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Purification and characterization of a novel thermo stable L-methioninase from *Streptomyces* sp. DMMMH4 and its evaluation for anticancer activity

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ABSTRACT

L-methioninase has been purified 2.55-fold from the crude extract of *Streptomyces* sp. DMMMH4. The purification procedure was carried out by heat treatment and gel filtration on Sephadex G-200 column chromatography. SDS-PAGE electrophoresis showed a migrating protein band molecular mass of 47 kDa. The kinetic properties determined for the purified enzyme displayed optimum activity at 70 ^{O}C and thermal stability were 70^{O}C for 30 min. The enzyme showed maximum activity at pH 6 using acetate buffer 0.05M and was relatively stable across a broad range of pH values (5.5-8 pH). The enzyme strongly inhibited by Cr^{+2}, Fe^{+2}, Ni^{+2}, Cd^{+2}, PMSF, β-mercaptoethanol and SDS while Hg^{+2},Cu^{+2} and iodoacetate completely inhibited the enzyme activity at a final concentration of 10mM. The purified enzyme exhibited a Km of 0.7, 0.15 and 0.25 mM for L-methionine, DL-ethionine and L-cystine respectively. Cytotoxicity test demonstrate that enzyme was active against liver HepG2, breast MCF-7, lung A549, prostate PC3 and colon HCT116 cancer cell lines and has negligible toxicity toward a normal melanocyte cell line HFB4.

INTRODUCTION

L-Methioninase (EC4.4.1.11; MGL), is a pyridoxal-5′-phosphate- dependent enzyme, catalyzes the γ-elimination of L-methionine to generate α-ketobutyrate, methanethiol, and ammonia as well as the α, β-replacement and β-elimination of *S*-substituted L-cysteines (Tanaka *et al*., 1983). A major potential therapeutic application of enzymes is in the treatment of cancer. Therefore, much attention has been paid to L-methioninase which have demonstrated antitumor efficacy *in vitro* as well as *in vivo* (Kahraman *et al*., 2011; Tan *et al*., 1998). L-methioninase is one of few microbial enzymes with high therapeutic value since it was reported as a potent anticancer agent against various types of tumor cell lines Breast, Lung, Colon, Kidney and Glioblastoma (Kokkinakis *et al*., 2001; Tan *et al*., 1998). Many human cancer cell lines and primary tumors have an absolute requirement for L-methionine as an essential amino acid, to survive and proliferate (Anderson, 1998). On the other hand, normal cells are methionine independent as they have active methionine synthase. Thus, they can grow on a medium supplemented with homocysteine, vitamin B12 and folic acid instead of methionine. The absence of methionine synthase in many tumors, in contrary to the normal cells, partially explains the inability of tumor cells to grow on homocystein (Kahraman *et al*., 2011 and Cellarier *et al*., 2003). Consequently, methionine is the main tumor specific target for therapeutic techniques. Therefore, therapeutic exploitation of L–methioninase to deplete plasma methionine seems to be a promising strategy (Sharma *et al*., 2014; Sundar and Nellaiah, 2013; Pinnamaneni *et al*., 2012; Yoshioka *et al*., 1998). L-methioninase was extensively characterized from various bacterial species especially *Pseudomonas*. Purification of L-methioninase from *Pseudomonas ovalis* was carried out by (Tanaka *et al*., 1976). They reported that, the purified enzyme had a molecular weight of 43KDa.

