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MOLECULAR IDENTIFICATION, GENE DETECTION, AND IMPROVING L-METHIONINASE PRODUCTION OF *SERRATIA SP.* ISOLATE

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ABSTRACT

L-methioninase has attracted a great deal of attention due to has potential use as an effective therapeutic agent for cardiovascular diseases, different types of human cancer, and other applications. Bacterial isolates were collected from marine samples obtained from the Jeddah coast In Saudi Arabia. Bacterial isolate's ability for L-methioninase productions was tested on a modified mineral salt M9 L-methionine agar medium using phenol red as the pH indicator. Colonies with pink-red and yellow zone around were selected as L-methionine degrading bacteria.Based on biochemical tests and 16S rDNA sequencing, the highest L-methioninase producing strain was identified as *Serratia proteamaculans* strain. The nutrition,fermentation period, temperature, and pH value affecting L-methioninase production by the strain were optimized.Maximum L-methioninase production by *S. proteamaculans* was (0.102U\min\ml) obtained after 24 and 48 h respectively at 35±2 °C of incubation in a constant incubator in a culture supplemented with fructose (0.7%) and free of L-methionine medium, at pH 7.5.

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Introduction

L-methioninase (EC 4.4.1.11) is a pyridoxal phosphate (PLP)-dependent hydrolytic enzyme and also known by different names. The enzyme catalyzes the direct conversion of L-methionine to α-ketobutyrate, methanethiol, and ammonia by an α,γ-elimination reaction. [1]

Optimization of the production of L-methioninase was done by many kinds of research, from *Bacillus subtilis*was optimum assay parameters and activity of 17.4Unit was estimated for L-methioninase. [2] L-methioninase from diverse microorganisms exhibits significant reductions in methionine in vivo and efficacy against a broad spectrum of transplantable animal and solid human tumors. [3] The therapeutic potential of metabolism-derived microbial products is mostly not explored, besides the astonishing number of different microorganisms that inhabit the earth. [4] However, microbial anti-cancer enzymes have been proven to be active, and economic agents for cancer treatment. [5]

It has been extensively studied from terrestrial and marine microbes. [6] L-methioninase has been reported from both gram-positive and gram-negative bacterial species from various sources [7] such as anaerobic *Porphyromonas gingivalis* [8] and *Treponema denticola*, [9] in eukaryotic pathogens such as *Entamoeba histolytica*, [10] bacteria such as *Pseudomonas putida*, *Aeromonas* sp., *Citrobacter freundii*, *Lactococcus lactis*, [11] *Clostridium sporogenes*, [12]*Salmonella, Mycobacterium, Bacillus, Listeria*, [13] *and Brevibacterium linens*. [14]

L-methioninase of many bacterial species was purified and characterized from several microorganisms such as *Bacillus subtilis, Aeromonas* sp., *Citrobacter freundii*, *Bacillus linens*, *Lactococcus lactis*,and *Clostridium sporogenes*. [2, 15, 16]. [17] found that *Bacillus linens* which is a normal flora present in the whey of curd is a rich source of L-methionine γ-lyase (MGL). Some factors influence L-methioninase production. The optimal conditions for the production of the enzyme in*Streptomyces* DMMMH60 give the maximum enzyme production (60.7 U/mg). [18]Optimization of the production of L-methioninase was done by many types of research, from *Bacillus subtilis*was optimum assay parameters and activity of 17.4 Unit was estimated for L-methioninase. [2]

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Materials and Methods

Collection of samples

Different samples were collected from the west coast at the Red Sea. The seashell sample was collected from the marine environment of the west coast at a depth of 1 to 7m and placed in a zip-locked plastic bag. 16 bacterial isolates obtained from the marine samples, only four isolates had L-methioninase activity.

Qualitative screening of L-methioninase production of isolated bacteria

The bacterial isolates were screened for the ability to L-methioninase production by a qualitative rapid plate assay using a modified mineral salt M9 medium ($Na_2HPO_4.2H_2O$ 6.0 g/l, KH_2PO_4 3.0 g/l, NaCl 0.5 g/l, L-methionine 1 g/l, $MgSO_4.7H_2O$ 0.25 g/l, $CaCl_2$ 0.014 g/l, Glucose 2 g/l, at pH 7±0.2). Phenol red was added to the medium as an indicator of pH at a final concentration of 0.009% just before pouring the plates (El-Sayed, 2009). 0.1 ml of the serial dilution of different separately diluted samples were inoculated on a sterile modified M9 medium. The plates were incubated in an inverted position at 35±2ºC. for 24 to 48 hours. L-methioninase producing colonies were selected on the basis of the formation of the yellow or pink zone around. The colonies purified and subcultured from each plate for further studies. [19]

Quantitative estimation of L-methioninase by rapid plate method

The submerged fermentation SMF was carried out on a modified M9 medium supplemented with L-methionine and other L methionine + Glucose. The pH of the medium was adjusted at 7±0.2. The inoculated medium was incubated at 35±2°C under constant and shaking incubators at 150 rpm for 72h. The medium without L-methionine (substrate) was set as control. The bacterial cell-free filtrate culture CFF were harvested by centrifugation at 6000rpm min-1 for 30min. L-methionine agar plates were prepared by pouring 20ml of molten media into a sterile Petri dish. After plates solidified 7mm wells were punched using sterilized cork borer, 100µl of CFF was loaded in the wells, and the plates were kept in an upright position at 35±2°C for 48h. [20]

Assay of L-methioninase byNesslerization method

L-methioninase was determined by estimating the amount of ammonia liberated from L-methionine spectrophotometrically following the method of Nesslerization [21] with some modifications. The bacterial cell-free filtrate culture CFF were harvested by centrifugation at 6000rpm for 30min. One unit of L-methioninase was defined as the amount of enzyme that liberates ammonia at 1µmol/h under optimal assay conditions. The specific activity of L-methioninase was expressed as the activity of the enzyme in terms of units per milligram of protein [22].

Molecular identification of bacterial isolate based on 16S rDNA

Molecular characterization of bacterial isolate

Genomic DNA isolation was performed using GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific. Bacterial isolates were identified by16S rDNA universal oligonucleotide primers designed by [23].PCR mixture included 2µL of template DNA, 1µL of each primer, 12.5µL Master mix, and 9.5µL sterile dH_2O. PCR amplification conditions were as follows; 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72 °C, and a final extension of 10 min at 72°C.

Primer Design

For L-methioninase bacterial isolates, 16S rDNA universal oligonucleotide primers were designed. [23]Primers for amplifying the L-methioninase gene of *Serratia proteamaculans*strain were ordered according to the sequence of *Serratia proteamaculans*L-methioninasegene (NCBI accession number CP000826.1).(Tables 1, 2, and 3)**.**

Table 1: Primers designed for PCR amplification.

