EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY
G

ISSN
2090-0872
WWW.EAJBS.EG.NET
Vol. 15 No. 1 (2023)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.14 (2) pp.77- 86 (2022)*
DOI: 10.21608/EAJBSG.2023.289460

Egypt. Acad. J. Biolog. Sci., 15(1):77-86(2023)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg

Screening for the Production of MetNase and Its Optimum Conditions from Streptomycete Isolate

Mohamed O. Abdelmonem [1], Mervat G. Hassan [1], Mohamed E. Elawady [2], Manal M. Mohamed [2] and Reem I. Hilal [1]

1-Department of Botany and Microbiology, Faculty of Science, Benha University, Benha 33516, Egypt.
2-Department of Genetic Engineering and Biotechnology National Research Center.
*E.Mail: dr.prof2011@gmail.com

ARTICLE INFO

Article History
Received:21/12/2022
Accepted:27/2/2023
Available:3/3/2023

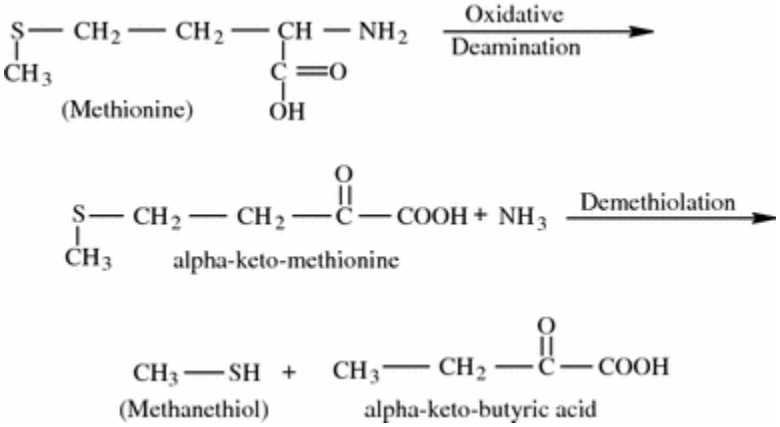
***Keywords*:**
Streptomycetes, MetNase, isolation, screening, optimization.

ABSTRACT

MetNase might be a valuable source for different medical applications. MetNase has the potential to be used against a wide range of tumour cell types as an anticancer agent. The purpose of this research is to screen and develop optimal conditions of streptomycetes isolates for MetNase production. For this study, 34 randomly selected streptomyces isolates from soil and marine water were used. The isolates that can produce MetNase were qualitatively selected using a quick plate assay method. When the pH was set to 7.0, the incubation temperature was set to 30 °C, and the carbon and nitrogen sources were glucose and peptone, respectively, the highest levels of MetNase synthesis were attained.

INTRODUCTION

MetNase, also known as L-methionine-diamino-mercaptomethane-lyase (EC 4.4.1.11), is a pyridoxal-L-phosphate-dependent enzyme that facilitates the oxidative deamination and demethiolation processes necessary to convert L-methionine to methanethiol, ketobutyrate, and ammonia (Khalaf and El-Sayed, 2009) as follows:



Microbial MetNase has garnered a lot of attention due to reports that it has antitumor properties against various types of malignant cells. In this regard, methionine serves as a growth factor that tumour cells must have indefinitely; when this resource is depleted, growth ceases, which results in cell death (El-Sayed, 2010).

MetNase from fungi is an extracellular enzyme, while that from bacteria is an intracellular enzyme. Recent studies have focused a lot of attention on the discovery of the MetNase enzyme from fungal sources due to its higher L-methionine substrate specificity and lower immunogenicity to the human immune system (Salim *et al*., 2019). It has been discovered that methionine deprivation is a potentially effective treatment for methionine auxotrophic cancers. Additionally, MetNase demonstrates a range of medicinal properties, such as antioxidant activity by lowering polyamine concentrations, which include spermidine, spermine, and putrescine. (Carr and Arduino, 1981; Moinard *et al*., 2005; Gilmour, 2007) in the food industry as well by releasing volatile sulphur compounds to improve flavour (Bonnarme *et al*., 2000; Amarita *et al*., 2004). In this respect, some bacteria that garner a lot of attention for producing MetNase are *Streptomyces*, *Pseudomonas*, *Bacillus subtilis*, *Serratia marcescens*, *Citrobacter freundii*, *Brevibacterium* linens, and *Arthrobacter* sp (Nakayama *et al*., 1984; Bonnarme *et al*., 2000; Amarita *et al*., 2004; Manukhov *et al*., 2005; Sundar and Nellaiah, 2013; Selim *et al*., 2015a, Selim *et al*., 2015b; Singh and Kharayat, 2018). ASNase is currently used extensively to treat lymphosarcoma and acute lymphoblastic leukemia. (Rani *et al*., 2012), Hodgkin's disease (Sundar and Nellaiah, 2013) and additional kinds of tumors (Sadraeian *et al*., 2013). Administration of ASNase causes anaphylactic reactions, which is a related issue. MetNase, however, has not detected this issue. Numerous efforts have been made to produce MetNase from microorganisms, such as fungi and bacteria. Although using fungi to produce MetNase is an appealing option, it is difficult to avoid the possible anaphylactic reactions brought on by fungus sources. (El-Sayed, 2010). And hence, bacteria were chosen as MetNase producers are of significant interest.

