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 wide-spread 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 21 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 *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-flourouracil 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 (Noftsger *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. DMMMH4.

Forty-five *Streptomycetes* 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* DMMMH60 (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 hansenii* 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 (≈2225U/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.

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