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A similar enzyme from a different clone of *Pseudomonas putida* was isolated and purified by (Ito *et al*., 1976). The purified enzyme from *Pseudomonas putida* ICR 3460 was published by (Nakayama *et al*., 1984) has a molecular weight 43KDa. Furthermore, pure L-methioninase was also obtained from other species of bacteria such as *Colstridium sporogens* (Kreis and Hession, 1973), *Brevibacterium linens* (Pinnamaneni *et al*., 2012). On the other hand, a few studies on the purification and characterization of L-methioninase from fungi such as *Aspirgillus flvips* reported by (El-Sayed, 2011), the purified enzyme had a molecular mass 47 KDa. In addition, Purification and characterization of this enzyme from the yeast was carried out by (Selim *et al*., 2015a). To our knowledge, no investigation has yet been performed on purification of this enzyme from *Streptomyces* species. Although, the only reported on the production of this enzyme by *Streptomyces* Sp was published through us (Selim *et al*., 2015b). In the present work, we purified and characterized of a new L-methioninase from *Streptomyces* sp. DMMMH4 and were to evaluate in-vitro anticancer activity.

MATERIAL AND METHODS

Materials

Chemicals and reagents

The source of chemicals and reagents used in this study were as follows: L-methionine from (Merk, Germany). Methanethiol used as sodium methanethiolate; Pyridoxal-5-phosphate (PLP); 5,5-Dithiobis-2-nitrobenzoicacid (DTNB); Commassi Brilliant Blue G-250 and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Sigma, St. Louis, USA). Sephadex G-200 and DEAE-cellulose were purchased from Pharmica Biotechnology (Sweden). Cancer and normal cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All other chemicals were of the highest analytical grade.

Methods

Streptomyces strain and production conditions:

Streptomyces sp. DMMMH4 was deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession number: LC021308. This strain was previously isolated from Egyptian soil and chosen as a most potent L-methioninase producer (Selim *et al*., 2015b). The enzyme production medium used is a modified starch medium. It's contained (g/L): Starch, 20; L-methionine, 3; $MgCl_2$ anhydrous, 1.5; $CaCO_3$, 3 and yeast extract. In addition, growth medium was adjusted to 7 using potassium phosphate buffer 0.075 M. After four days of growth with shaking (150 rbm, New Brunswick, USA) cells were harvested by centrifugation (5500 rpm, Herml, Germany). The clear supernatant was used as a crude enzyme.

Protein determination

Protein concentration was determined according to the method of (Bradford, 1976) and its determination in the purified fractions using method of (Schleif and Wensink, 1981) with bovine serum albumin as standard.

L-methioninase assay

L-Methioninase activity was determined by the method of (Arfi *et al*., 2003; Laakso *et al*., 1976) using L-methionine as a substrate. Methanethiol (MTL) produced from substrate reacted with 5,5 dithio-bis-2-nitrobenzoicacid added (DTNB) to form thionitrobenzoic acid was detected spectrophotometrically at 412 nm (Agilent UV/Vis. Cary-100). The assay mixture contained 20mM of L-methionine in 0.1M potassium phosphate buffer pH 7.0, 0.02mM PLP, 0.25mM DTNB and the supernatant in a final volume of 1 ml. One unit (U) of L-methioninase was expressed as the amount of enzyme that releases 1µmole of methanethiol per minute under optimal assay conditions.

In addition, Deaminating activity was carried out by measuring on releasing ammonia from L-methionine according to (Spinnler *et al*., 2001) using Nessler's reagent. One unit (U) of enzyme activity was expressed as the amount of enzyme that releases one µmol of ammonia per minute under optimal assay conditions.

L-methioninase Purification

The clear supernatant obtained was used as the crude enzyme preparation for the subsequent steps. According to the method of (Selim *et al*., 2015a), the enzyme was partially purified by heating the supernatant at different temperatures (60-70^{O}C) for different times (10-25min.) intervals. After cooling on ice the denatured proteins were removed using cooling centrifuge (Sigma, Germany at 5500 RPM) for 15 min. The active fraction was dialyzed overnight against the same volume of 0.05 M potassium phosphate buffer, pH.7 amended with 0.02mM PLP.