Many tumour cells' reliance on methionine, an exogenous source of the sulphur amino acid (Cellarier *et al*., 2003; Mecham *et al*., 1983; Guo *et al*., 1993) making dietary methionine restriction (MR) a promising possible cancer treatment method. As long as homocysteine is present, restricting methionine has no impact on healthy cells, but MR prevents the growth and proliferation of a number of cancerous cell types. (Cellarier *et al*., 2003). In addition to preventing the growth of cancer cells, MR has been demonstrated in animal models to improve the efficacy of chemotherapy and radiation treatment. (Gao *et al*., 2019).

MATERIALS AND METHODS

Sample Collection:

Samples of the soil and water were taken from several Egyptian governorates between August and December 2021. Water samples were obtained from Alexandria and the South Sinai Sea and kept at 4°C until processing, while soil samples were taken from Giza, Dakahlia, and Gharbia. All samples were poised in sterile screw-capped tubes, and care was taken to ensure that the points of collection encompassed wide variations in characteristics such as soil colour and topographical distribution.

Isolation of Bacterial Strains:

Five grams of soil from each sample were suspended in forty-five sterile saline water (0.85% w/v aqueous NaCl solution). Serial dilutions were prepared up to 10^{-7} and 100 microliters were spread on starch nitrate agar and incubated at 37°C for 5 days. Also, 10 ml of water samples were added to 90 ml of sterile saline water. Petri dishes were made using the dilution procedure, to remove the moisture layer from the agar surface **(Janata *et al*.,2018)**. Each plate received a 0.1 ml inoculation of the correct dilution, which was dispersed using a sterile glass rod.

Streptomycetes were isolated using starch nitrate agar. The agar was composed of 2% agar, pH 6.5 (w/v), 0.5% starch, 1% peptone, 0.33% K2HPO4, and 0.5% NaCl. By streaking, multiple agar plates were inoculated with bacteria as part of the purification process. Single colonies were chosen for sub-culturing on agar slants. (Hayakawa and Nonomura, 1987).

Screening of Bacterial Isolates for Methionase Enzyme:

Using a qualitative rapid plate assay, the obtained bacterial isolates were examined for their ability to produce the methioninase enzyme. (Gulati, 1997). The modified medium contained disodium hydrogen phosphate (6 g/L), potassium dihydrogen phosphate (3 g/L), sodium chloride (0.5 g/L), L-methionine (5 g/L), magnesium sulfate (0.24 g/L), calcium chloride (0.011 g/L), and glucose (2 g/L) in the specified concentrations. The pH of the medium was adjusted to 7.0 just before being poured into the plates, and a final concentration of 0.007% (w/v) of phenol red was included as an indicator. Subsequently, the plates were observed for a duration of 24 to 48 hours.

Screening of Streptomycetes Isolates for MetNase Production:

The rapid plate assay technique was used to test the bacterial isolates for their aptitude to yield MetNase. Methionine agar medium containing 0.3 ml of 0.07% phenol red, pH 7.0, was used as the testing medium. The pinkish zone surrounding the gatherings was measured after two to three days of incubation at 37 °C, and an enzyme index was determined using the formula: enzyme index = pink zone (mm)/colony diameter (mm), (Li *et al*., 2011).

