

Microbial sources and some therapeutic applications of L-methioninase

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Abstract: Natural products are produced by a wide range of different organisms including microorganisms. It is a source of compounds opening promising avenues for the treatment of a great variety of diseases accurately in a proper manner to specifically target cancer cells. Over the past 50 years, cancer has become a problem that threatens human health. According to the world health organization WHO website, there were 9.6 million people died from cancer and 18.1 million new cancer cases worldwide in 2018, with 60% of world's total new annual cases occurring in Africa, Asia, and Central and South America. The percentage of cancer deaths in Asia and Africa (57.3% and 7.3%, respectively) are higher than the ratios of incident cases (48.4% and 5.8%, respectively). L-methioninase is attracted a great deal of attention due to has potential application as an active therapeutic agent against cardiovascular diseases and different types of cancer in human beings and other applications. L-methioninase from diverse microorganisms exhibits significant reductions in L-methionine in vivo and efficacy against a broad spectrum of transplantable animal and solid human tumors.

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Review

Over the past 50 years, cancer has become a problem that threatens human health. According to the world health organization WHO website, there were 9.6 million people died from cancer and 18.1 million new cancer cases worldwide in 2018, with 60% of world's total new annual cases occurring in Africa, Asia, and Central and South America. The percentage of cancer deaths in Asia and Africa (57.3% and 7.3%, respectively) are higher than the ratios of incident cases (48.4% and 5.8%, respectively). Malignant growths of the lung and female breast are the main sorts worldwide as far as the number of new cases; for every one of these sorts, during 2018, roughly 2.1 million diagnoses are assessed, offering about 11.6% of the total cancer occurrence burden. Colorectal cancer is the third most regularly diagnosed cancer (1.8 million cases, 10.2% of the total), prostate cancer (1.3 million cases, 7.1%) is the fourth and stomach cancer (1.0 million cases, 5.7%) is the fifth (Miller *et al.*, 2016).

L-methioninase is attracted a great deal of attention due to has potential application as an active therapeutic agent against cardiovascular diseases and different types of cancer in human beings and other applications (El-Sayed and Shindia, 2011). L-methioninase from diverse microorganisms exhibits significant reductions in L-methionine in vivo and efficacy against a broad spectrum of transplantable animal and solid human tumors (Hoffman, 2015).

Overview of cancer:

The normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be acquired or inherited as discussed by (Haber and Fearon, 1998). However, carcinogenesis is a complex multistage process, usually involving more than one genetic change as well as other epigenetic factors (hormonal, carcinogenic and tumor-promoter effects) that do not themselves produce cancer, but increase the likelihood of the genetic mutations resulting eventually in cancer (Sundar, 2014).

The cancer cells proliferate abnormally. Therefore, they required a high amount of amino acids as nutrients because they are the building blocks for protein synthesis. So without amino acid, tumor cells fail to function because proteins cannot be synthesized.

According to this concept recent research has targeted on amino acid metabolic enzymes that deregulate specific amino acid metabolism that is essential for cancer cell proliferation (Supriya and Prajapati, 2018).

The chemotherapy of cancer:

Since the 1950s, unique advances have been made in the chemotherapeutic administration of disease. Unfortunately, the chemotherapy of solid tumors with a few exceptions has had only limited effectiveness. Thus, the majority of disseminated solid cancers are generally not responsive to current chemotherapeutic regimens (Sundar, 2014).

Cytotoxic drugs are not cancer-selective and are therefore active against both a tumor and normal cells,

which gives the drugs limited efficacy and significant toxicity (Minchinton and Tannock, 2006). So, it is of critical importance to identify targets and agents which are tumor-selective.

The role of microbial agents in cancer therapy:

Natural products are produced by a wide range of different organisms including microorganisms. It is a source of compounds opening promising avenues for the treatment of a great variety of diseases accurately in a proper manner to specifically target cancer cells (Justo *et al.*, 2011).

Because the microorganisms are most diverse (both structurally and metabolically) and account for 60% of the earth's biomass. Therefore, they constitute a rich source of a potential anti-inflammatory agent such as pseudopterosins, topsentins, scytonemin, and manoalide, antitumor compounds as bryostatins, discodermolide, eleutherobin, and sarcodictyin, and antibiotics substance as marinone, and products of antibacterial protein as bacteriocin (Justo *et al.*, 2011).

Lactic acid bacteria produce several natural antimicrobials, including organic acids (lactic acid, and acetic acid), carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocin, reuterin, and reutericyclin. Therefore, bacteriocins produced by LAB have the potential to cover a vast field of application, including both the food industry and the medical sector (De Vuyst and Leroy, 2007).

The potential use of bacteriocins in anti-cancer therapy is due to their inhibition of DNA and membrane protein synthesis, inducing apoptosis or cytotoxicity in tumor cells (Joo *et al.*, 2012). Supplements of bacteriocin-producing probiotics may be another way to prevent cancer occurrence. Also colicins could act as an anti-cancer drug of moderate potential. In a recent study, (Joo *et al.*, 2012) found that nisin had capabilities to inhibit cancer cell growth.

Microbial anti-cancer enzymes:

Microbial enzymes are known to act a critical role as metabolic catalysts, leading to their use in various industries and applications. The end-user market for industrial enzymes is extremely widespread with numerous industrial, commercial applications (Adrio and Demain, 2005).

The therapeutic potential of metabolism-derived microbial products is mostly not explored, besides the astonishing number of different microorganisms that inhabit the earth (Knight *et al.*, 2003). However, microbial anti-cancer enzymes have been proven to be active and economic agents for cancer treatment (Jesuraj *et al.*, 2017).