Bacteria used	Primer name	Nucleotide sequence	Size of PCR product
L-methioninase bacteria	16S27-F	AGAGTTTGATCCTGGCTCAG	1500
	16S1522-R	AAGGAGGTGATCCAGCCGCA	
Serratia proteamaculans	Sp-F	GGTGACTTACTGGCCGGTCT	422
	Sp-R	GAGTGAGTCATACTGGCGGG	

Table 2: PCR amplification mixtures.

Template DNA	Primers	PCR mixtures.
DNA of L- methioninase bacteria	16S27-F and 16S1522-R	Total volume of 25µl containing 2µL DNA, 1µL of each primer, 12.5µL Master mix, and 9.5µL sterile dH_2O.
Genomic DNA of *Serratia proteamaculans*	Sp-F and Sp-R	Total volume of 25µl containing 2µL DNA, 1µL of each primer, 12.5µL Master mix, and 9.5µL sterile dH_2O.

Table 3: Amplified Sequences/genes and PCR conditions.

Primers	Template DNA	PCR condition
16S27-F and 16S1522-R	DNA of L-methioninase bacteria	Initial denaturation for 5min at 94°C, 35 cycles of denaturation for 1 min at 94°C, annealing for 1min at 55°C, and extension for 2min at 72°C, andfinal extension for 10min at 72°C in a thermal cycler (Bibby Scientific, UK).
Sp-F and Sp-R	Genomic DNA of *Serratia proteamaculans*	Initial denaturation for 5min at 94°C, 35 cycles of denaturation for 1 min at 94°C, annealing for 1min at 54°C, and extension for 2min at 72°C, and final extension for 10min at 72°C in a thermal cycler (Bibby Scientific, UK).

Optimization of L-methioninase production

Various parameters that were known to influence the production of microbial metabolites during SMF were optimized. The strategy adopted for standardization of fermentation parameter was to evaluate the effect of an individual parameter and incorporate it at a standard level before standardizing the next parameter. The process parameters optimized in this study included incubation in shaking (150rpm) or static incubator, incubation period (24-72h.), incubation temperature (25-40°C), pH (4-8), carbon sources (glucose, mannitol, sucrose, xylose, maltose, lactose, galactose, fructose, and glycerol), best carbon source concentration, and nitrogen sources (tryptone, peptone and yeast extract and different amino acids (L-form; methionine, asparagine, and glutamine), concentration of best nitrogen source, and the effect of mixed nitrogen sources (% w/v).

Results:

Isolation and qualitative estimation of L-methioninase producing bacterial isolates

Four bacterial isolates obtained from seashells had varying abilities of L-methioninase activity on solid medium. The highest L-methioninase activity was reported for the bacterium Sh2.

Quantitative estimation of L-methioninase by rapid plate method

The quantitative estimation of L-methioninase was done byagar well diffusion assay of cell-free filtrate (CFF) of culture supplemented with L-methionine as the sole organic source, and other with L-methionine as nitrogen source and glucose as carbon source.

Results revealed that the isolate produced L-methioninase in cultures supplemented only with L-methionine as the sole organic source. The biggest diameter of the zone of diffusion for isolate Sh2 CFF incubated in the constant incubator was 35mm, and it was 16mm in shaking incubator (150rpm). The diameters of L-methioninase diffusion zones for isolate Sh2 were 22.5mm and 20mm in CFF of culture supplemented with L-methionine and glucose in constant and shaking incubators, respectively **(Table 4; Figures 1 and 2).**

Table 4: Quantitative estimation of L-methioninase production by rapid plate method.

Source of sample	**Sample**	**Bacterial isolates**	**Type of Incubator**	**L-methionine**	**L-methionine + Glucose**
				The diameter of enzyme diffusion (in mm)	
Marine sample	Seashell	Sh1	Constant	17	23
			Shaking	30	15
		Sh2	Constant	35	22.5
			Shaking	16	20
		Sh3	Constant	-	17
			Shaking	-	-
		Sh4	Constant	16	20
			Shaking	30	15

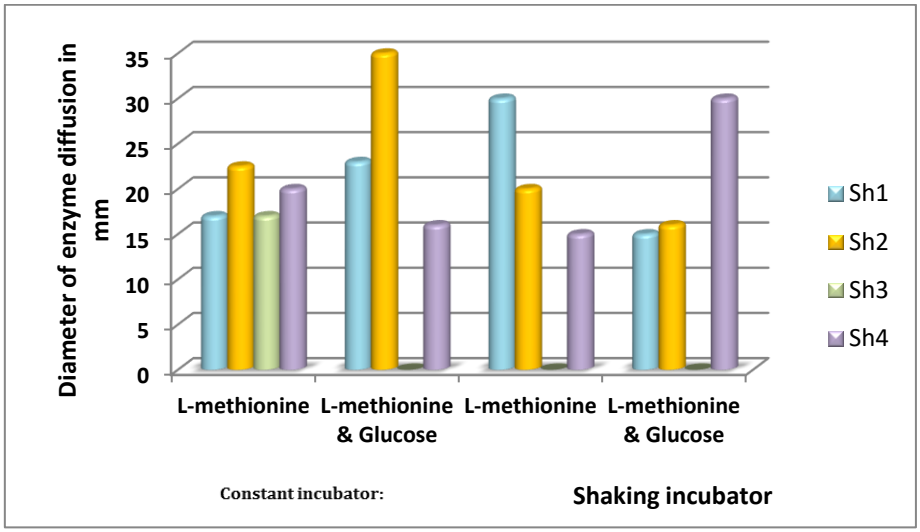

Figure 1: Quantitative estimation of L-methioninase production by rapid plate method.

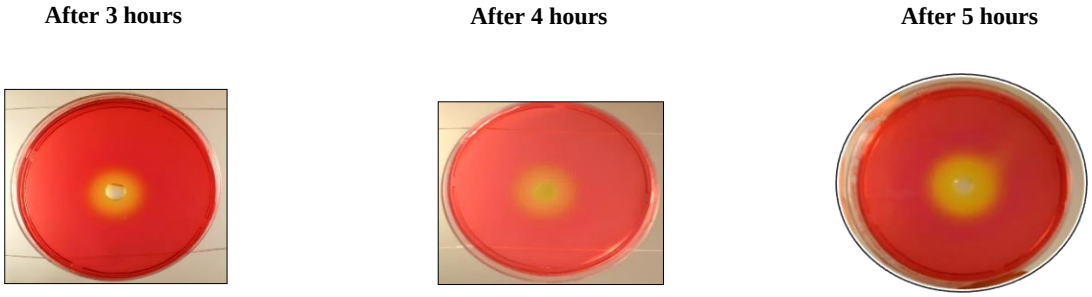

Figure 2: Quantitative estimation of L-methioninase production by rapid plate method of bacterial isolate Sh2and the pink-red and yellow color clearly showed the zone size.