MetNase Assay:

The Nesslerization method was used to measure the deamination activity, (Khalaf and El-Sayed, 2009). One ml of 50 mM Tris hydrochloride buffer (pH 7.0), 0.1 ml of 20 mM L-methionine solution, and 0.9 ml of deionized water were mixed in a clean test tube and equilibrated at 37°C for 5 minutes. To this mixture, 0.1 ml of enzyme solution was added and mixed immediately by vortex, after which the test tube was incubated at 37°C for 30 minutes. After incubation, 0.1 ml of 1.5M TCA (Trichloroacetic acid) was added to the test tube and mixed. The mixture was then centrifuged for 2 minutes to clarify, after which 0.2 ml of the supernatant, 4.3 ml of deionized water, and 0.5 ml of Nessler's reagent were mixed in a test tube, and the absorbance of the mixture measured at 436 nm using a spectrophotometer. A blank was also prepared without the enzyme, along with the experimental tubes. The amount of enzyme that releases one micromole of ammonia per minute under ideal assay conditions was used to express one unit of enzyme activity.

Optimizing the Production Medium for MetNase:

The study focused on optimizing the media components to achieve maximum production of methininase by the Streptomycetes strain. This was accomplished by analyzing a number of factors, such as the duration of incubation (4, 5, 6, 7, 8, and 9 days), the pH level (from 3 to 9 adjusted with 1 N HCl or 1 N NaOH), the temperature (between 20 and 40 C), the concentration of methionine (between 0 and 1.6%), and additional carbon sources. Additionally, different nitrogen sources were tested.

RESULTS

Isolation and Screening of *Streptomycetes*:

The results of the isolation of MetNase-producing organisms from a variety of samples collected from a variety of locations in a variety of Egyptian governorate regions are summarized in (Table 1). The colonies of MetNase-producing organisms were identified by a pink halo, as seen in Figure 1. A total of 34 isolates of the *streptomycetes* genus were randomly found in the sediment, seawater, and soil. Dakahlia produced the most *streptomycetes* isolates, followed by Gharbia and Giza, whereas Alexandria and South Sinai produced the least (Sharm-Elsheikh). Five *Streptomyces* isolates (D2, D10, G6, G8, and GH2) were screened for enzyme production by the presence of a pink hue around their colonial

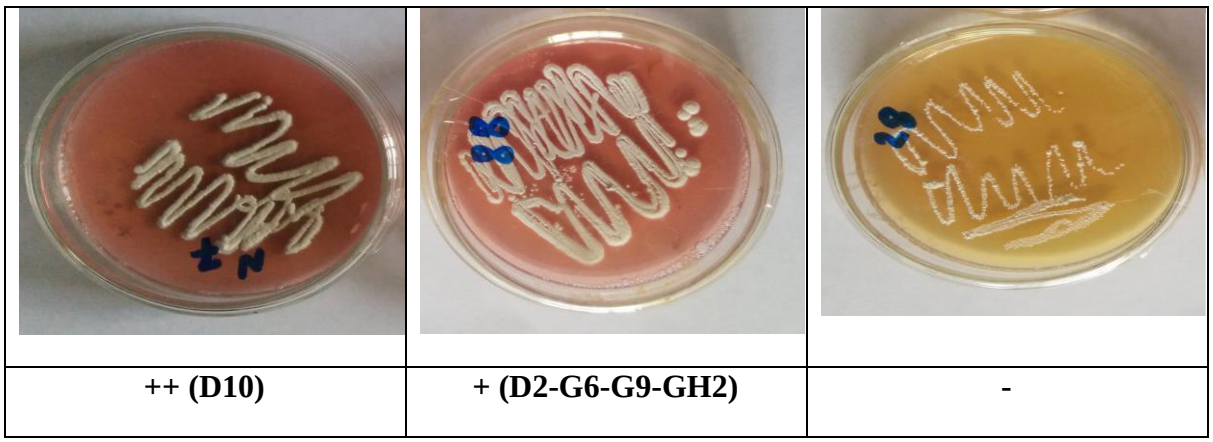
growth as evidence for the synthesis of extracellular MetNase. Production of methioninase was tested quantitatively and validated by a rapid plate assay method (Table 2).

Table 1: Total number of streptomycetes isolates.

Source	Locality	No. of isolates	Incidence percent (%)
Soil	Dakahlia	11	**32.35**
	Gharbia	9	**26.47**
	Giza	8	**23.52**
Marine	Alexandria	4	**11.76**
	South Sinai (Sharm El-Sheikh)	2	**5.88**
	Total	**34**	**100**

Table 2. Screening for production of MetNase from streptomycetes isolated by qualitative rapid plate assay test.