Exploitation of enzymes as anticancer, anti-cardiovascular, anticoagulants, and antioxidants (Vellard, 2003) was approved by Food and Drug Administration (FDA). In the last fifty years, the evolution in the field of biotechnology and proteomics

create a new therapeutic field (Enzyme-therapy) for treatment of various types of disease (El-Sayed and Shindia, 2011). Antibody-directed enzyme prodrug therapy (ADEPT) illustrates further applications of enzymes as therapeutic agents in cancer. In this, a monoclonal antibody carries an enzyme specifically to cancer cells, where the enzyme activates a prodrug, destroying these cells, but not the normal cells as reported by (Jung, 2001; Xu *et al.*, 2001). This approach is being utilized to discover and develop a class of cancer therapeutics based on tumor-targeted enzymes every organ of the body. Therefore, current efforts to cure cancer have been focusing on drugs, biological molecules and immune-mediated therapies.

To date, cancer remains one of the most life-threatening diseases. Even today the mortality rate or survival time for metastatic cancer has not been prolonged as reported by (Chong *et al.*, 2006).

Some tumors require the extracellular sources of some amino acids, which are considered as non-essential in normal cells, due to metabolic deficiencies (Kuo *et al.*, 2010). So enzymatic degradation of these amino acids can be an effective strategy in the suppression of such tumors (Shen *et al.*, 2006). However, various enzymes have been utilized as medicine and practically, almost of tumor cells were reported to be auxotrophs for L-methionine, L-glutamine, L-asparagine and L-arginine, due to the absence of intrinsic enzymatic systems that synthesizing these amino acids the cancer cell depends for growth and proliferation on the exogenous supply of these amino acids, usually from diets (Pasut *et al.*, 2008).

Thus, L-methioninase, L-glutaminase, L-asparaginase and arginine deiminase were frequently used as common anticancer agents (Cheng *et al.*, 2005). Among the different types of cancers, the hematological type is physiologically L-glutamine and L-asparagine dependent. Thus, L-glutaminase and L-asparaginase were successfully used as potent anti-leukemic agents (Abdallah *et al.*, 2012; Nimkande *et al.*, 2015; Piatkowska-Jakubas *et al.*, 2008).

PEGylated arginine deiminase (ADI-PEG20) is a novel anticancer enzyme that produces depletion of arginine is a novel anticancer enzyme that produces certain depletion tumors such as malignant melanoma and hepatocellular carcinoma, are auxotrophic for arginine. It is generally expressed in bacteria, fungi, yeast, actinomycetes, algae, and plants. (Feun and Savaraj, 2006; Unissa *et al.*, 2015).

L-methioninase for cancer therapy:

L-methionine-gamma-lyase (MGL, EC 4.4.1.11), is also known by other names such as L-methionase, methioninase, methionine lyase and methionine demethylase (Suganya *et al.*, 2017). It's dependent on pyridoxal-5-phosphate (PLP) that catalyzes the

elimination reactions of L-methionine to α -ketobutyrate, methanethiol and ammonia (Percudani and Peracchi, 2003).

In general, physiologically, normal cells can grow on homocysteine, instead of L-methionine, due to their active L-methionine synthase (Mecham *et al.*, 1983). So, the cancer cells are deprived of these amino acids, they starve to death, since they can't synthesize these amino acids (Lishko *et al.*, 1993a). Nutritional starvation can be done by two ways, one by controlling the dietary intake of these amino acids and the other by decreasing the serum concentration of these amino acids.

Recombinant L-methionine α,γ -lyase (rMETase), an L-methionine depleting enzyme cloned from *Pseudomonas putida*, was shown to have efficacy on a broad series of cancer cell lines (Tan *et al.*, 1997). A methionine-cleaving enzyme would lower L-methionine levels more than L-methionine starvation and, thereby, could have better therapeutic effects. Studies of the anticancer efficacy of recombinant L-methioninase (rMETase) in vitro and in vivo on human tumors xenografted in nude mice pretend that all types of human tumors tested, including those from the lung, colon, kidney, brain, prostate and melanoma, were sensitive to rMETase. In contrast, normal cells were insensitive to rMETase in vitro. No toxicity was detected in vivo at the effective doses as reported by (Tan *et al.*, 1997). Overexpression, cloning and large-scale production protocols for rMETase have enabled rMETase to be used as a tumor-selective therapeutic with broad indications and a high promise for effective, low-toxicity human cancer therapy. The most significant promise for L-methioninase, however, is most possibly in combination therapy, where it has the potential to selectively sensitize tumor cells to many classes of currently used chemotherapy. In this way, methioninase may act not only as a universal cancer drug but also as a universal modulator of other chemotherapy drugs.

The enzyme is promising as an antitumor agent because L-methionine is required for the growth of malignant cells (Cellarier *et al.*, 2003). Numerous human cancer cell lines have an absolute requirement for L-methionine to survive and proliferate as an essential amino acid, whereas normal cells are L-methionine independent (Kahraman, 2015). Due to there are reports suggesting that L-methionine may be a tumor-specific target since some malignant cell lines were identified that had an absolute requirement for L-methionine as they would not grow on homocysteine. Therefore tumors are L-methionine-dependent. On the contrary, normal cells and tissues were found to be able to use homocysteine in place of L-methionine for proliferation, and are therefore L-methionine-independent (Sundar, 2014).

In fact, L-methionine can be recycled by re-methylation of homocysteine in normal cells (Delgado-Reyes *et al.*, 2001). Most cancer cells do not have L-methionine cycle enzymes intact, though. As a result, they need to have L-methionine available for growth processes (Cavuoto and Fenech, 2012). In vitro, there is direct evidence that L-methionine restriction leads to selective death of cancer cells versus normal cells (Fu *et al.*, 2003). Several cancer cell types cannot survive in media freed of L-methionine even when homocysteine is present (Pavillard *et al.*, 2004). L-methionine was first investigated as a tumor-selective therapeutic target in *in vitro* experiments (Sundar, 2014).

