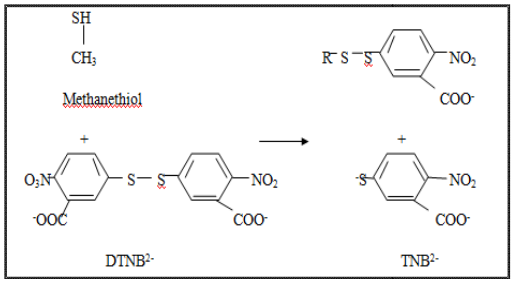

Fig 4 Methioninase quantitative detection by DTNB

Biotechnological Applications of Microbial L-Methioninase

Role of L-Methioninase in Cancer

Cancer is the uncontrolled cellular growth causing change in expression of tumor suppressing genes and tumor promoting genes. Majority of cancers results from alterations in DNA integrity caused by environmental genotoxic factors and endogenous metabolism (65). L-methioninase has been isolated from rumen bacteria*, Pseudomonas putida, Pseudomonas ovalis* and *Clostridium sporogenes* and *Escherichia coli*. Among these microbial strains, L-methioninase from *Clostridium sporogenes* showed inhibitory effect against carcinoma cell culture of ascites and Walker carcinoma in rats (7, 28). L-methioninase dependent antitumor drugs have been developed by targeting the inhibition of exogenous supply of L-methionine for cancer cell cultures. Therefore, depletion of methionine from the reinjection of homocysteine in blood plasma is the basis of chemotherapeutic treatment for tumours (66). Currently retroviral vectors gene therapy has been studied by transduction of microbial L-methioninase gene into cancer cells (67,68,69,70,71). A recombinant adenovirus having the methioninase gene showed a strong antitumor activity against fibrosarcoma and human ovarian cancer (70, 71).

Most of the researcher found that in vivo therapeutic effects of the enzyme L-methioninase usually have some minimal complications like vomiting, weight loss, mild anemia (72, 73). Besides the potential antitumor efficacy of methioninase, it has quick plasma clearance, short biological half-life time and high immunogenicity (74). Half life and depletion of L-methioninase in the mouse plasma was improved by conjugation with polyethylene glycol (PEG), pyridoxal 5'-phosphate, oleic acid, dithiothreitol (6). Some therapeutic drugs of L-methioninase are modulated by activating the suppressor of apoptosis-mediating genes to induce apoptosis and selectively kill the cancerous cells (75, 76). The anticancer activity of L-methioninase from *Pseudomonas putida* has been extensively investigated in plasma of mouse (77, 78). The antiproliferative property of this enzyme has been already reported against Lewis lung and human colon carcinoma (9), glioblastoma (8), and neuroblastoma (9). Recombinant methioninase from *Pseudomonas putida* (PpMGL) has been currently developed as effective anticancer drug to deplete methionine level in cancer cells. The conjugation of these enzymes with cisplatin, 5-fluorouracil (5-FU), 1-3-bis(2-chloroethyl)-1- nitrosourea (BCNU), and vincristine has improved the efficacy and synergy in mouse models against colon cancer, lung cancer, and brain cancer. Selenomethionine (SeMET) acts as suicide prodrug substrate for L-methioninase and effectively inhibit the tumour growth in rodents and prolonged their survivals (69, 70). These SeMET biomolecules enhanced oxidative stress in tumour cells which result exposure of phosphatidylserine on the surface of the vascular endothelium of blood vessels in tumours but not on normal. The injection of fusion protein (FP) consisting of L-methioninase conjugated to human annexin-V into the bloodstream will bind to marker on vascular endothelial cells of the tumour cells. These biomolecules catalyzed the conversion of nontoxic selenomethionine into toxic methylselenol which inhibited the growth of tumpr cells by blocking exogenous source of methionine uptake by tumour cells (71, 76).