Sample	Results	Sample	Results	Sample	Results
D 1	-	G 2	-	GH 5	-
D 2	+	G 3	-	GH 6	-
D 3	-	G 4	-	GH 7	-
D 4	-	G 5	-	GH 8	-
D 5	-	G 6	+	Alex 1	-
D 6	-	G 7	-	Alex 2	-
D 7	-	G 8	+	Alex 3	-
D 8	-	G 9	-	Alex 4	-
D 9	-	GH 1	-	SH 1	-
D 10	++	GH 2	+	SH 2	-
D 11	-	GH 3	-		
G 1	-	GH 4	-		



++ (D10)	**+ (D2-G6-G9-GH2)**	**-**

Fig. 1: MetNase production screening using rapid plate assays.

Confirmation of the Screening for MetNase - Production by Streptomycetes:

The assessments of specific activity, enzyme synthesis, and protein estimate for each isolate revealed that isolate (D10) had the highest specific activity, as shown in Table 3. The isolates that exhibited typical MetNase-producing capacity were subsequently put through further identification, characterization, and optimization processes.

Table 3: Quantitative screening of *Streptomyces* isolates for MetNase formation

Samples	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
D2	48.5	3.7	22.83
D10	86.1	3.1	27.77
G6	39.4	2.7	14.59
G8	33.8	3.2	10.56
GH2	60.1	3.9	15.41

Factors Affecting the Production of MetNase:

Effect of Time:The generation of MetNase by Streptomycete isolate (D10) had an impact on the incubation time test. Results in (Fig. 2) indicated that MetNase synthesis increased progressively up to 7 days, at which point it peaked at 4.65 U/ml of enzyme production. Thereafter, enzyme activity decreased.

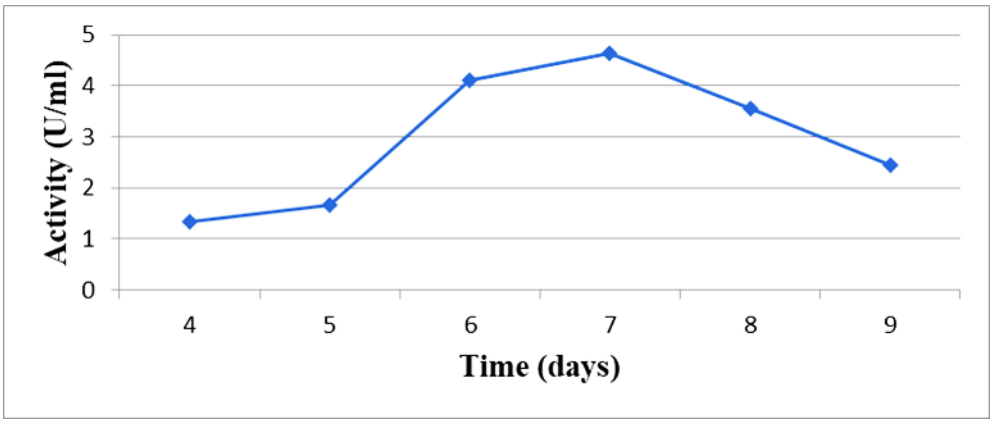


Fig. 2. The impact of incubation time on MetNase production

Effect of pH: Streptomycete isolate (D10) production of MetNase was influenced by pH. The pH of the fermentation medium has an impact on enzyme synthesis, as shown in **(**Fig. 3). Thus, at pH 7.0 (5.21 U/ml), the maximum enzyme production was observed. Reduced enzyme synthesis was caused by either an overabundance of enzymes or a reduction in the pH of the medium.

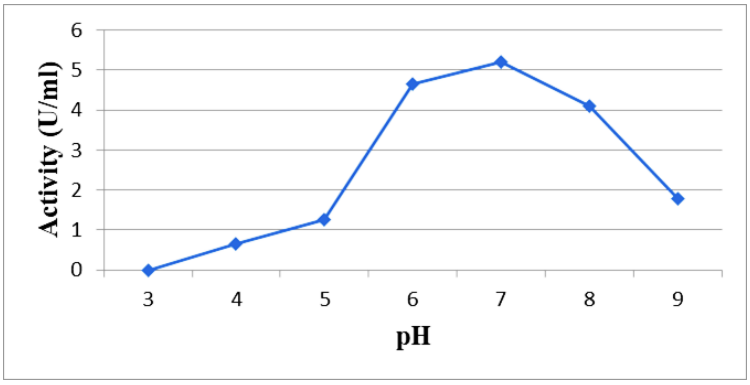


Fig. 3. Effect of pH on MetNase production.