This metabolic difference in L-methionine usage may allow for a targetable vulnerability in cancer cells and the normal cells should be capable of surviving without L-methionine, while cancer cells would not. It has been demonstrated in animal models of various cancers (Kokkinakis *et al.*, 2004). Therefore, in the fight against cancer, enzyme-therapy is the most effective strategy recently, it's unlike traditional approaches, it seems to be the promising therapeutic technology for their high specificity and affinity towards a clue substrate on specific metabolic pathway, and it may enhance drug efficacy or directly directed to cancer treatment and may diminish chemotherapy toxicity (El-Sayed, 2010; El-Sayed and Shindia, 2011).

Role of L-methioninase and L-methionine starvation as a target for cancer therapy:

L-methioninase catalysis the breakdown of L-methionine to α -ketobutyric acid, methanethiol and ammonia (Ronda *et al.*, 2011). L-methionine is an essential amino acid with several critical functions, that plays a crucial role in protein synthesis, cellular processes, glutathione is a tripeptide that reduces reactive oxygen species, thereby protecting cells from oxidative stress and L-methionine is required for the formation of the polyamines, spermine and spermidine, which has far-ranging effects on nuclear and cell division. Also, in DNA and protein methylation by serving as the methyl-group donor, thus regulating the gene expression (Cavuoto and Fenech, 2012; Cellarier *et al.*, 2003; Davis and Uthus, 2004; Laird, 2003; Takakura *et al.*, 2006).

Research on physiological competences of L-methioninase was the understudy for more than half century, and a real bound forward was accomplished in 1953 when Wiesendanger and Nisman revealed the presence of L-methioninase in rumen bacteria (Wiesendanger and Nisman, 1953). Because, most cancer cells are dependent on exogenous, preformed L-methionine and do not grow, even in the presence of homocysteine. Therefore, L-methioninase acts as an antitumor agent against various type of solid tumor

cell lines: breast MCF7, lung A549, colon HCT116, prostate PC3, liver HepG2, kidney, glioblastoma and neuroblastoma (Benavides *et al.*, 2007; Hu and Cheung, 2009; Kokkinakis *et al.*, 2001).

Studies on the mechanism of altered L-methionine metabolism in cancer have indicated that L-methionine-dependent tumor cells generally synthesize L-methionine at a normal rate from homocysteine as reported by (Hoffman *et al.*, 1976), although there may be some exceptions in some cancer cell types where vitamin B12 metabolism is altered as reported by (Fiskerstrand *et al.*, 1994). Seems to be an abnormally high rate of L-methionine utilization in L-methionine dependent tumor cells for methylation reactions appearing to be an abnormally high rate that requires more L-methionine than the cell can synthesize from homocysteine during L-methionine starvation as reported by (Stern and Hoffman, 1984; Tisdale, 1980). Some tumors are also altered in the L-methionine salvage pathway, which may also impact L-methionine dependence.

When L-methionine-dependent tumor cells in vitro are deprived of L-methionine in a homocysteine-containing medium, they reversibly arrest in the late S/G2 phase of the cell cycle as reported by (Guo *et al.*, 1993; Guo *et al.*, 1993). The tumor-selective cell-cycle arrest allows L-methionine depletion to modulate the efficacy of many currently used chemotherapeutic agents as reported by (Stern and Hoffman, 1986). Dietary L-methionine starvation extended the lifespan of tumor-bearing animals and lowered the metastatic rate of L-methionine-dependent tumors. L-methionine-free total parenteral nutrition doubled the response and survival rate of high-stage gastric patients treated with 5-fluorouracil and mitomycin C, compared with patients treated with these drugs and given L-methionine-containing total parenteral nutrition. Clinical trials have demonstrated that L-methionine depletion has clinical activity.

However, dietary L-methionine starvation is insufficient to deplete serum L-methionine entirely and, therefore, does not wholly arrest tumor growth.

Other therapeutic uses of L-methioninase:

Currently, retroviral vectors gene therapy has been studied by transduction of microbial L-methioninase gene cells (Gupta *et al.*, 2003; Miki *et al.*, 2000; Miki *et al.*, 2001; Yamamoto *et al.*, 2003). There are still more therapeutic uses of L-methioninase, among which the more specific ones are for heart disease, elevated serum total homocysteine (tHcy) levels have emerged as a significant cardiovascular risk factor as mentioned by (McCully, 1969), aging, and obesity as reported by (Guo *et al.*, 1996; Poirson-Bichat *et al.*, 1997). The dietary L-methionine deprivation after adding L-methioninase

supplement has been studied to control weight gain exquisitely in the rats (Fan *et al.*, 1997).

L-methioninase finds application in the pharmaceutical industry as it has antioxidant activity which helps in downregulation of polyamines spermine and spermidine, which has far-ranging effects on nuclear and cell division. L-methionine is the significant sources of methyl groups for methylation of DNA and other molecules (Bondar *et al.*, 2005). The limited distribution of L-methioninase as intracellular enzyme among all microbial pathogens but not in humans makes this enzyme a promising drug target for antibacterial, antifungal and antiprotozoal therapies (Ali and Nozaki, 2007; D Sato and Nozaki, 2009). As well as in the food industry by improving the aroma via a release of volatile Sulphur compounds (Bonnarme *et al.*, 2001). L-methionine as amino acid a nutritive feed additive have been investigated. It was observed for poultry that the stability of shells decreases just as the milk production in cow does (Noftsgger *et al.*, 2005).