The dialyzed supernatant was applied to a (2.5×50 cm) column of Sephadex G-200 pre-equilibrated with 0.05 M potassium phosphate buffer pH 7 containing 0.02mM PLP. The enzyme fractions were eluted with the same buffer at a flow rate 1ml/5min. Eliot (5 ml fractions) was collected separately for the measurement of enzyme activity and protein content. The active fractions were combined and the solution was concentrated and collected prior to confirm the purity of enzyme and determination of molecular mass by (SDS-PAGE).

Determination of purity and molecular mass of L-methioninase

The homogeneity of purified L-methioninase was checked using dissociating polyacrylamide gel electrophoresis (SDS-PAGE) according to a protocol proposed by (Lammeali, 1970) with standard marker proteins.

Statistical analysis

Results are expressed as the mean ± S.D, calculated using excel 2010.

Effect of pH on the enzyme activity and stability

The optimum pH for pure enzyme activity was determined using 0.05 M of different buffers (i.e.) acetate (pH 4.0-6.0); potassium phosphate (pH 6.5- 8.0) and Glycine-NaOH (8.5-10). After incubation with different buffers, each reaction enzymatic activity was estimated. The pH stability of the enzyme was determined by preincubating the enzyme solution at different pH values ranging from 4.0 to 10 for 18 h at 4^{O}C and at room temperature 33^{O}C. At the end of preincubation time the pH value of enzyme solution was readjusted to pH 7 i.e. (optimum pH used in standard assay method) and then residual enzyme activity was assayed by the standard method.

Effect of temperature on the enzyme activity and stability

The effect of reaction temperature on pure enzyme activity was determined by incubating the reaction mixture at different temperatures ranging (30-90^{O}C). On the other hand, the thermal stability of the purified enzyme was determined by preincubating the enzyme solution at various temperatures (30-90^{O}C) for different times (0-100 min) in the absence of substrate. Followed by aliquots was removed, cooled of enzyme solution and the residual activity was measured by the standard assay method as previously mentioned.

Effect of some metal ions and chemical reagents on the enzyme activity

Metal ions with chloride salt and some chemical reagents tested for their effects on pure enzyme activity by incubating enzyme solution with 1 and 10mM final concentrations for 4h before adding the substrate. After preincubation time, enzymatic activity was determined under optimal assay conditions.

Determination of kinetic parameters

In this experiment, the kinetic parameters such as Michalis-Menten constant (Km) and maximum velocity (Vmax) of purified L-methioninase was determined by incubating the enzyme using different concentrations of each substrate in the range of (1-70 mM) under optimum assay conditions. The apparent Km and Vmax of purified enzyme were calculated from a Lineweaver-Burk plot.

In-Vitro anticancer activity assay

To determine the effect of Purified L-methioninase as anticancer activity, we tested the viability of cancer cell lines exposed to various concentrations of pure enzyme. Anticancer activity was expressed by median growth inhibitory concentration (IC_{50}). The antiproliferative activity of pure enzyme was evaluated against liver HepG2, breast MCF-7, lung A549, prostate PC3 and colon HCT116 cancer cell lines as well as the normal cell line (human normal melanocyte, HFB4) in comparison with doxorubicin as reference drug. The cells were grown and maintained in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100µg/ml). The cells were maintained at 37^{O}C in humidified atmosphere containing 5% CO_2. The cancer cell lines were suspended in medium at cells concentration of 5 x 10^4 cell/well in Corning® 96-well tissue culture plates grown in a 25 cm^2 flask in 5 ml of culture medium, and then incubated for 24 h. The tested enzyme was then added into 96 well plates (six replicates) to achieve eight concentrations of enzyme. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubation for 24 h, the numbers of viable cells were determined by the MTT test (Mosmann, 1983).

RESULTS AND DISCUSSION

Purification of L-methioninase

Purification of L-methioninase to homogeneity was accomplished in two successive steps Table (1). Nine eighty percent of L-methioninase activity was obtained from heat treatment at 70^{O}C for 10 min. Separation of enzyme from other proteins by gel filtration using Sephadex G-200 Fig. (1) resulted in a homogenous enzyme which was purified 2.55 fold with an activity yield of 49.1.