Effect of shaking incubation on L-methioninase production by bacterial isolates

The effect of shaking incubator on L-methioninase production by bacterial isolate after 48 hours of incubation at 35±2°C was studied. The results of the enzyme assay by the Nesslerization method showed that the enzyme production was 0.0359U/min/ml in the presence of glucose under contestant incubation by bacterial isolate Sh2 that isolated from the seashell sample. Without glucose, its production was equal under the constant and shaking conditions (0.02U/min/ml) (**Figure 3).**The bacterial isolate Sh2 was chosen for further studies.

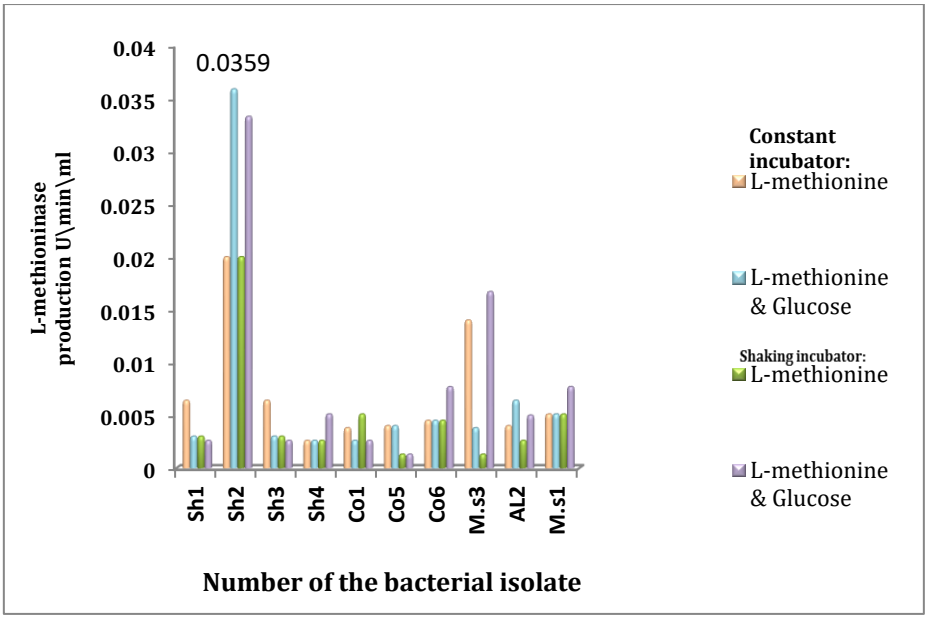

Figure 3: Effect of shaking incubation on L-methioninase production.

Molecular identification of bacterial isolates based on 16S rDNA

DNA sequences analyzed using Nucleotide BLAST alignment tools revealed the highest L-methioninase producing isolate, which was identified as *Serratia proteamaculans.* The sequence was submitted to the Bacterial or Archaeal 16S ribosomal RNA sequences database under the accession number WSk2 (**Table 5; Figure 4).**

Table 5: Identity percentage of 16S rDNA between the L-methioninase producing strain isolated from seashell and related strains in GeneBank.

Bacterial Isolates	**Name and Accession of No. of the most related strain in NCBI GenBank.**		**Identity**	**Coverage**	**Suggested Name and Accession No. of the isolates obtained in work.**	
Seq2	WSk2	*Serratia proteamaculans*	100%	100%	WSk2	*Serratia proteamaculans*

Phylogenetic tree analysis of *Serratia proteamaculans*isolates based on 16S rDNA

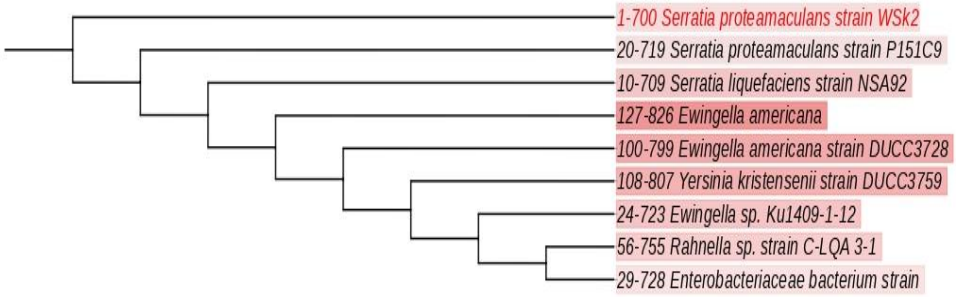

Figure 4: Neighbor-joining tree showing the phylogenetic position of *Serratia proteamaculans*strain and their related species based onpartial 16S rRNA gene sequences. The strain used in this study, WSk2, is marked in red color.

Molecular detection of L-methioninase gene in *S. proteamaculans* strain by PCR

The gel electrophoresis image shows the L-methioninase gene presented in *Serratia proteamaculans.* The positive result of L-methioninase gene presence in *Serratia proteamaculans* is presented as *a* 422bp PCR product (**Figure 5).**

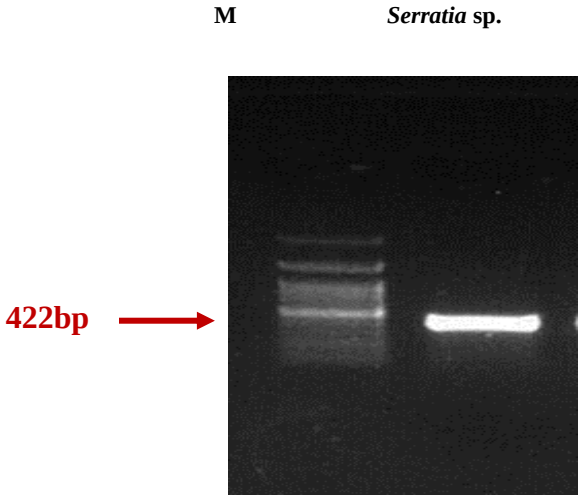

Figure 5: PCR amplification of the L-methioninase gene from *S. proteamaculans* strain. Lane 1: 100bp ladder; Lane 2: 422bp partial L-methioninase gene.

Optimization of environmental conditions for maximum L-methioninaseproduction

Effect of incubation periods and different temperatures on L-methioninase production by the selected bacterial isolates

Results in Figures (6) shows the effect of different incubation periods and temperatures (24, 48, and 72 hours at 40±2°C, 35±2°C, 30±2°C, and 20±2°C) on L-methioninase production by *Serratia proteamaculans*. The highest amount of the enzyme (0.04U\min\ml) was observed at 35±2°C.