Production of L-methioninase by microorganisms:

Presence of L-methioninase has been reported in several organisms including plants as *Arabidopsis thaliana* (Rébeillé *et al.*, 2006). Optimal culture conditions for the production of L-methioninase diverse methods have been reported for the production and purification of L-methioninase from various organisms include solid-state fermentation (SSF) by using several agro-industrial residues: corn, tea waste, soya bean, palm oil, sesame oil and wheat bran. At the same time, L-methioninase can be done by submerged fermentation (SmF) (Abu-Tahon and Isaac, 2016; Khalaf and El-Sayed, 2009). The natural agro-industrial residues were utilized as substrates for enzyme production and it's a favored environmentally and economically. For the high expense of enzyme purification from the microbial cultures, immobilization is a promising technique for enzyme stabilization and continuous production of methanethiol (El-Sayed and Shindia, 2011). Permeabilization treatment proved that L-methioninase was found to be extracellularly produced in bacteria (Selim *et al.*, 2015; Swathi, 2015).

From bacteria

It has been extensively studied from terrestrial and marine microbes (Suganya *et al.*, 2017). L-methioninase has been reported from both gram-positive and gram-negative bacterial species from various sources (Rodionov *et al.*, 2004), some of which are anaerobic *Porphyromonas gingivalis* (Yoshimura *et al.*, 2000) and *Treponema denticola* (Sharma *et al.*, 2014), in eukaryotic pathogens such as *Entamoeba histolytica* (Tokoro *et al.*, 2003), farther more, the bacteria as *Pseudomonas putida*, *Aeromonas* sp., *Citrobacter freundii* and *Lactococcus lactis*

(Swathi, 2015); *Clostridium sporogenes* (Krishnaveni *et al.*, 2009); *Salmonella*, *Mycobacterium*, *Bacillus*, *Listeria* (Bernardes *et al.*, 2010) and *Brevibacterium linens* (Pavani and Saradhi, 2014).

L-methioninase from many bacterial species was purified and characterized from several microorganisms such as *B. subtilis*, *Aeromonas sp.*, *C. freundii*, *B. linens*, *L. lactis* and *Clo. sporogenes* (El-Sayed, 2010; El-Sayed and Shindia, 2011; Singh and Kharayat, 2018).

Takakura *et al.* (2006) when its discovery in *Escherichia coli* and *Proteus vulgaris* (Onitake, 1938) a series of research has been carried out to explore the enzyme and this enzyme has been found in various bacteria and is considered as a key enzyme in the bacterial metabolism of L-methionine. L-methioninase has been found in bacteria, some of which are anaerobic, *Porphyromonas gingivalis* (Yoshimura *et al.*, 2000) and *Treponema denticola* (Fukamachi *et al.*, 2005).

L-methioninase have been isolated, purified, and characterized from several microorganisms such as *P. putida* (El-Sayed, 2010; Esaki and Soda, 1987; Esaki *et al.*, 1979; Ito *et al.*, 1976; Lishko *et al.*, 1993b; Nakayama *et al.*, 1984; Tanaka *et al.*, 1977; Tanaka *et al.*, 1976), *Clo. sporogenes* (Tanaka *et al.*, 1977), *Aeromonas sp.* (Nakayama *et al.*, 1984), *Citrobacter intermedius* (Faleev *et al.*, 1996), *B. linens* (Dias and Weimer, 1998) *Trichomonas vaginalis* (Lockwood and Coombs, 1991) and *Porphyromonas gingivalis* (Yoshimura *et al.*, 2000). Pinnamaneni *et al.* (2012) found that *B. linens* which are a normal flora present in the whey of curd are a rich source of L-methionine γ -lyase (MGL).

From actinomycetes

Selim *et al.* (2015) Found that, only 60 isolates of *Streptomyces* tested; only 40 isolates were capable of utilizing L-methionine as the only main origin of nitrogen in the medium. Also, 24 of these isolates could grow in medium amended with L-methionine as a source of nitrogen and carbon, the enzyme purified from the crude extract of *Streptomyces sp.* DMMM4.

Forty-five *Streptomyces* isolates were screened for production of L-methioninase. Among them best nine isolates have a higher productive of extracellular L-methioninase. These isolates were quantitatively checked of L-methioninase production and the promising isolate was subjected to identification showed that the strain named *Streptomyces variabilis* 3MA2016 (El Awady *et al.*, 2017). *Actinomycetes* as *Streptomyces sp.*, *A. carneus* and *Streptomyces* DMMM460 (Abdelraof *et al.*, 2019; Khalaf and El-Sayed, 2009; Nwachukwu and Ekwealor, 2009).

From filamentous Fungi and yeast

Swathi, (2015), investigated the production and optimization of extracellular L-methioninase enzyme using several agro-industrial residues by *Aspergillus flavipes* MTCC 6337 using solid-state fermentation (SSF). Fungal species as an intracellular and extracellular enzyme (El-Sayed, 2009). Fungi such as *Trichoderma harzianum* (Salim *et al.*, 2019), *Geotrichum candidum* (Bonnarme *et al.*, 2001) and *Penicillium notatum* (Khalaf and El-Sayed, 2009); archaea as *Ferroplasma acidarmanus* (Baumler *et al.*, 2007) and the protozoan *Entamoeba histolytica* (Sato *et al.*, 2006).

Some studies were reported on the partial characterization of L-methioninase from fungi including *Penicillium sp.*, *Aspergillus sp.*, *Humicola fuscoatra* and *A. flavipes* (Swathi, 2015), describe filtrates L-methioninase in the culture of yeast such as *Geotrichum candidum*, *Debaromyces hanseii* and *Saccharomyces cerevisiae* (Bonnarme *et al.*, 2001). It is noteworthy that reports describe L-methioninase in the culture filtrates of a few yeasts including *Geotrichem candidum*, *Debaromyces hasenii* and *Saccharomyces cerevisiae* (Bonnarme *et al.*, 2001). A large number of isolated yeast from various locations including Egyptian soils, marine water or cheese products were quantitatively screened for their L-methioninase activity. *Candida tropicalis* was the most active isolate. Results showed that the enzyme was intracellular produced (Selim *et al.*, 2015).

Sharma *et al.*, 2014, described L-methioninase in the culture filtrates of a few yeasts including *Geotrichem candidum*, *Debaromyces hasenii* and *Saccharomyces cerevisiae*.