Role of L-Methioninase in Other Therapeutic Treatment

Aging has been associated with changes in DNA methylation and hypermethylation of the estrogen receptor gene in aging colons which cause hematopoietic neoplasms (79). Promoter controlling methylation of the IGF2 gene is greatly increased in human colon as a function of aging (79). The dietary methionine deprivation after adding methioninase as food supplement has been studied to control weight gain exquisitely in the rats. The study showed that reduction of L-methionine in diet from 0.86 to 0.17% results in 30% longer life span of male Fischer 344 rats (80). In recent year, recombinant methioninase has been studied for pathogenekharayat30sis of obesity in mice by targeting melanocyte- stimulating hormone (MSH), melanocortin-4 receptor and its peptide ligand (81, 82, 83, 84). This enzyme has been also studied for treatment of parkinsons disease in rats (85). S-adenosyl-L-methionine has been considered as rate limiting endogenous methyl donor for the methylation of dopamine. The drug levodopa has been formulated for Parkinson disease by blocking the metabolic pathway regulated by S-adenosyl-L-methionine using catechol-O-methyltransferase (COMT) and L-dopa decarboxylase (86). In one study, L-methioninase has also lowered the level of homocysteine in plasma of rat suffered from Parkinson's disease (85).

Elevated level of serum total homocysteine (tHCY) has been reported as important risk factor for cardiovascular disease. In recent year, recombinant methioninase (rMETase) have been reported to cause depletion in level of serum methionine and homocysteine. Methioninase from *Trichomonas vaginalis* also showed high substrate specificity and high catalytic efficiency towards HCY-induced cardiovascular disease (82).

Role of L-Methioninase in Food Industry

L-methioninase has important role in food industry by imparting a specific aroma in variety of cheese like limburger, camembert, blue cheeses and cheddar (89). The enzymatic catalysis of L-methionine in presence of L-methioninase from yeast strains secreted a volatile sulfur component of methanethiol which has been used as flavor enhacer for cheese industry (24, 90). The demethiolating activity of *Brevibacterium linens* is used commercially as cheese ripening bacterium (88). Some yeast strains (*Kluyveromyces lactis, Kluyveromyces marxianus* and *Saccharomyces cerevisia)* and some bacterial strains (*Lactobacilli*, *Lacococci*) are considered as safe organisms and used commercially as food additive (87, 90).

CONCLUSION

The biotechnology importance of microbial L-methioninase in food and pharmaceutical sectors has been extensively studied by scientists from last few decades. The microbial production of enzyme is cost effective and easily produced, which has drawn significant attention to explore new strain for producing L-methioninase having therapeutic values. Therefore, in this review, we briefly describe the historical aspects of microbial L-methioninase, their biochemical characterization and their industrial application.