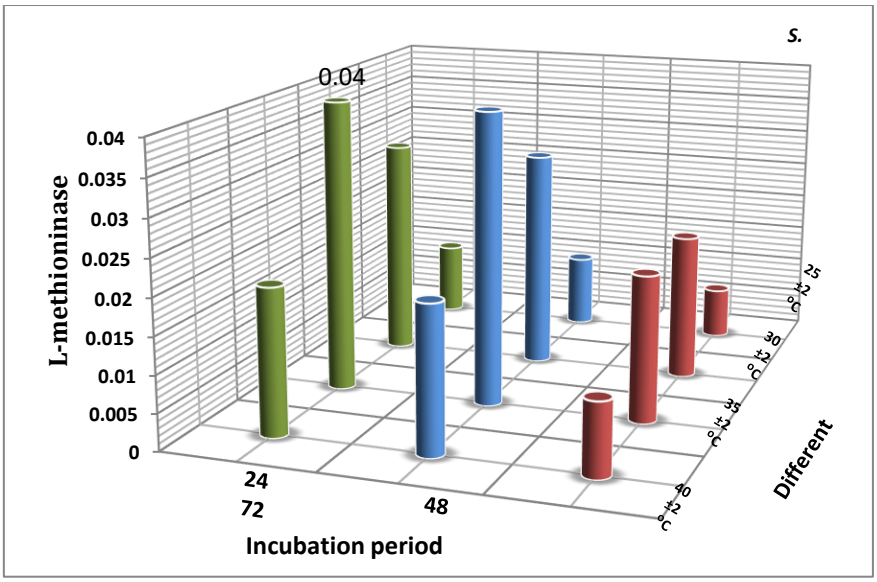

Figure 6: Effect of incubation period and different temperatures on L-methioninase production by *Serratia proteamaculans*

Effect of different pH values on L-methioninase production by *S. proteamaculans*

The productivity of L-methioninase increased by increasing the alkalinity of the medium. The maximum L-methioninase production (0.091U/min/ml) was observed at pH 7 (**Figure 7).**

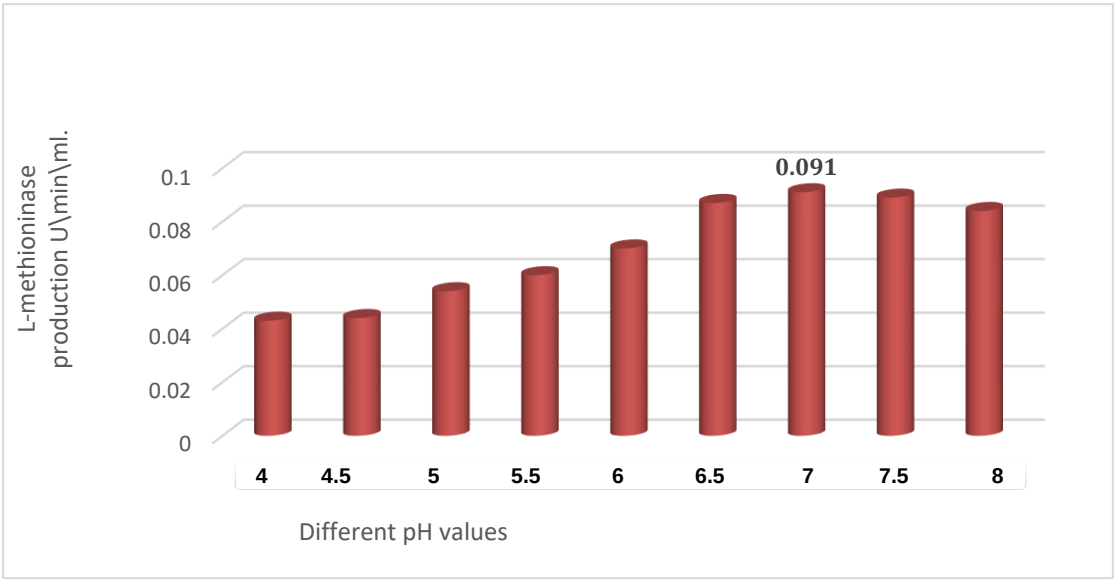

Figure 7: Effect of pH values on L-methioninase production by *S. proteamaculans*

Effect of different carbon sources on L-methioninase production by *S. proteamaculans*

The highest production of L-methioninasewas 0.04U/min/ml, and it was recorded in contestant incubator in cultures supplemented with fructose as the best carbon source followed by xylose (0.042 U/min/ml) and galactose (0.041 U/min/ml), compared to control (0.01 U\min\ml) **(Figure 8).**

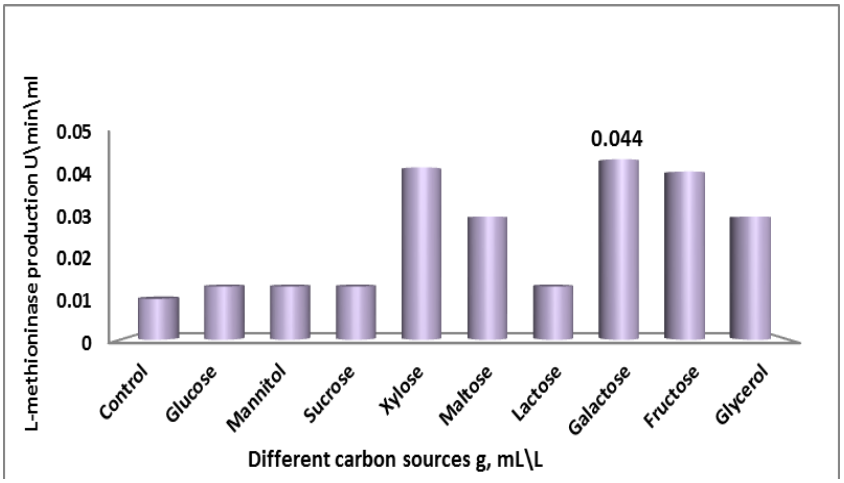

Figure 8: Effect of different carbon sources on L-methioninase production by *S. proteamaculans*

Effectof different concentrations of carbon source on L-methioninase production by the selected bacterial isolates.
To assess the impacts of various concentrations of carbon source on the production of L-methioninase by *Serratia proteamaculans*, different concentrations (1-9 g\L) of the best carbon source were assessed for each isolate (**Figure 9)** The highest L-methioninase production was 0.07U/min/ml when 7g/L of fructose was added to the fermentation medium.