Optimization of L-methioninase production by microorganisms:

Optimization of the production of L-methioninase was done by many researches, from *Bacillus subtilis* was optimum assay parameters and activity of 17.4 Unit was estimated for L-methioninase (Singh and Kharayat, 2018).

Aspergillus ustus AUMC 10151 displayed the highest yield of enzyme (10.8 U/mg protein), followed by *A. ochraceus* and *Fusarium proliferatum* upon optimization of the submerged fermentation (SmF) conditions, the maximum enzyme yield (18.23 U/mg protein), And Seven agro-industrial by-products were screened as substrates for L-methioninase production under solid-state fermentation (SSF). Wheat bran resulted 38.1 U/mg protein, followed by rice bran (27.6 U/mg protein) and soya bean meal (26.6 U/mg protein) (Abu-Tahon and Isaac, 2016).

Chaetomium globosum was the most efficacious isolate and a dematiaceous filamentous fungi, it is produce L-methioninase with predicted specific

activity of (≈ 2225 U/mg) (Hamed *et al.*, 2016). The production and optimization of extracellular L-methioninase enzyme using several agro-industrial residues by *Aspergillus flavipes* MTCC 6337. The organism produced high levels of L-methioninase under optimized culture conditions (Swathi, 2015). Some factors influencing L-methioninase production by *Candida tropicalis* isolate (Mohsen *et al.*, 2013). *Aspergillus flavipes* had the most methioninolytic activity, giving the highest yield of L-methioninase (10.78 U/mg protein), followed by *Scopulariopsis brevicaulis* and *A. carneus* (Khalaf and El-Sayed, 2009).

From *Streptomyces* sp. the promising isolate named *Streptomyces variabilis* 3MA2016. Ultimate L-methioninase production by *S. variabilis* 3MA2016 giving the highest yield of L-methioninase (El Awady *et al.*, 2017; Selim *et al.*, 2015). Therefore, further experimentation is required in order to utilize the potential of the bacterial isolates to produce L-methioninase which is an enzyme gaining therapeutic application.

References

1. Abdallah, N. A.; Amer, S. K. and Habeeb, M. K. (2012). Screening of L-Glutaminase produced by actinomycetes isolated from different soils in Egypt. *International Journal of Chem Tech Research*, 4(4), 1451-1460.
2. Abu-Tahon, M. A. and Isaac, G. S. (2016). 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*, 59.
3. Adrio, J. L. and Demain, A. L. (2005). Microbial Cells and Enzymes A Century of Progress. In *Microbial Enzymes and Biotransformations* (pp. 1-27): Springer.
4. Ali, V. and Nozaki, T. (2007). Current therapeutics, their problems, and sulfur-containing-amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. *Clinical microbiology reviews*, 20(1), 164-187.
5. Baumler, D. J.; Hung, K.-F.; Jeong, K. C. and Kaspar, C. W. (2007). Production of methanethiol and volatile sulfur compounds by the archaeon "Ferroplasma acidarmanus". *Extremophiles*, 11(6), 841-851.
6. Benavides, M. A.; Oelschlager, D. K.; Zhang, H.-G.; Stockard, C. R.; Vital-Reyes, V. S.; Katkooi, V. R.; Manne, U.; Wang, W.; Bland, K. I. and Grizzle, W. E. (2007). Methionine inhibits cellular growth dependent on the p53 status of cells. *The American journal of surgery*, 193(2), 274-283.
7. Bernardes, N.; Seruca, R.; Chakrabarty, A. M. and Fialho, A. M. (2010). Microbial-based therapy of cancer: current progress and future prospects. *Bioengineered bugs*, 1(3), 178-190.
8. 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), 4585-4591.
9. Bonnarme, P.; Lapadatescu, C.; Yvon, M. and Spinnler, H.-E. (2001). L-methionine degradation potentialities of cheese-ripening microorganisms. *Journal of Dairy Research*, 68(4), 663-674.
10. Cavuoto, P. and Fenech, M. F. (2012). A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension. *Cancer treatment reviews*, 38(6), 726-736.
11. Cheng, P.; Leung, Y.; Lo, W.; Tsui, S. and Lam, K. (2005). Remission of hepatocellular carcinoma with arginine depletion induced by systemic release of endogenous hepatic arginase due to transhepatic arterial embolisation, augmented by high-dose insulin: arginase as a potential drug candidate for hepatocellular carcinoma. *Cancer letters*, 224(1), 67-80.
12. Chong, S.; Lee, K. S.; Chung, M. J.; Han, J.; Kwon, O. J. and Kim, T. S. (2006). Neuroendocrine tumors of the lung: clinical, pathologic, and imaging findings. *Radiographics*, 26(1), 41-57.
13. De Vuyst, L. and Leroy, F. (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *Journal of molecular microbiology and biotechnology*, 13(4), 194-199.
14. Delgado-Reyes, C. V.; Wallig, M. A. and Garrow, T. A. (2001). Immunohistochemical detection of betaine-homocysteine S-methyltransferase in human, pig, and rat liver and kidney. *Archives of Biochemistry and Biophysics*, 393(1), 184-186.
15. Dias, B. and Weimer, B. (1998). Purification and Characterization of Methionine γ -Lyase from *Brevibacterium linens* BL2. *Applied and environmental microbiology*, 64(9), 3327-3331.
16. El-Sayed, A. S. (2010). Microbial L-methioninase: production, molecular characterization, and therapeutic applications. *Applied microbiology and biotechnology*, 86(2), 445-467.
17. El-Sayed, A. S. and Shindia, A. A. (2011). PLP-Dependent Enzymes: a Potent Therapeutic