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References

1. Sharma, B., Singh, S. and Kanwar, S.S., 2014. L-methionase: a therapeutic enzyme to treat malignancies. *BioMed research international*, *2014*.
2. Tanaka, H., Esaki, N. and Soda, K., 1985. A versatile bacterial enzyme: L-methionine γ-lyase. *Enzyme and Microbial Technology*, 7(11), pp.530-537.
3. Kallio, R.E. and Larson, A.D., 1955. Methionine degradation by a species of *Pseudomonas.* In A symposium on amino acid metabolism*. Johns Hopkins Press, Baltimore* , pp. 616-631.
4. Miwatani, T., Omukai, Y. and Nakada, D., 1954. Enzymatic cleavage of methionine and homocysteine by bacteria. *Medical journal of Osaka university 5*, pp.347-352.
5. Wiesendanger, S. and Nisman, B., 1953. L-Methionine demercapto-deaminase; a new enzyme requiring pyridoxal-phosphate. *Comptes rendus hebdomadaires des seances de l'Academie des sciences*, 237(14), pp.764-765.
6. Sun, X., Yang, Z., Li, S., Tan, Y., Zhang, N., Wang, X., Yagi, S., Yoshioka, T., Takimoto, A., Mitsushima, K. and Suginaka, A., 2003. In vivo efficacy of recombinant methioninase is enhanced by the combination of polyethylene glycol conjugation and pyridoxal 5′-phosphate supplementation. *Cancer research*, 63(23), pp.8377-8383.
7. Kreis, W. and Hession, C., 1973a .Biological effects of enzymatic deprivation of L-methionine in cell culture and an experimental tumor. *Cancer Research*, 33(8), pp.1866-1869.
8. Kokkinakis, D.M., Hoffman, R.M., Frenkel, E.P., Wick, J.B., Han, Q., Xu, M., Tan, Y. and Schold, S.C., 2001. Synergy between methionine stress and chemotherapy in the treatment of brain tumor xenografts in athymic mice. *Cancer Research*, 61(10), pp.4017-4023.
9. Tan, Y., Sun, X., Xu, M., An, Z., Tan, X., Tan, X., Han, Q., Miljkovic, D.A., Yang, M. and Hoffman, R.M., 1998. Polyethylene glycol conjugation of recombinant methioninase for cancer therapy. *Protein Expression and Purification*, 12(1), pp.45-52.
10. Davis, C.D. and Uthus, E.O., 2004. DNA methylation, cancer susceptibility, and nutrient interactions. *Experimental Biology and Medicine*, 229(10), pp.988-995.
11. Baylln, S.B., Herman, J.G., Graff, J.R., Vertino, P.M. and Issa, J.P., 1997. Alterations in DNA methylation: a fundamental aspect of neoplasia. In *Advances in cancer research*, 72, pp. 141-196. Academic Press.
12. Goseki, N., Yamazaki, S., Shimojyu, K., Kando, F., Maruyama, M., Endo, M., Koike, M. and Takahashi, H., 1995. Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. *Cancer Science*, 86(5), pp.484-489
13. Guo, H., Lishko, V.K., Herrera, H., Groce, A., Kubota, T. and Hoffman, R.M., 1993. Therapeutic tumor-specific cell cycle block induced by methionine starvation in vivo. *Cancer research*, 53(23), pp.5676-5679.
14. Kokkinakis, D.M., Schold Jr, S.C., Hori, H. and Nobori, T., 1997. Effect of long-term depletion of plasma methionine on the growth and survival of human brain tumor xenografts in athymic mice.
15. Kallio RE, Larson AD (1955) Methionine degradation by a species of *Pseudomonas*. A symposium on amino acid metabolism. *Johns Hopkins Press, Baltimore*, pp. 616–631.
16. Tan Y, Xu M, Guo H, Sun X, Kubota T, Hoffman RM (1996) Anticancer efficiency of methioninase in vivo. *Anticancer Res,* 16, pp.3931–3936.
17. Faleev, N.G., Troitskaya, M.V., Paskonova, E.A., Saporovskaya, M.B. and Belikov, V.M., 1996. L-Methionine-γ-lyase in *Citrobacter intermedius* cells: stereochemical requirements with respect to the thiol structure. *Enzyme and Microbial Technology*, 19(8), pp.590-593.
18. Chin, H.W. and Lindsay, R.C., 1994. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. *Food Chemistry*, 49(4), pp.387-392.
19. Huang, T., Joshi, V. and Jander, G., 2014. The catabolic enzyme methionine gamma-lyase limits methionine accumulation in potato tubers. *Plant biotechnology journal*, 12(7), pp.883-893.
20. Sato, D. and Nozaki, T., 2009. Methionine gamma-lyase: The unique reaction mechanism, physiological roles, and therapeutic applications against infectious diseases and cancers. *International Union of Biochemistry and Molecular Biology Life* , 61(11), pp.1019-1028.
21. El-Sayed, A.S., 2009. L-methioninase production by *Aspergillus flavipes* under solid-state fermentation. *Journal of basic microbiology*, 49(4), pp.331-341.
22. Ruiz-Herrera, J. and Starkey, R.L., 1969. Dissimilation of methionine by fungi. *Journal of Bacteriology*, 99(2), pp.544-551.
23. Nakayama, T., Esaki, N., Tanaka, H. and Soda, K., 1988 a. Chemical modification of cysteine residues of L-methionine γ-lyase. *Agricultural and biological chemistry*, 52(1), pp.177-183.
24. Bonnarme, P., Arfi, K., Dury, C., Helinck, S., Yvon, M. and Spinnler, H.E., 2001. Sulfur compound production