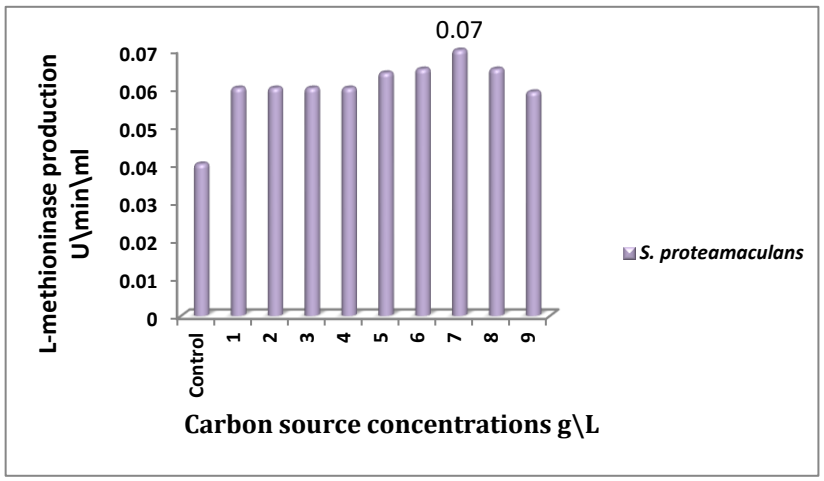

Figure 9: Effectdifferent concentrations of carbon source on L-methioninase production by *Serratia proteamaculans*

Effect of different nitrogen sources on L-methioninase production by *S. proteamaculans*
In the presence of nitrogen sources L-methionine and peptone the amounts of L-methioninase were 0.076 and 0.066 U/min/ml, respectively, which were lower compared with the control (0.1 U/min/ml) that did not supplement with nitrogen source in fermentation medium **(Figure 10).**

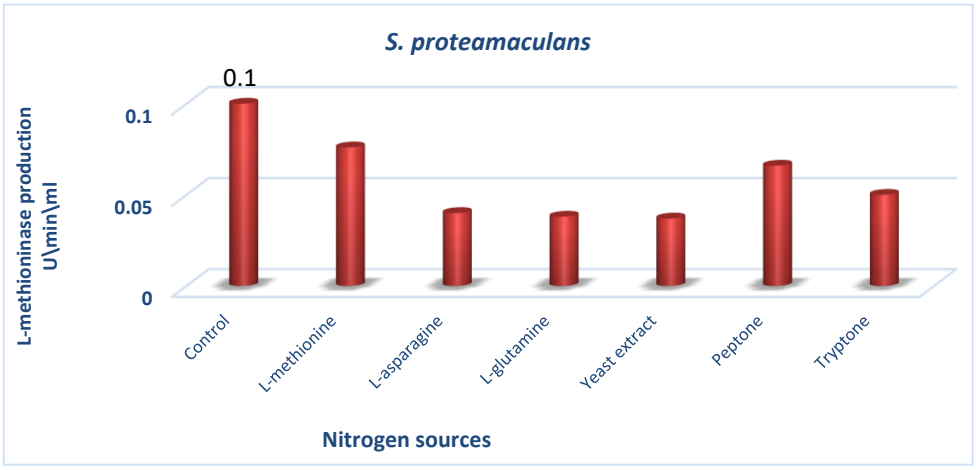

Figure 10: Effect of different nitrogen sources on L-methioninase production by *S. proteamaculans.*

Effect of inorganic nitrogen sources on L-methioninase production by the selected bacterial isolate

The effect of inorganic nitrogen sources including KNO_3, $NaNO_3$, and $(NH_4)2SO_4$ on L-methioninase production was assessed. The higher production of L-methioninase (0.102 U/min/ml) by *Serratia proteamaculans* was reported when $NaNO_3$ was added (**Figure 11).**

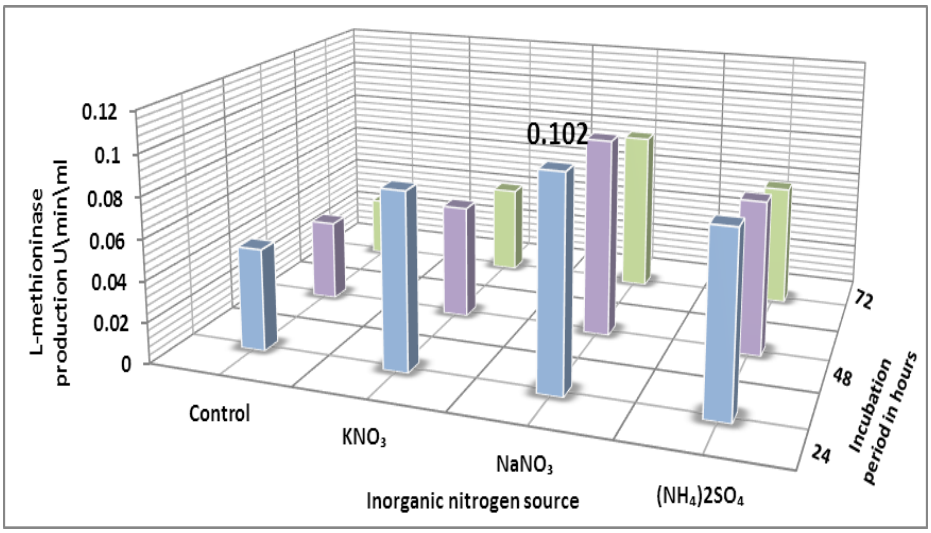

Figure 11: The effect of inorganic nitrogen source on L-methioninase production by *Serratia proteamaculans*.

Discussion

Phenol red shows the basic pH change, which is red in alkaline condition, turning to yellow under acidic condition. [24] Similarly, this technique is used to study the dissimilation of L-methionine by L-methioninase. Therefore, the isolates showing the yellow zone on the modified M9 medium were selected for primary screening. This results in a reduction in pH due to the formation of α-keto butyric acid; hence pH change leads to change in color to yellow. The result agrees with [19]. The ammonia formation raises the alkalinity of the L-methionine agar medium. The color changed to pink-red. These results agreed with [16, 25, 26].

The fermentation is a metabolic process in many microorganisms and involves oxide-reduction reactions resulting in the breakdown of complex organic compounds into simpler by-products and energy. The microbes carry out this metabolic action by the release of 27 extracellular enzymes [19]. Culture broths produced on L-methionine glucose media using *Aspergillus flavipes* have shown good L-methioninase production under submerged conditions. Culture broths have shown good L-methioninase production in modified M9 medium under submerged conditions. [22]

The quantitative assay of the CFF culture can be carried out by agar well diffusion technique, in which the wells are punctured on the agar containing the substrate and indicator. Then the well is loaded with the CFF culture to be tested. The isolates that showed the activity in the CFF culture on L-methionine agar plates were considered potent producers. The result also showed that the enzyme production was enhanced in the presence of carbon source, as the carbon source acts as a growth-supporting. This result agrees with [22, 25, 27, 28].