- Approach for Cancer and Cardiovascular Diseases. In *Targets in Gene Therapy*: InTech.
18. El- Sayed, A. S. (2009). L- methioninase production by *Aspergillus flavipes* under solid- state fermentation. *Journal of basic microbiology*, 49(4), 331-341.
 19. 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), 906-921.
 20. Esaki, N. and Soda, K. (1987). L-Methionine γ -lyase from *Pseudomonas putida* and *Aeromonas*. In *Methods in enzymology* (Vol. 143, pp. 459-465): Elsevier.
 21. Esaki, N.; Tanaka, H.; Uemura, S.; Suzuki, T. and Soda, K. (1979). Catalytic action of L-methionine. gamma.-lyase on selenomethionine and selenols. *Biochemistry*, 18(3), 407-410.
 22. Faleev, N.; Troitskaya, M.; Paskonova, E.; Saporovskaya, M. and Belikov, V. (1996). L-Methionine- γ -lyase in *Citrobacter intermedius* cells: stereochemical requirements with respect to the thiol structure. *Enzyme and Microbial technology*, 19(8), 590-593.
 23. Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruba, V. J. and Cone, R. D. (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature*, 385(6612), 165.
 24. Feun, L. and Savaraj, N. (2006). Pegylated arginine deiminase: a novel anticancer enzyme agent. *Expert opinion on investigational drugs*, 15(7), 815-822.
 25. Fiskerstrand, T.; Christensen, B.; Tysnes, O. B.; Ueland, P. M. and Refsum, H. (1994). Development and reversion of methionine dependence in a human glioma cell line: relation to homocysteine remethylation and cobalamin status. *Cancer research*, 54(18), 4899-4906.
 26. Fu, Y. M.; Yu, Z. X.; Li, Y. Q.; Ge, X.; Sanchez, P. J.; Fu, X. and Meadows, G. G. (2003). Specific amino acid dependency regulates invasiveness and viability of androgen-independent prostate cancer cells. *Nutrition and cancer*, 45(1), 60-73.
 27. 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), 127-131.
 28. Guo; Hui-Yan; Herrera, H.; Groce, A. and Hoffman, R. M. (1993). Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. *Cancer research*, 53(11), 2479-2483.
 29. Guo, H.; Tani, Y. and Moossa, T. k. A. (1996). Methionine depletion modulates the antitumor and antimetastatic efficacy of ethionine. *Ratio*, 1(20), 60.
 30. Gupta, A.; Miki, K.; Xu, M.; Yamamoto, N.; Moossa, A. and Hoffman, R. (2003). Combination efficacy of doxorubicin and adenoviral methioninase gene therapy with prodrug selenomethionine. *Anticancer research*, 23(2B), 1181-1188.
 31. Haber, D. A. and Fearon, E. R. (1998). The promise of cancer genetics. *The Lancet*, 351, SIII-SII8.
 32. Hamed, S. R.; Elsoud, M. M. A.; Mahmoud, M. G. and Asker, M. M. (2016). Isolation, screening and statistical optimizing of L-methioninase production by *Chaetomium globosum*. *African Journal of Microbiology Research*, 10(36), 1513-1523.
 33. Hoffman; L. M. and Levine, L. E. (1976). Early sex differences in empathy. *Developmental Psychology*, 12(6), 557.
 34. Hoffman, R. M. (2015). Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. *Expert opinion on biological therapy*, 15(1), 21-31.
 35. Ito, S.; Nakamura, T. and Eguchi, Y. (1976). Purification and characterization of methioninase from *Pseudomonas putida*. *The Journal of Biochemistry*, 79(6), 1263-1272.
 36. Jesuraj, S. A. V.; Sarker, M. M. R.; Ming, L. C.; Praya, S. M. J.; Ravikumar, M. and Wui, W. T. (2017). Enhancement of the production of L-glutaminase, an anticancer enzyme, from *Aeromonas veronii* by adaptive and induced mutation techniques. *PLoS one*, 12(8), e0181745.
 37. Joo, N. E.; Ritchie, K.; Kamarajan, P.; Miao, D. and Kapila, Y. L. (2012). Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC 1. *Cancer medicine*, 1(3), 295-305.
 38. Jung, D. I. (2001). Transformational and transactional leadership and their effects on creativity in groups. *Creativity Research Journal*, 13(2), 185-195.
 39. Justo, G. Z.; Souza, A. C.; de Fátima, Â.; Pedrosa, M. F.; Ferreira, C. V. and Rocha, H. A. (2011). The medicinal value of biodiversity: new hits to fight cancer. In *Biological Diversity and Sustainable Resources Use*: InTech.