Effect of Temperature: It is known that the fermentation medium's incubation temperature has an impact on how any microbial strain grows. Therefore, 30°C was shown to have the highest enzyme production (5.42 U/ml). As seen in (Fig. 4), any variation in temperature causes a reduction in MetNase synthesis.

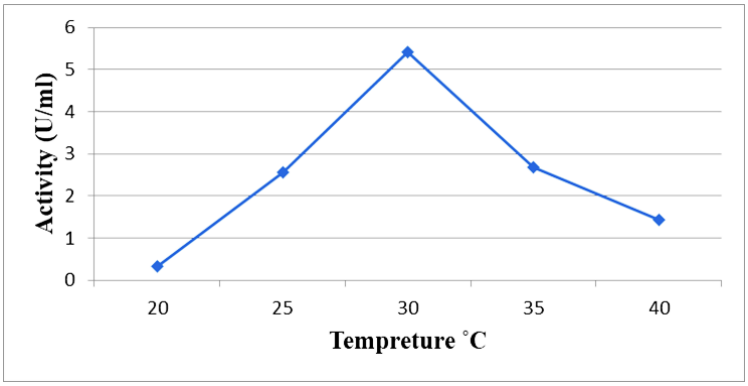


Fig. 4. The impact of Temperature on MetNase production

Effect of Methionine Concentration: MetNase synthesis is induced by the amino acid methionine. Therefore, mixing different methionine concentrations into the medium for producing enzymes was done. According to the data in (Fig. 5), The highest level of enzyme production (5.50 U/ml) was achieved at 0.4% concentration. However, there was a decreased ion in enzyme synthesis as a result of the rise in methionine concentration.

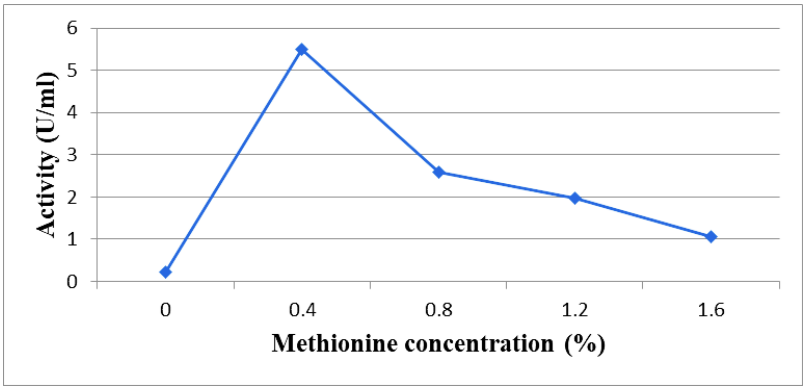


Fig. 5. Effect of asparagine concentration on MetNase production.

Effect of Different Carbon Sources: The synthesis of enzymes was increased by 1% by the addition of various carbon sources to the medium. All other carbon sources also demonstrated a sizable quantity of enzyme production, although glucose provided the highest enzyme output (6.35 U/ml). Arabinose had the lowest efficiency (0.41 U/ml) as a carbon source (Fig. 6).

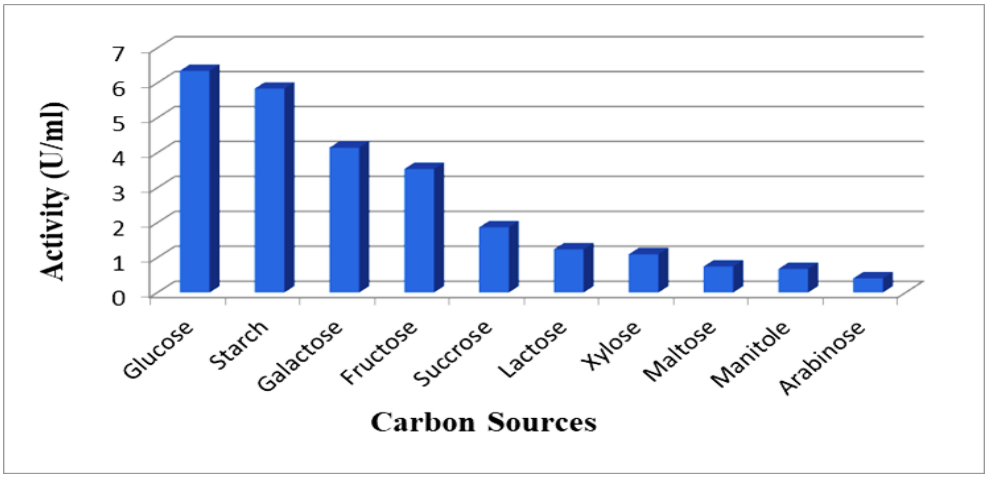


Fig. 6. Effect of different carbon sources on MetNase production.