Physiologically, SSF has the potential for enzyme manufacturing. This scheme provides countless benefits, including elevated volumetric productivity, comparatively greater product concentration, lower wealth generation, and easy fermentation machinery requirement. [29] In addition, limited amino acids (solid substrates) are chemically more stable than free ones; hence, the former is chosen as a substrate for the mass production of economically precious compounds for wide technological applications. [30, 31]The variation in the inductivity on the production of alkaline L-methioninase *of A. ustus* depended on the type and nature of solid substrate, and changes in the chemical composition of these compounds. [22]

The most significant physical variable in the SMF and SSF was fermentation time. Production of enzyme decreased after passing 24 and 48 hours, which could be due to either the inactivation of the enzyme owing to the presence of some kind of proteolytic activity or growth of the organism could have reached a phase from which its steady growth could no longer be balanced with the supply of nutrient resources. [32]

The L-methioninase production was reduced gradually with an increase in incubation temperature. This may be due to the denaturation of microbial strain at higher temperatures. The optimum temperature of the selected isolate was at 35±2°C, and the result agrees with [25].*P. putida* had optimal activity at 35±2°C for different times reported by [33] and *Aeromonas* sp. Reported by [34]. Similar ranges were reported for several bacteria such as *Brevibacterium Linens* [17] and *Citrobacter intermedius* [35]. In addition, (El-Sayed, 2011) [36] reported that the optimum temperature for L-methioninase activity obtained from *Aspergillus flavipes* was observed at 35±2°C. Furthermore, enteric protozoan, such as *Entamoeba histolytica* and, *Treponema denticola*. [37, 38] Moreover, the maximum production of L-methioninase from *Streptomyces Variabilis*and *Aspergillus flavipes* were at 30 ±2°C and 45±2°C, respectively. [11, 39]

Many microorganisms including bacteria attack L-methionine but do not grow on it, perhaps because of their inability to metabolize the deaminated (α-keto methionine) and demethylated (α-keto butyric acid and methaniol) residues of L-methionine. The inability of bacteria to grow on L-methionine may be partially overcome by the use of a growth-supporting organic compound such as glucose, or equivalent carbohydrates designated as a co dissimilater. [27]

Carbon sources in culture medium were considered as critical factors for growth as well as metabolites' production by microorganisms. Fructose was the best carbon source by *S. proteamaculans* followed by xylose and galactose. Similar results obtained for L-methioninase production by *Pseudomonas ovalis* [40] and *Trichoderma vaginalis*. [27] This result was similar to that reported by Khalaf and El-Sayed (2009). [41] In contrast, Ruiz-Herrera and Stakey (1969b) [28]and Khalaf and El-Sayed (2009) [41] reported that glucose was the favored carbon source for L-methioninase production by filamentous fungi.

Growth and metabolism, along with enzyme production, are governed by a significant factor called pH. The important characteristic of most microorganisms is their strong dependence on the extracellular pH of the medium, which has a great effect on cell growth and enzyme production. [25] The maximum L-methioninase production by ***S. proteamaculans*** was increased by increasing the alkalinity of the medium. The production by ***S. proteamaculans*** was noticed at pH 7 and 7.5, and any increase or decrease in the pH of the medium resulted in a decline in the production of the enzyme. It was stated that maximum L-methioninase productivity was obtained at pH 8 by *Aspergillusflavipes* MTCC 6337, [11] *P.putida*, *P. ovalis*, [33, 40, 42]*Aeromonas* sp., [34]*Citrobacter freundii*, [43]*Trichoderma harzianum*, [44] *Aspergillus* Rs-1a [45] and *Aspergillusflavipes*. [22]*Serratia marcescens*, *Citrobacter freundii*, and *Lactococcus lactis* at pH 8, [43, 46, 47] and at pH 8.5 by *Aspergillus ustus* AUMC 10151. [25]

The physical properties for L-methioninase from different microbes are relatively similar. For example, pH optima ranged from 7.0 to 8.0. [15] Similar results were obtained for L-methioninase production by *Trichoderma harzianum* at pH 7, [44]*Brevibacteriumlinens* and *Brevibacterium linens* BL2 at pH 7 and 7.5, respectively. [48, 49]

It has been noted that among the various nitrogen sources supplemented, L-methionine promoted maximal L-methioninase production by *A. flavipes*. [11, 22] L-methioninase biosynthesis by *Geotrichum candidum* and *Pseudomonas putida* were found to be L-methionine-independent. [50, 51] The results revealed that L-methionine concentrations have no significant effect on L-methioninase production by *S. proteamaculans*. The production of L-methioninase regardless of the presence of its inducer L-methionine in the culture medium may indicate that L-methioninase was L-methionine-independent. In both cases, the strain was productive. Different species belonging to the *Serratia* genus are well-known plant growth-promoting bacteria (PGPR), and strains have been isolated from the rhizosphere of crops. Based on that, *Serratia* sp. is considered one of PGPR strains by fixing nitrogen. This explained the ability to produce the L-methioninase in nitrogen-source-free medium. [52] The lower enzyme production with higher concentrations of L-methionine may be imputed to the downregulation of GATA gene transcription that blocked the gene expression of L-methioninase, [53, 54] L-methionine catabolic suppression, or the trans inhibition phenomenon. [55]

Supplementation of the SMF medium of *A. ustus* with various nitrogen sources showed that none of the incorporated nitrogenous compounds had a stimulatory effect on alkaline production, except L-methionine. It could be concluded that the induction of L-methioninase by *A. ustus* was L-methionine-dependent (inducible enzyme) and was not only nitrogen regulated. These results were in accordance with that for L-methioninase production by *Aspergillus* sp., [45]*Yarrwia lipolytica*, [56] and *A. flavipes*. [41] However, L-methioninase production by *Pseudomonas putida* [57] and *Geotricum candidum* [50]was L-methionine-independent.

Members of the *Serratia* genus have a ubiquitous nature. Furthermore, they thrive very competitively in water and soil environments. [58]***S. proteamaculans*** is well known for contaminating and spoiling protein-rich food, like raw meat and seafood, [59, 60] using a strong capacity to resist CO_2-enriched modified atmosphere (MA), [61] and to metabolize protein and amino acids for growth. [62] Therefore, the secretion of potent lytic enzymes is characteristic gnomically of *S. proteamaculans*. [63] Accordingly, maybe *S. proteamaculans* have the ability of enzyme production in salt media (control).

References

1. Tanaka, H.; Esaki, N.; Soda, K., A versatile bacterial enzyme: L-methionine γ-lyase. Enzyme and Microbial Technology 1985, 7 (11), 530-537.
2. Singh, P.; Kharayat, B., Statistical optimization of reaction condition of L-methioninase from Bacillus subtilis. Research Reports 2018, 2.
3. Hoffman, R. M., Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. Expert opinion on biological therapy 2015, 15 (1), 21-31.
4. Knight, V.; Sanglier, J.-J.; DiTullio, D.; Braccili, S.; Bonner, P.; Waters, J.; Hughes, D.; Zhang, L., Diversifying microbial natural products for drug discovery. Applied microbiology and biotechnology 2003, 62 (5-6), 446-458.
5. Jesuraj, S. A. V.; Sarker, M. M. R.; Ming, L. C.; Praya, S. M. J.; Ravikumar, M.; Wui, W. T., Enhancement of the production of L-glutaminase, an anticancer enzyme, from Aeromonas veronii by adaptive and induced mutation techniques. PloS one 2017, 12 (8), e0181745.