by *Geotrichum candidum* from L-methionine: importance of the transamination step. *FEMS Microbiology Letters*, 205(2), pp.247-252.
25. Hullo, M.F., Auger, S., Soutourina, O., Barzu, O., Yvon, M., Danchin, A. and Martin-Verstraete, I., 2007. Conversion of methionine to cysteine in *Bacillus subtilis* and its regulation. *Journal of bacteriology*, 189(1), pp.187-197.
26. Amarita, F., Yvon, M., Nardi, M., Chambellon, E., Delettre, J. and Bonnarme, P., 2004. Identification and functional analysis of the gene encoding methionine-γ-lyase in *Brevibacterium linens*. *Applied and environmental microbiology*, 70(12), pp.7348-7354.
27. Manukhov, I.V., Mamaeva, D.V., Morozova, E.A., Rastorguev, S.M., Faleev, N.G., Demidkina, T.V. and Zavilgelsky, G.B., 2006. L-methionine γ-lyase from *Citrobacter freundii*: cloning of the gene and kinetic parameters of the enzyme. *Biochemistry (Moscow)*, 71(4), pp.361-369.
28. Kreis, W. and Hession, C., 1973b. Isolation and purification of L-methionine-α-deamino-γ-mercaptomethane-lyase (L-methioninase) from *Clostridium sporogenes*. *Cancer Research*, 33(8), pp.1862-1865.
29. Ohigashi, K., Tsunetoshi, A. and Ichihara, K., 1951. The role of pyridoxal in methylmercaptan formation, partial purification and resolution of methioninase. *Medical Journal of Osaka University*, *2*(2), pp.111-117.
30. Tanaka, H., Esaki, N., and Soda, K., 1976. Purification and properties of methioninase from *Pseudomonas ovalis. FEBS letters*, 66(2), pp. 307-311.
31. Chen, S.S., Walgate, J.H. and Duerre, J.A., 1971. Oxidative deamination of sulfur amino acids by bacterial and snake venom L-amino acid oxidase. *Archives of biochemistry and biophysics*, 146(1), pp.54-63.
32. Bondar, D.C., Beckerich, J.M. and Bonnarme, P., 2005. Involvement of a branched-chain aminotransferase in production of volatile sulfur compounds in *Yarrowia lipolytica*. *Applied and environmental microbiology*, 71(8), pp.4585-4591.
33. Abu-Seidah, A.A. and Youssef, M.S., 2000. Characterization of L-methionine g-lyase from *Cladosporium cladosporioides*. *Bulletin of the Faculty Science Assiut University Egypt*, *130*, pp.83-91.
34. Stahl, W.H., McQue, B., Mandels, G.R. and Siu, R.G.H., 1949. Studies on the microbiological degradation of wool. I. Sulfur metabolism. *Archieves of Biochemistry.*, 20(2).
35. Akobe, K., 1936. Darstellung von d-und 1-α-Oxy-γ-methiobuttersaure und damit ausgefuhrte Ernahrungsversuche. *Hoppe-Seyler´ s Zeitschrift für physiologische Chemie*, 244(1-2), pp.14-18.
36. Challenger F (1959) Aspects of the organic chemistry of sulfur. Academic, New York.
37. El-Sayed and A.S., 2010. Microbial L-methioninase: production, molecular characterization, and therapeutic applications. *Applied Microbiology and Biotechnology*, 86(2), pp.445-467.
38. Tokoro, M., Asai, T., Kobayashi, S., Takeuchi, T. and Nozaki, T., 2003. 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*, 278(43), pp.42717-42727