Effect of Different Nitrogen Sources:A considerable increase in enzyme synthesis was seen in response to the addition of extra organic and inorganic nitrogen sources to the medium. Peptone supported the highest enzyme production (6.81 U/ml) out of all the other nitrogen sources examined. The synthesis of enzymes was present in varied degrees in all the other nitrogen sources. According to (Fig. 7), ammonium oxalate was the least efficient nitrogen source (0.75 U/ml).

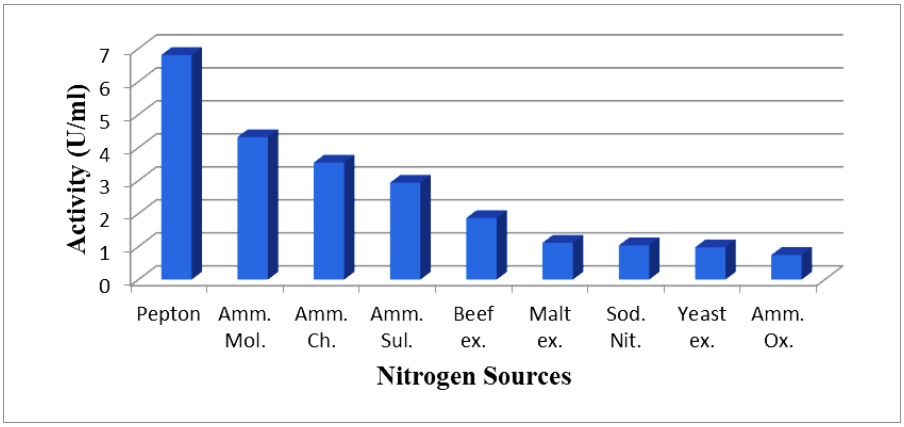


Fig. 7. Effect of different nitrogen sources on MetNase production

DISCUSSION

Microorganisms are the primary and most useful sources of commercial enzyme production due to their ability to grow on inexpensive media and produce enzymes rapidly. Enzymes usually work well under mild conditions of pH, pressure, and temperature, reducing the need for energy and capital investment in corrosion-resistant equipment. Moreover, enzyme-catalyzed reactions exhibit high reaction rates and easy catalytic regulation, leading to increased productivity and reduced overhead and production costs. (Mala *et al*., 1998). Global enzyme demand will rise 6.3% yearly through 2013. The value of the worldwide sales of

industrial enzymes is expected to be $5.1 billion in 2013 and 2018 (Taddei *et al*., 2006).

For that reason, a modified agar medium containing starch was utilized to evaluate the viability of 34 isolates of Streptomycetes. L-methionine was incorporated into the medium to provide a nitrogen source for the production of MetNase. Previous studies have suggested that bacteria and other microorganisms also utilize L-methionine for their metabolic processes. (Ferchichi *et al*., 1985), yeast (Selim *et al.*, 2013) and fungi (Khalaf and El-Sayed, 2009) possessing the capacity to produce the enzyme MetNase and using methionine as a nitrogen source. Only 5 of the *Streptomyces* isolates tested (isolates D2, D10, G6, G8, and GH2) were able to develop and use L-methionine as the solitary nitrogen cradle in the medium. On an agar plate medium with 0.5% L-methionine as the only source of nitrogen and carbon, the ability of these isolates to thrive was tested., according to (Selim *et al.*, 2015).

These isolates noticed results that demonstrated discernible growth. As a result, these isolates were employed for additional research. These isolates produce extracellular MetNase, as seen by the pink tint surrounding their colonial development. This occurrence concurs with what (Hendy *et al.*, 2022) observed about *Thermo-tolerant Fungi;* who claimed that the pink hue of the colonies was proof that the MetNase producers were present. These outcomes matched those that were reported by (Selim *et al.*, 2015) who used a rapid method to detect the production of MetNase and found that 18 isolates had pink colour around their colonial growth, indicating that these isolates were producing extracellular MetNase.