6. Suganya, K.; Govindan, K.; Prabha, P.; Murugan, M., An Extensive Review on L-Methioninase and Its Potential Applications. Biocatalysis and Agricultural Biotechnology 2017.
7. Rodionov, D. A.; Vitreschak, A. G.; Mironov, A. A.; Gelfand, M. S., Comparative genomics of the methionine metabolism in Gram-positive bacteria: a variety of regulatory systems. Nucleic acids research 2004, 32 (11), 3340-3353.
8. Yoshimura, M.; Nakano, Y.; Yamashita, Y.; Oho, T.; Saito, T.; Koga, T., Formation of Methyl Mercaptan froml-Methionine by Porphyromonas gingivalis. Infection and immunity 2000, 68 (12), 6912-6916.
9. Sharma, B.; Singh, S.; Kanwar, S. S., L-methionase: a therapeutic enzyme to treat malignancies. BioMed research international 2014, 2014.
10. Tokoro, M.; Asai, T.; Kobayashi, S.; Takeuchi, T.; Nozaki, T., 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 2003.
11. Swathi, A., Optimization of process parameters for L-methioninase production in Solid state fermentation by Aspergillus flavipes from Sesame oil cake. Yeast 2015, 2, 1.0.
12. Krishnaveni, R.; Rathod, V.; Thakur, M.; Neelgund, Y., Transformation of L-tyrosine to L-DOPA by a novel fungus, Acremonium rutilum, under submerged fermentation. Current microbiology 2009, 58 (2), 122-128.
13. Bernardes, N.; Seruca, R.; Chakrabarty, A. M.; Fialho, A. M., Microbial-based therapy of cancer: current progress and future prospects. Bioengineered bugs 2010, 1 (3), 178-190.
14. Pavani, K.; Saradhi, S. V., Cloning and expression of methionine--lyase (MGL) of Brevibacterium linens. International Journal of Current Microbiology and Applied Sciences 2014, 3, 615-631.
15. El-Sayed, A. S., Microbial L-methioninase: production, molecular characterization, and therapeutic applications. Applied microbiology and biotechnology 2010, 86 (2), 445-467.
16. El-Sayed, A. S.; Shindia, A. A., PLP-Dependent Enzymes: a Potent Therapeutic Approach for Cancer and Cardiovascular Diseases. In Targets in Gene Therapy, InTech: 2011.
17. Pinnamaneni, R.; Gangula, S.; Koona, S.; Potti, R., Isolation screening and assaying of methioninase of Brevibacterium linens. Int J Sci & Nat 2012, 3 (4), 773-779.
18. Abdelraof, M.; Selim, M. H.; Elsoud, M. M. A.; Ali, M. M., Statistically optimized production of extracellular L-methionine γ-lyase by Streptomyces Sp. DMMMH60 and evaluation of purified enzyme in sub-culturing cell lines. Biocatalysis and Agricultural Biotechnology 2019, 101074.
19. Bahl, C.; Saxena, S. G.; Sharma, S. G. Screening endophytic fungal broth for l-Methioninase activity. 2012.
20. Jain; Richa; Zaidi; KU; Verma; Yogita; Saxena; Pooja, L-asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia. 2012.
21. Imada, A.; Igarasi, S.; Nakahama, K.; Isono, M., Asparaginase and glutaminase activities of micro-organisms. Microbiology 1973, 76 (1), 85-99.
22. El-Sayed, A. S., L-methioninase production by Aspergillus flavipes under solid-state fermentation. Journal of basic microbiology 2009, 49 (4), 331-341.
23. Buonaurio, R.; Stravato, V.; Cappelli, C., Brown spot caused by Sphingomonas sp. on yellow Spanish melon fruits in Spain. Plant pathology 2001, 50 (3), 397-401.
24. Theantana, T.; Hyde, K.; Lumyong, S., Asparaginase production by endophytic fungi isolated from some Thai medicinal plants. KMITL Science and Technology Journal 2007, 7.31, 13-18.
25. Abu-Tahon, M. A.; Isaac, G. S., 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 2016, 59.
26. Selim, M.; Elshikh, H.; El-Hadedy, D.; Saad, M.; Eliwa, E.; Abdelraof, M., 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 2015, 13 (2), 129-137.
27. Lockwood, B. C.; Coombs, G. H., Purification and characterization of methionine γ-lyase from Trichomonas vaginalis. Biochemical Journal 1991, 279 (3), 675-682.
28. Ruiz-Herrera, J.; Starkey, R. L., Dissimilation of methionine by fungi. Journal of bacteriology 1969, 99 (2), 544-551.
29. Sharma, D.; Niwas, S.; Behera, B., Solid state fermentation of bagasse for the production of cellulase enzyme from cellulolytic fungi and extent of simultaneous production of reducing sugars in the fermenter. Journal of Microbial Biotechnology 1991, 6 (1), 7-14.
30. Delgado-Andrade, C.; Seiquer, I., Maillard reaction products consumption: Magnesium bioavailability and bone mineralization in rats. Food chemistry 2008, 107 (2), 631-639.
31. El-Sayed, A. S.; Shouman, S. A.; Nassrat, H. M., Pharmacokinetics, immunogenicity and anticancer efficiency of Aspergillus flavipesl-methioninase. Enzyme and microbial technology 2012, 51 (4), 200-210.
32. Tunga, R.; Banerjee, R.; Bhattacharya, B., Some studies on optimization of extraction process for protease production in SSF. Bioprocess Engineering 1999, 20 (6), 485-489.