40. Kahraman, H. (2015). L-Methionine, L-Methionine γ -Lyase, *Cancer. therapy*, 4, 6.
41. Khalaf, S. A. and El-Sayed, A. S. (2009). L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions. *Current microbiology*, 58(3), 219-226.
42. Knight, V.; Sanglier, J.-J.; Di Tullio, D.; Braccili, S.; Bonner, P.; Waters, J.; Hughes, D. and Zhang, L. (2003). Diversifying microbial natural products for drug discovery. *Applied microbiology and biotechnology*, 62(5-6), 446-458.
43. Kokkinakis, D. M.; Liu, X.; Chada, S.; Ahmed, M. M.; Shareef, M. M.; Singha, U. K.; Yang, S. and Luo, J. (2004). Modulation of gene expression in human central nervous system tumors under methionine deprivation-induced stress. *Cancer research*, 64(20), 7513-7525.
44. Krishnaveni, R.; Rathod, V.; Thakur, M. and Neelgund, Y. (2009). Transformation of L-tyrosine to L-DOPA by a novel fungus, *Acremonium rutilum*, under submerged fermentation. *Current microbiology*, 58(2), 122-128.
45. Kuo, M. T.; Savaraj, N. and Feun, L. G. (2010). Targeted cellular metabolism for cancer chemotherapy with recombinant arginine-degrading enzymes. *Oncotarget*, 1(4), 246.
46. Laird, P. W. (2003). Early detection: the power and the promise of DNA methylation markers. *Nature Reviews Cancer*, 3(4), 253.
47. Lishko, V. K.; Lishko, O. V. and Hoffman, R. M. (1993a). Depletion of serum methionine by methioninase in mice. *Anticancer research*, 13(5A), 1465-1468.
48. Lishko, V. K.; Lishko, O. V. and Hoffman, R. M. (1993b). The preparation of endotoxin-free l-methionine- α -deamino- γ -mercaptomethane-lyase (l-methioninase) from *Pseudomonas putida*. *Protein expression and purification*, 4(6), 529-533.
49. Lockwood, B. C. and Coombs, G. H. (1991). Purification and characterization of methionine γ -lyase from *Trichomonas vaginalis*. *Biochemical Journal*, 279(3), 675-682.
50. McCully, K. S. (1969). Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *The American journal of pathology*, 56(1), 111.
51. Miki, K.; Al-Refaie, W.; Xu, M.; Jiang, P.; Tan, Y.; Bouvet, M.; Zhao, M.; Gupta, A.; Chishima, T. and Shimada, H. (2000). Methioninase gene therapy of human cancer cells is synergistic with recombinant methioninase treatment. *Cancer research*, 60(10), 2696-2702.
52. Miller, K. D.; Siegel, R. L.; Lin, C. C.; Mariotto, A. B.; Kramer, J. L.; Rowland, J. H.; Stein, K. D.; Alteri, R. and Jemal, A. (2016). Cancer treatment and survivorship statistics, 2016. *CA: a cancer journal for clinicians*, 66(4), 271-289.
53. Minchinton, A. I. and Tannock, I. F. (2006). Drug penetration in solid tumours. *Nature Reviews Cancer*, 6(8), 583.
54. Nakayama, T.; Esaki, N.; Sugie, K.; Beresov, T. T.; Tanaka, H. and Soda, K. (1984). Purification of bacterial L-methionine γ -lyase. *Analytical biochemistry*, 138(2), 421-424.
55. NAKAYAMA, T.; Esaki, N.; Lee, W.-J.; Tanaka, I.; Tanaka, H. and Soda, K. (1984). Purification and properties of l-methionine γ -lyase from *Aeromonas* sp. *Agricultural and biological chemistry*, 48(9), 2367-2369.
56. Nimkande, K. D.; Khan, Z. H.; Mular, S. M. and Kunjwani, S. S. (2015). Isolation, Purification & Characterization of L-Asparaginase from dry seeds of *Pisum sativum* and *Vigna radiata*. *IJAR*, 1(7), 628-631.
57. Noftsker, S.; St-Pierre, N. and Sylvester, J. (2005). Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. *Journal of dairy science*, 88(1), 223-237.
58. Nwachukwu, R. and Ekwealor, I. (2009). Methionine-producing *Streptomyces* species isolated from Southern Nigeria soil. *African Journal of Microbiology Research*, 3(9), 478-481.
59. Onitake, J. (1938). On the formation of methylmercaptan from L-cystine and L-methionine by bacteria. *J. Osaka Med. Assoc*, 37, 263-270.
60. Pasut, G.; Sergi, M. and Veronese, F. M. (2008). Anti-cancer PEG-enzymes: 30 years old, but still a current approach. *Advanced drug delivery reviews*, 60(1), 69-78.
61. Pavani, K. and Saradhi, S. V. (2014). Cloning and expression of methionine-lyase (MGL) of *Brevibacterium linens*. *International Journal of Current Microbiology and Applied Sciences*, 3, 615-631.
62. Pavillard, V.; Drbal, A. A.; Swaine, D. J.; Phillips, R. M.; Double, J. A. and Nicolaou, A. (2004). Analysis of cell-cycle kinetics and sulfur amino acid metabolism in methionine-dependent tumor cell lines; the effect of homocysteine supplementation. *Biochemical pharmacology*, 67(8), 1587-1599.
63. Percudani, R. and Peracchi, A. (2003). A genomic overview of pyridoxal-phosphate-dependent enzymes. *EMBO reports*, 4(9), 850-854.