39. Yoshimura, M., Nakano, Y., Yamashita, Y., Oho, T., Saito, T. and Koga, T., 2000. Formation of Methyl Mercaptan froml-Methionine by *Porphyromonas gingivalis*. *Infection and immunity*, 68(12), pp.6912-6916.
40. Fukamachi, H., Nakano, Y., Okano, S., Shibata, Y., Abiko, Y. and Yamashita, Y., 2005. High production of methyl mercaptan by L-methionine-α-deamino-γ-mercaptomethane lyase from *Treponema denticola*. *Biochemical and biophysical research communications*, 331(1), pp.127-131.
41. Lockwood, B.C. and Coombs, G.H., 1991. Purification and characterization of methionine γ-lyase from *Trichomonas vaginalis*. *Biochemical Journal*, 279(3), pp.675-682.
42. Clausen, T., Huber, R., Laber, B., Pohlenz, H.D. and Messerschmidt, A., 1996. Crystal Structure of the Pyridoxal-5′-phosphate Dependent Cystathionine β-lyase from *Escherichia coli* at 1.83 Å. *Journal of molecular biology*, 262(2), pp.202-224.
43. Johnston, M., Jankowski, D., Marcotte, P., Tanaka, H., Esaki, N., Soda, K. and Walsh, C., 1979. Suicide inactivation of bacterial cystathionine. gamma-synthase and methionine. gamma-lyase during processing of L-propargylglycine. *Biochemistry*, 18(21), pp.4690-4701.
44. Nikulin, A., Revtovich, S., Morozova, E., Nevskaya, N., Nikonov, S., Garber, M. and Demidkina, T., 2008. High-resolution structure of methionine γ-lyase from *Citrobacter freundii*. *Acta Crystallographica Section D: Biological Crystallography*, 64(2), pp.211-218.
45. Kudou, D., Misaki, S., Yamashita, M., Tamura, T., Takakura, T., Yoshioka, T., Yagi, S., Hoffman, R.M., Takimoto, A., Esaki, N. and Inagaki, K., 2007. Structure of the antitumour enzyme l-methionine γ-lyase from *Pseudomonas putida* at 1.8 Å resolution. *Journal of biochemistry*, 141(4), pp.535-544.
46. Nakayama, T., Esaki, N., Tanaka, H. and Soda, K., 1988 b. Specific labeling of the essential cysteine residue of L-methionine. gamma.-lyase with a cofactor analog, N-(bromoacetyl) pyridoxamine phosphate. *Biochemistry*, 27(5), pp.1587-1591.
47. Sato, D., Yamagata, W., Harada, S. and Nozaki, T., 2008. Kinetic characterization of methionine γ-lyases from the enteric protozoan parasite *Entamoeba histolytica* against physiological substrates and trifluoromethionine, a promising lead compound against amoebiasis. *The FEBS journal*, 275(3), pp.548-560.
48. McKie, A.E., Edlind, T., Walker, J., Mottram, J.C. and Coombs, G.H., 1998. The Primitive Protozoon *Trichomonas vaginalis* contains Two Methionine γ-Lyase genes that encode members of the γ-Family of Pyridoxal 5′-Phosphate-dependent Enzymes. *Journal of Biological Chemistry*, 273(10), pp.5549-5556.
49. Goyer, A., Collakova, E., Shachar-Hill, Y. and Hanson, A.D., 2007. Functional characterization of a methionine γ-lyase in *Arabidopsis* and its implication in an alternative to the reverse trans-sulfuration pathway. *Plant and Cell Physiology*, *48*(2), pp.232-242.