33. Takakura, T.; Mitsushima, K.; Yagi, S.; Inagaki, K.; Tanaka, H.; Esaki, N.; Soda, K.; Takimoto, A., Assay method for antitumor L-methionine γ-lyase: comprehensive kinetic analysis of the complex reaction with L-methionine. Analytical biochemistry 2004, 327 (2), 233-240.
34. Nakayama, T.; Esaki, N.; Sugie, K.; Beresov, T. T.; Tanaka, H.; Soda, K., Purification of bacterial L-methionine γ-lyase. Analytical biochemistry 1984, 138 (2), 421-424.
35. Faleev, N.; Troitskaya, M.; Paskonova, E.; Saporovskaya, M.; Belikov, V., L-Methionine-γ-lyase in Citrobacter intermedius cells: stereochemical requirements with respect to the thiol structure. Enzyme and Microbial Technology 1996, 19 (8), 590-593.
36. El-Sayed, A. S., Purification and characterization of a new L-methioninase from solid cultures of Aspergillus flavipes. The Journal of Microbiology 2011, 49 (1), 130-140.
37. Sato, D.; Yamagata, W.; Harada, S.; Nozaki, T., 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 2008, 275 (3), 548-560.
38. Fukamachi, H.; Nakano, Y.; Okano, S.; Shibata, Y.; Abiko, Y.; Yamashita, Y., High production of methyl mercaptan by L-methionine-α-deamino-γ-mercaptomethane lyase from Treponema denticola. Biochemical and biophysical research communications 2005, 331 (1), 127-131.
39. El Awady, M. E.; Selim, M. S.; Abd El-Razek, A. S.; Asker, M., Production, Purification and Characterization of L-Methioninase from Streptomyces Variabilis 3MA2016. RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES 2017, 8 (3), 906-921.
40. Tanaka, H.; Esaki, N.; Yamamoto, T.; Soda, K., Purification and properties of methioninase from Pseudomonas ovalis. FEBS letters 1976, 66 (2), 307-311.
41. Khalaf, S. A.; El-Sayed, A. S., L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions. Current microbiology 2009, 58 (3), 219-226.
42. Lishko, V. K.; Lishko, O. V.; Hoffman, R. M., The preparation of endotoxin-free l-methionine-α-deamino-γ-mercaptomethane-lyase (l-methioninase) from Pseudomonas putida. Protein expression and purification 1993, 4 (6), 529-533.
43. Manukhov, I. V.; Mamaeva, D. V.; Rastorguev, S. M.; Faleev, N. G.; Morozova, E. A.; Demidkina, T. V.; Zavilgelsky, G. B., A gene encoding l-methionine γ-lyase is present in Enterobacteriaceae family genomes: identification and characterization of Citrobacter freundii l-methionine γ-lyase. Journal of bacteriology 2005, 187 (11), 3889-3893.
44. Salim, N.; Santhiagu, A.; Joji, K., 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 2019, 17, 299-308.
45. Ruiz-Herrera, J.; Starkey, R. L., Dissimilation of methionine by a demethiolase of Aspergillus species. Journal of bacteriology 1969, 99 (3), 764-770.
46. Sundar, A. W. A., Isolation Of Methioninase Producers Optimization Of Enzyme Production And Purification Of L Methioninase. 2014.
47. Martínez-Cuesta, M. C.; Peláez, C.; Eagles, J.; Gasson, M. J.; Requena, T.; Hanniffy, S. B., YtjE from Lactococcus lactis IL1403 is a CS lyase with α, γ-elimination activity toward methionine. Appl. Environ. Microbiol. 2006, 72 (7), 4878-4884.
48. Dias, B.; Weimer, B., Purification and Characterization ofl-Methionine γ-Lyase from Brevibacterium linens BL2. Appl. Environ. Microbiol. 1998, 64 (9), 3327-3331.
49. Arfi, K.; Landaud, S.; Bonnarme, P., Evidence for distinct L-methionine catabolic pathways in the yeast Geotrichum candidum and the bacterium Brevibacterium linens. Appl. Environ. Microbiol. 2006, 72 (3), 2155-2162.
50. Bonnarme, P.; Arfi, K.; Dury, C.; Helinck, S.; Yvon, M.; Spinnler, H.-E., Sulfur compound production by Geotrichum candidum from L-methionine: importance of the transamination step. FEMS microbiology letters 2001, 205 (2), 247-252.
51. Tan, Y.; Xu, M.; Tan, X.; Tan, X.; Wang, X.; Saikawa, Y.; Nagahama, T.; Sun, X.; Lenz, M.; Hoffman, R. M., Overexpression and Large-Scale Production of Recombinantl-Methionine-α-deamino-γ-mercaptomethane-lyase for Novel Anticancer Therapy. Protein expression and purification 1997, 9 (2), 233-245.
52. George, P.; Gupta, A.; Gopal, M.; Thomas, L.; Thomas, G. V., Multifarious beneficial traits and plant growth promoting potential of Serratia marcescens KiSII and Enterobacter sp. RNF 267 isolated from the rhizosphere of coconut palms (Cocos nucifera L.). World Journal of Microbiology and Biotechnology 2013, 29 (1), 109-117.
53. Caddick, M. X.; Peters, D.; Platt, A., Nitrogen regulation in fungi. Antonie Van Leeuwenhoek 1994, 65 (3), 169-177.
54. Mitchell, A. P.; Magasanik, B., Regulation of glutamine-repressible gene products by the GLN3 function in Saccharomyces cerevisiae. Molecular and Cellular Biology 1984, 4 (12), 2758-2766.
55. Pall, M. L., Amino acid transport in Neurospora crassa IV. Properties and regulation of a methionine transport system. Biochimica et Biophysica Acta (BBA)-Biomembranes 1971, 233 (1), 201-214.

56. Bondar, D. C.; Beckerich, J.-M.; Bonnarme, P., Involvement of a branched-chain aminotransferase in production of volatile sulfur compounds in Yarrowia lipolytica. Applied and environmental microbiology 2005, 71 (8), 4585-4591.
57. Tan, Y.; Sun, X.; Xu, M.; An, Z.; Tan, X.; Tan, X.; Han, Q.; Miljkovic, D. A.; Yang, M.; Hoffman, R. M., Polyethylene glycol conjugation of recombinant methioninase for cancer therapy. Protein expression and purification 1998, 12 (1), 45-52.
58. Petersen, L. M.; Tisa, L. S., Friend or foe? A review of the mechanisms that drive Serratia towards diverse lifestyles. Canadian journal of microbiology 2013, 59 (9), 627-640.
59. Säde, E.; Murros, A.; Björkroth, J., Predominant enterobacteria on modified-atmosphere packaged meat and poultry. Food microbiology 2013, 34 (2), 252-258.
60. Fougy, L.; Desmonts, M.-H.; Coeuret, G.; Fassel, C.; Hamon, E.; Hézard, B.; Champomier-Vergès, M.-C.; Chaillou, S., Reducing salt in raw pork sausages increases spoilage and correlates with reduced bacterial diversity. Appl. Environ. Microbiol. 2016, 82 (13), 3928-3939.
61. Schuerger, A. C.; Ulrich, R.; Berry, B. J.; Nicholson, W. L., Growth of Serratia liquefaciens under 7 mbar, 0 C, and CO2-enriched anoxic atmospheres. Astrobiology 2013, 13 (2), 115-131.
62. Borch, E.; Kant-Muermans, M.-L.; Blixt, Y., Bacterial spoilage of meat and cured meat products. International journal of food microbiology 1996, 33 (1), 103-120.
63. Fougy, L.; Coeuret, G.; Champomier-Vergès, M.-C.; Chaillou, S., Draft genome sequence of Serratia proteamaculans MFPA44A14-05, a model organism for the study of meat and seafood spoilage. Genome Announc. 2017, 5 (23), e00491-17.