64. Piatkowska-Jakubas, B.; Krawczyk-Kuliś, M.; Giebel, S.; Adamczyk-Cioch, M.; Czyz, A.; Lech, M. E.; Paluszewska, M.; Pałynyczko, G.; Piszcz, J. and Hołowicki, J. (2008). Use of L-asparaginase in acute lymphoblastic leukemia: recommendations of the Polish Adult Leukemia Group. *Pol Arch Med Wewn*, 118(11), 664-669.
65. Pinnamaneni, R.; Gangula, S.; Koonna, S. and Potti, R. (2012). Isolation screening and assaying of methioninase of *Brevibacterium linens*. *Int J Sci & Nat*, 3(4), 773-779.
66. Poirson-Bichat, F.; Lopez, R.; Gonçoes, R. B.; Miccohi, L.; Bourgeois, Y.; Demerseman, P.; Poisson, M.; Dutrillaux, B. and Poupon, M. (1997). Methionine deprivation and methionine analogs inhibit cell proliferation and growth of human xenografted gliomas. *Life sciences*, 60(12), 919-931.
67. Rébeillé, F.; Jabrin, S.; Bligny, R.; Loizeau, K.; Gambonnet, B.; Van Wilder, V.; Douce, R. and Ravanel, S. (2006). Methionine catabolism in *Arabidopsis* cells is initiated by a γ -cleavage process and leads to S-methylcysteine and isoleucine syntheses. *Proceedings of the National Academy of Sciences*, 103(42), 15687-15692.
68. Rodionov, D. A.; Vitreschak, A. G.; Mironov, A. A. and Gelfand, M. S. (2004). Comparative genomics of the methionine metabolism in Gram-positive bacteria: a variety of regulatory systems. *Nucleic acids research*, 32(11), 3340-3353.
69. Ronda, L.; Bazhulina, N. P.; Morozova, E. A.; Revtovich, S. V.; Chekhov, V. O.; Nikulin, A. D.; Demidkina, T. V. and Mozzarelli, A. (2011). Exploring methionine γ -lyase structure-function relationship via microspectrophotometry and X-ray crystallography. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1814(6), 834-842.
70. Salim, N.; Santhiagu, A. and Joji, K. (2019). 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. *Biocatalysis and Agricultural Biotechnology*, 17, 299-308.
71. Sato, D. and Nozaki, T. (2009). Methionine gamma- lyase: The unique reaction mechanism, physiological roles, and therapeutic applications against infectious diseases and cancers. *IUBMB life*, 61(11), 1019-1028.
72. Sato, D.; Yamagata, W.; Kamei, K.; Nozaki, T. and Harada, S. (2006). Expression, purification and crystallization of l-methionine γ -lyase 2 from *Entamoeba histolytica*. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, 62(10), 1034-1036.
73. Selim, M.; Elshikh, H.; El-Hadedy, D.; Saad, M.; Eliwa, E. and Abdelraof, M. (2015). L-Methioninase from some *Streptomyces* isolates I: Isolation, identification of best producers and some properties of the crude enzyme produced. *Journal of Genetic Engineering and Biotechnology*, 13(2), 129-137.
74. Sharma, B.; Singh, S. and Kanwar, S. S. (2014). L-methionase: a therapeutic enzyme to treat malignancies. *BioMed research international*, 2014.
75. Shen, L.-J.; Beloussow, K. and Shen, W.-C. (2006). Modulation of arginine metabolic pathways as the potential anti-tumor mechanism of recombinant arginine deiminase. *Cancer letters*, 231(1), 30-35.
76. Stern, P. H. and Hoffman, R. M. (1984). Elevated overall rates of transmethylation in cell lines from diverse human tumors. *In vitro*, 20(8), 663-670.
77. Stern, P. H. and Hoffman, R. M. (1986). Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. *JNCI: Journal of the National Cancer Institute*, 76(4), 629-639.
78. Suganya, K.; Govindan, K.; Prabha, P. and Murugan, M. (2017). An Extensive Review on L-Methioninase and Its Potential Applications. *Biocatalysis and Agricultural Biotechnology*.
79. Sundar, A. W. a. (2014). Isolation of methioninase producers optimization of enzyme production and purification of L methioninase.
80. Supriya, N. R. and Prajapati, B. (2018). REVIEW ON ANTICANCER ENZYMES AND THEIR TARGETED AMINO ACIDS. *World Journal of Pharmaceutical Research*, 6(12), 268-284.
81. Swathi, A. (2015). Optimization of process parameters for L-methioninase production in Solid state fermentation by *Aspergillus flavipes* from Sesame oil cake. *Yeast*, 2, 1.0.
82. Takakura, T.; Ito, T.; Yagi, S.; Notsu, Y.; Itakura, T.; Nakamura, T.; Inagaki, K.; Esaki, N.; Hoffman, R. M. and Takimoto, A. (2006). High-level expression and bulk crystallization of recombinant L-methionine γ -lyase, an anticancer agent. *Applied microbiology and biotechnology*, 70(2), 183-192.
83. Takakura, T.; Takimoto, A.; Notsu, Y.; Yoshida, H.; Ito, T.; Nagatome, H.; Ohno, M.; Kobayashi, Y.; Yoshioka, T. and Inagaki, K. (2006). Physicochemical and pharmacokinetic characterization of highly potent recombinant L-methionine γ -lyase conjugated with polyethylene

- glycol as an antitumor agent. *Cancer research*, 66(5), 2807-2814.
84. Tan, Y.; Zavala, S. J.; Han, Q.; Xu, M.; Sun, X.; Tan, X.; Magana, R.; Geller, J. and Hoffman, R. (1997). Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. *Anticancer research*, 17(5B), 3857-3860.
 85. Tanaka, H.; Esaki, N. and Soda, K. (1977). Properties of L-methionine γ -lyase from *Pseudomonas ovalis*. *Biochemistry*, 16(1), 100-106.
 86. Tanaka, H.; Esaki, N.; Yamamoto, T. and Soda, K. (1976). Purification and properties of methioninase from *Pseudomonas ovalis*. *FEBS letters*, 66(2), 307-311.
 87. Tisdale, M. (1980). Effect of methionine deprivation on methylation and synthesis of macromolecules. *British journal of cancer*, 42(1), 121.
 88. 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 transsulfuration pathways. *Journal of Biological Chemistry*.
 89. Unissa, R.; Sudhakar, M. and Reddy, A. S. K. (2015). Selective isolation and molecular identification of L-arginase producing bacteria from marine sediments. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(06), 998-1006.
 90. Vellard, M. (2003). The enzyme as drug: application of enzymes as pharmaceuticals. *Current Opinion in Biotechnology*, 14(4), 444-450.
 91. Xu, J.; Xin, S. and Du, W. (2001). *Drosophila* Chk2 is required for DNA damage- mediated cell cycle arrest and apoptosis. *FEBS letters*, 508(3), 394-398.
 92. Yamamoto, N.; Gupta, A.; Xu, M.; Miki, K.; Tsujimoto, Y.; Tsuchiya, H.; Tomita, K.; Moossa, A. and Hoffman, R. (2003). Methioninase gene therapy with selenomethionine induces apoptosis in bcl-2-overproducing lung cancer cells. *Cancer gene therapy*, 10(6), 445.
 93. Yoshimura, M.; Nakano, Y.; Yamashita, Y.; Oho, T.; Saito, T. and Koga, T. (2000). Formation of Methyl Mercaptan from L-Methionine by *Porphyromonas gingivalis*. *Infection and immunity*, 68(12), 6912-6916.

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