50. Dias, B. and Weimer, B., 1998 a. Conversion of methionine to thiols by *lactococci, lactobacilli,* and *brevibacteria*. *Applied and Environmental Microbiology*, *64*(9), pp.3320-3326.
51. Khalaf, S.A. and El-Sayed, A.S., 2009 a. L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions. *Current microbiology*, 58(3), pp.219-226.
52. Khalaf, S.A. and El-Sayed, A.S., 2009 b. L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions. *Current microbiology*, 58(3), pp.219-226.
53. El-Sayed, A.S., 2011. Purification and characterization of a new L-methioninase from solid cultures of *Aspergillus flavipes*. *The Journal of Microbiology*, 49(1), pp.130-140.
54. Suwabe, K., Yoshida, Y., Nagano, K. and Yoshimura, F., 2011. Identification of an L-methionine γ-lyase involved in the production of hydrogen sulfide from L-cysteine in *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586. *Microbiology*, 157(10), pp.2992-3000.
55. Dias, B. and Weimer, B., 1998 b. Purification and Characterization ofl-Methionine γ-Lyase from *Brevibacterium linens* BL2. *Applied and Environmental Microbiology*, 64(9), pp.3327-3331.
56. El Awady, M.E., Selim, M.S., Abd El-Razek, A.S. and Asker, M., 2017. Production, Purification and Characterization of L-Methioninase from *Streptomyces Variabilis* 3MA2016. *Research journal of Pharmaceutical Biological and Chemical sciences*, 8(3), pp.906-921.
57. Riddles, P.W., Blakeley, R.L. and Zerner, B., 1979. Ellman's reagent: 5, 5′-dithiobis (2-nitrobenzoic acid)—a reexamination. *Analytical biochemistry*, 94(1), pp.75-81.
58. Frenzel, W., Oleksy-Frenzel, J. and Morlen, J., 1992. Spectrophotmetric determination of phenolic compounds by flow-injection analysis. *Analytica chimica acta*, 261(1-2), pp.253-259.
59. Soda, K., 1967. A spectrophotometric microdetermination of keto acids with 3-methyl-2-benzothiazolone hydrazone. *Agricultural and Biological Chemistry*, 31(9), pp.1054-1060.
60. Takakura, T., Mitsushima, K., Yagi, S., Inagaki, K., Tanaka, H., Esaki, N., Soda, K. and Takimoto, A., 2004. Assay method for antitumor L-methionine γ-lyase: comprehensive kinetic analysis of the complex reaction with L-methionine. *Analytical biochemistry*, 327(2), pp.233-240.
61. Johnston, M., Raines, R., Chang, M., Esaki, N., Soda, K. and Walsh, C., 1981. Mechanistic studies on reactions of bacterial methionine-lyase with olefinic amino acids. *Biochemistry*, *20*, pp.4325-4333.
62. Krug, F.J., Ruzicka, J. and Hansen, E.H., 1979. Determination of ammonia in low concentrations with Nessler's reagent by flow injection analysis. *Analyst*, *104*(1234), pp.47-54.
63. Sun, X., Tan, Y., Yang, Z., Li, S. and Hoffman, R.M., 2005. A rapid HPLC method for the measurement of ultra-low plasma methionine concentrations applicable to methionine depletion therapy. *Anticancer research*, 25(1A), pp.59-62.
64. Kominami, G., Agou, T., Kanda, A. and Ohno, M., 2002. Immunoenzymometric assay for recombinant methioninase in biological fluids. *Journal of pharmaceutical and biomedical analysis*, 30(3), pp.733-738.
65. Stratton, M.R., Campbell, P.J. and Futreal, P.A., 2009. The cancer genome. *Nature*, 458(7239), p.719.
66. Breillout, F., Antoine, E. and Poupon, M.F., 1990. Methionine dependency of malignant tumors: a possible approach for therapy. *JNCI: Journal of the National Cancer Institute*, 82(20), pp.1628-1632.
67. Gupta, A., Miki, K., Xu, Mingxu., Yamamoto, N., Moossa, A.R. and Hoffman, R.M., 2003. Combination efficacy of doxorubicin and adenoviral methioninase gene therapy with prodrug selenomethionine. *Anticancer research*, 23(2B), pp.1181-1188.
68. Yamamoto, N., Gupta, A., Xu, M., Miki, K., Tsujimoto, Y., Tsuchiya, H., Tomita, K., Moossa, A.R. and Hoffman, R.M., 2003.Methioninase gene therapy with selenomethionine induces apoptosis in bcl-2-overproducing lung cancer cells. *Cancer Gene Therapy*, 10(6), p.445.
69. Miki, K., Xu, M., Gupta, A., Ba, Y., Tan, Y., Al-Refaie, W., Bouvet, M., Makuuchi, M., Moossa, A.R. and Hoffman, R.M., 2001. Methioninase cancer gene therapy with selenomethionine as suicide prodrug substrate. *Cancer research*, 61(18), pp.6805-6810.
70. Miki, K., Al-Refaie, W., Xu, M., Jiang, P., Tan, Y., Bouvet, M., Zhao, M., Gupta, A., Chishima, T., Shimada, H. and Makuuchi, M., 2000. Methioninase gene therapy of human cancer cells is synergistic with recombinant methioninase treatment. *Cancer research*, 60(10), pp.2696-2702.
71. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S. and Wang, X., 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell*, *91*(4), pp.479-489.
72. Yang, Z., Wang, J., Lu, Q., Xu, J., Kobayashi, Y., Takakura, T., Takimoto, A., Yoshioka, T., Lian, C., Chen, C. and Zhang, D., 2004 a. PEGylation confers greatly extended half-life and attenuated immunogenicity to recombinant methioninase in primates. *Cancer research*, 64(18), pp.6673-6678.
73. Yang, Z., Wang, J., Yoshioka, T., Li, B., Lu, Q., Li, S., Sun, X., Tan, Y., Yagi, S., Frenkel, E.P. and Hoffman, R.M., 2004b. Pharmacokinetics, methionine depletion, and antigenicity of recombinant methioninase in primates. *Clinical Cancer Research*, 10(6), pp.2131-2138.
74. Vellard, M., 2003. The enzyme as drug: application of enzymes as pharmaceuticals. *Current Opinion in Biotechnology*, 14(4), pp.444-450.
75. Merlo, A., Herman, J.G., Mao, L., Lee, D.J., Gabrielson, E., Burger, P.C., Baylin, S.B. and Sidransky, D., 1995. 5′ CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nature Medicine*, 1(7), p.686.