The ideal values for the incubation period, temperature, pH, methionine concentration, carbon source, and nitrogen source were 7 days, 30°C, 7, 0.4%, glucose, and peptone, respectively, according to a desirability analysis of the model variables and numerical optimization. Streptomyces yields MetNase at an ideal temperature of 30 °C, which is similar to that of many fungi, including *A. ustus*. (Abu-Tahon and Isaac, 2016) and *A. flavipes* (El-Sayed and Shindia, 2011) and bacteria *P. putida* (Takakura *et al*., 2004)

MetNase is produced at its maximum level by l-methionine, which is the best nitrogen supplement for *A. flavipes*. In (Abu-Tahon and Isaac, 2016) and (Khalaf and El-Sayed, 2009), l-methionine (0.4%) was found to be present in *A. ustus*. Different nitrogen sources, such as peptone and yeast extract in the medium, were previously shown to increase MetNase production by *G. candidum* (Arfi *et al*., 2006) and *C. tropicalis* (Selim *et al*., 2015).

Conclusion

This study describes the discovery of MetNase from Streptomycetes isolated from different locations in Egypt. Then, screen for the production of MetNase by a rapid assay method. Therefore, the potent one (D10) was subjected to optimization to increase the productivity of MetNase.

Competing interest: It is asserted that the authors do not have any conflicts of interest.

REFERENCES

Abu-Tahon M.A., Isaac G.S. (2016). Comparative study of a new alkaline MetNase production by Aspergillus ustus AUMC 10151 in submerged and solid-state fermentation. *Brazilian Archive Biological Technology*, p. 59

Amarita, F., Yvon, M., Nardi, M., Chambellon, E., Delettre, J., Bonnarme, P. (2004). Identification and functional analysis of the gene encoding methionine-γ-lyase in Brevibacterium linens. *Applied. Environmental. Microbiology*, 70 (12), 7348–7354.

Arfi K., Landaud S., Bonnarme P. (2006). Evidence for distinct L-methionine catabolic pathways in the yeast Geotrichum candidum and the bacterium Brevibacterium linens. *Applied Environmental Microbiology*, Mar;72(3):2155-62.

Bonnarme, P., Psoni, I., Spinnler, H. (2000). Diversity of L-methionine

catabolism in cheese ripening micro-organisms. *Applied. Environmental. Microbiology,* 66, 5514–5517.

Carr, K.A., Arduino, M.J. (1981). Isolation and characterization of bacterial MetNase as an effective. *Bios (Biological Honor Society)* 52 (1), 13–22.

Cellarier, E.; Durando, X.; Vasson, M.P.; Farges, M.C.; Demiden, A.; Maurizis, J.C.; Madelmont, J.C.; Chollet, P. (2003). Methionine dependency and cancer treatment. *Cancer Treat Review, 29,* 489–499.

El-Sayed AS. (2010). Microbial MetNase: Production, molecular characterization, and therapeutic applications. *Applied Microbiology Biotechnology*,86(2):445–67.

El-Sayed, A.S., Shindia, A.A. (2011). PLP-dependent enzymes: a potent therapeutic approach for cancer and cardiovascular diseases, in: Targets in Gene Therapy. Intech Open.

Ferchichi, M., Hemme, D., Nardi, M. and Pamboukdjian. N. (1985). Production of methanethiol from methionine by Brevibacterium linens CNRZ 918. Journal of Genetic.*Microbiology*,131:715-723.

Gao, X., Sanderson, S.M., Dai, Z., Reid, M.A., Cooper, D.E., Lu, M., Richie, J.P., Jr., Ciccarella, A., Calcagnotto, A., Mikhael, P.G. **(**2019**)**. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature, 572*, 397–401.

Gilmour, S.K. (2007). Polyamines and nonmelanoma skin cancer. *Toxicology and Applied Pharmacology,* 224 (3), 249–256.

Gulati R., Saxena R. K., and Gupta R. (1997). "A rapid plate assay for screening l-asparaginase producing micro-organisms," *Letters. Applied. Microbiology,* vol. 24, no. 1, pp. 23–26,

Guo, H.Y., Herrera, H., Groce, A., Hoffman, R.M. (1993). Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. *Cancer Research,* 53, 2479–2483.

Hayakawa M. and Nonomura H. (1987). "Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes," *Journal Fermentation Technology,* vol. 65, no. 5, pp. 501–509,

Khalaf S. A. and El-Sayed A. S. A. (2009). "MetNase production by filamentous fungi: I-screening and optimization under submerged conditions," *Current. Microbiology,* vol. 58, no. 3, pp. 219–226.

Khalaf, S.A., El-Sayed, A.S.A. (2009). MetNase Production by Filamentous Fungi: I-Screening and Optimization Under Submerged Conditions. *Current Microbiology,* 58, 219–226

Li A.-X., Guo L.-Z., Fu Q., and Lu W.-D. (2011). "A simple and rapid plate assay for screening of inulindegrading microorganisms using Lugol's iodine solution," *African Journal of Biotechnology,* vol. 10, no. 46, pp. 9518–9521,

Mala, B. R., Aparna, M. T., Mohini, S. G. and Vasanti, V. (1998). Molecular and biological aspects of proteases. *American Society Microbiology,* 62: 597-635.

Manukhov, I.V., Mamaeva, D.V., Rastorguev, S.M., Faleev, N.G., Morozova, E.A., Demidkina, T.V., Zavilgelsky, G.B. (2005). A gene encoding l-methionine γ-lyase is present in Enterobacteriaceae family genomes: identification and characterization of Citrobacter freundii l-methionine γ-lyase. *Journal. Bacteriology,* 187 (11), 3889–3893

Mecham, J.O., Rowitch, D., Wallace, C.D., Stern, P.H., Hoffman, R.M. **(**1983**)**. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. *Biochemical And Biophysical*

Research Communications. 117, 429–434.

Moinard, C., Cynober, L., de Bandt, J.-P. (2005). Polyamines: metabolism and implications in human diseases. *Clinical Nutrition,* 24 (2), 184–197.

Nakayama, T., Esaki, N., Lee, W.-J., Tanaka, I., Tanaka, H., Soda, K. (1984). Purification and properties of l-methionine γ-lyase from Aeromonas sp. *Agricultural and Biological Chemistry,* 48,(9) 2367–2369.

Rani, S.A., Sundaram, L., Vasantha, P.B. (2012). Isolation and screening of L-asparaginase producing fungi from soil samples. *International Journal of Pharmaceutics. Science,* 4 (1), 279–282.

Sadraeian, M., Rasoul-Amini, S., Mansoorkhani, M.J.K., Mohkam, M., Ghoshoon, M.B,. Ghasemi, Y. (2013). Induction of antitumor immunity against cervical cancer by protein HPV-16 E7 in fusion with ricin B chain in tumor-bearing mice. *International Journal Gynecologic Cancer,* 23 (5), 809–814.

Salim N, Santhiagu A, Joji K. (2019). Process modeling and optimization of high yielding MetNase from a newly isolated Trichoderma harzianum using response surface methodology and artificial neural network coupled genetic algorithm. *Biocatalysis and Agricultural Biotechnology*, [Internet];17:299–308.

Selim M. H., Elshikh H. H., El-Hadedy D. E., Saad M. M., Eliwa E., and Abdelraof M. (2015). "MetNase from some Streptomyces isolates I: Isolation, identification of best producers and some properties of the crude enzyme produced," *Journal of Genetic. Engineer Biotechnology,* vol. 13, no. 2, pp. 129–137,

Selim, M. H., Karm Eldin, E.-Z., Saad, M. M., Mostafa, E.-S. E., Shetia, Y. H., & Anise, A. A. H. (2015). Purification, Characterization of MetNase from *Candida tropicalis*, and Its Application as an Anticancer. *Biotechnology Research International*, *2015*, 173140.

Selim, M. H.; El Zahraa, A. K.; Moataza, M. S.; El-sayed, E. M.; Yosrea, H. S. and Amany, A. H. (2013). Some factors influencing MetNase formation by Candida tropicalis isolate. *Journal Applied Science Research*, 9 (4): 2842–2852.

Singh, P., Kharayat, B. (2018). Statistical optimization of reaction condition of MetNase from Bacillus subtilis. *Research Reports*, 2, e1–e9.

Sundar, W.A., Nellaiah, H. (2013). A rapid method for screening of methioninas producing Serratia marcescens species from soil. *International Journal of Pharmaceutics Science,* 5 (2), 426–427.

Taddei A, Rodríguez MJ, Márquez-Vilchez E, Castelli C. (2006). Isolation and identification of Streptomyces spp. from Venezuelan soils: morphological and biochemical studies. International. Microbiology Research.;161(3):222-31.

TakakuraT, K. Mitsushima, S. Yagi, K. Inag aki, H. Tanaka, N. Esaki, K. Soda, A. Takimoto. (2004). Assay method for antitumor L-methionine γ-lyase: comprehensive kinetic analysis of the complex reaction with L-methionine. *Analytical Biochemistry,* 327, pp. 233-240