76. Zingg, J.M., Shen, J.C., Yang, A.S., Rapoport, H. and Jones, P.A., 1996. Methylation inhibitors can increase the rate of cytosine deamination by (cytosine-5)-DNA methyltransferase. *Nucleic acids research*, 24(16), pp.3267-3275.
77. Smiraglia, D.J., Rush, L.J., Frühwald, M.C., Dai, Z., Held, W.A., Costello, J.F., Lang, J.C., Eng, C., Li, B., Wright, F.A. and Caligiuri, M.A., 2001. Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. *Human molecular genetics*, 10(13), pp.1413-1419.
78. Issa, J.P.J., Baylin, S.B. and Belinsky, S.A., 1996. Methylation of the estrogen receptor CpGisland in lung tumors is related to the specific type of carcinogen exposure. *Cancer Research*, 56(16), pp.3655-3658.
79. Orentreich, N., Matias, J.R., DeFelice, A. and Zimmerman, J.A., 1993. Low methionine ingestion by rats extends life span. *Journal of Nutrition*, 123(2), pp.269-274.
80. Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J. and Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature*, *385*(6612), p.165.
81. Erickson, J.C., Hollopeter, G. and Palmiter, R.D., 1996. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science*, *274*(5293), pp.1704-1707.
82. Bultman, S.J., Michaud, E.J. and Woychik, R.P., 1992. Molecular characterization of the mouse agouti locus. *Cell*, *71*(7), pp.1195-1204.
83. Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D. and Smith, F.J., 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell*, *88*(1), pp.131-141.
84. Crowell Jr, B.G., Benson, R., Shockley, D. and Charlton, C.G., 1993. S-adenosyl-L-methionine decreases motor activity in the rat: similarity to Parkinson's disease-like symptoms. *Behavioral and neural biology*, *59*(3), pp.186-193.
85. Allain, P., Le Bouil, A., Cordillet, E., Le Quay, L., Bagheri, H. and Montastruc, J.L., 1995. Sulfate and cysteine levels in the plasma of patients with Parkinson's disease. *Neurotoxicology*, 16(3), pp.527-529.
86. Ferchichi, M., Hemme, D., Nardi, M. and Pamboukdjian, N., 1985. Production of methanethiol from methionine by *Brevibacterium linens* CNRZ 918. *Microbiology*, 131(4), pp.715-723.
87. Cuer, A., Dauphin, G., Kergomard, A., Roger, S., Dumont, J.P. and Adda, J., 1979. Production of S-methyl thioacetate by *Brevibacterium linens. Applied Environmental Biology* 38(2) pp.332-334.
88. Weimer, B., Seefeldt, K. and Dias, B., 1999. Sulfur metabolism in bacteria associated with cheese. In *Lactic acid bacteria: Genetics, metabolism and applications* pp. 247-261, Springer, Dordrecht.
89. Arfi, K., Amárita, F., Spinnler, H.E. and Bonnarme, P., 2003. Catabolism of volatile sulfur compounds precursors by *Brevibacterium linens* and *Geotrichum candidum*, two microorganisms of the cheese ecosystem. *Journal of Biotechnology*, 105(3), pp.245-253.
90. Selhub, J., Jacques, P.F., Bostom, A.G., D'Agostino, R.B., Wilson, P.W., Belanger, A.J., O'Leary, D.H., Wolf, P.A., Schaefer, E.J. and Rosenberg, I.H., 1995. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *New England Journal of Medicine*, 332(5), pp.286-291.

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