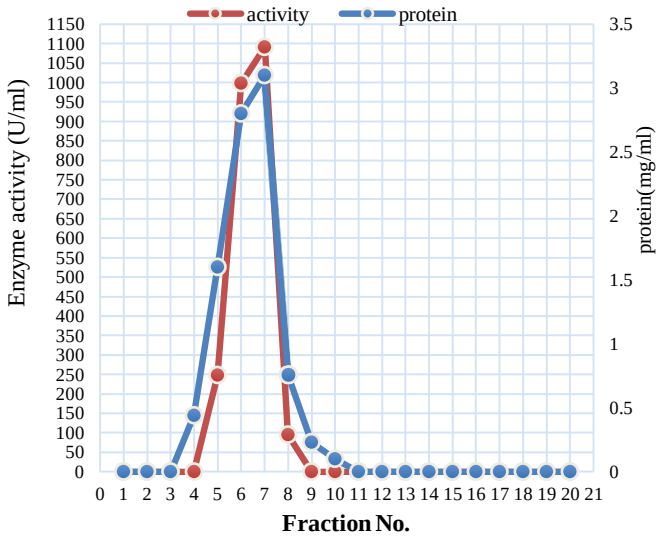

Fig. 1: Purification of L-methioninase using sephadex G-200.

On the other hand, when the gel was electrophoresd under denaturing conditions, a single band with an approximate molecular mass of 47 KDa was noted Fig. (2).

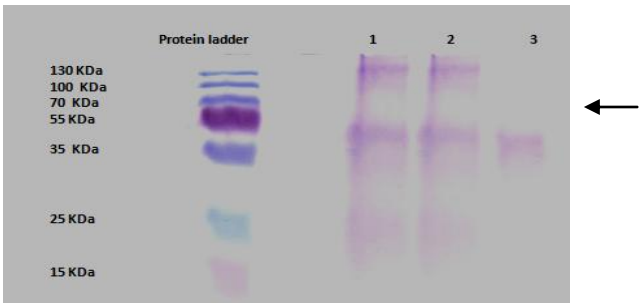

Fig. 2: Electrophoresis of *Streptomyces sp. DMMMH4* L-methioninase under the denaturing conditions of SDS-PAGE (12% acryl amide gel). Protein ladder: Molecular mass marker; gel lane 1: crude enzyme; gel lane 2: Heat treatment Gel lane 3: Sephadex G-200 column.

This enzyme has been purified from various sources but with several steps, L-methioninase from *Brevibacterium linens* was purified in five purification steps, including ammonium sulfate precipitation followed by several chromatographic procedures. Purified L-methioninase obtained from *Citrobacter freundii* by heat treatment at 60^{O}C followed by separation on DEAE-cellulose column and Sephacryl S-200HR column (Munkhov *et al*., 2005). Partial purification of this enzyme by heat treatment is consistent with published literature reported for purification from *Pseudomonas putida* (Tan *et al*., 1998; Hori *et al.,* 1996). In contrast, *Streptomyces* sp. DMMMH4 L-methioninase was purified using only two steps compared to other microbial sources. Accordingly, our results of molecular mass of the enzyme are in agreement with reported by L-methioninases purified from different sources, the molecular mass of L-methioninase purified from *Citrobacter freundii* (Munkhov *et al*., 2005) was found to range from 43.0 to 45.0 kDa per subunit. while, the purified enzyme from *Aspergillus flavipes* had a molecular mass 47 kDa (El-Sayed, 2011).

Effect of pH on the enzyme activity and stability:

Results in Fig. (3) indicate that somewhat acidic and neutral pH values (6-7 pH) using acetate and potassium phosphate buffers 0.05M were the most favorable for enzyme activity, the maximum activity of enzyme was obtained at pH 6 when acetate buffer 0.05M was used (103.9%).

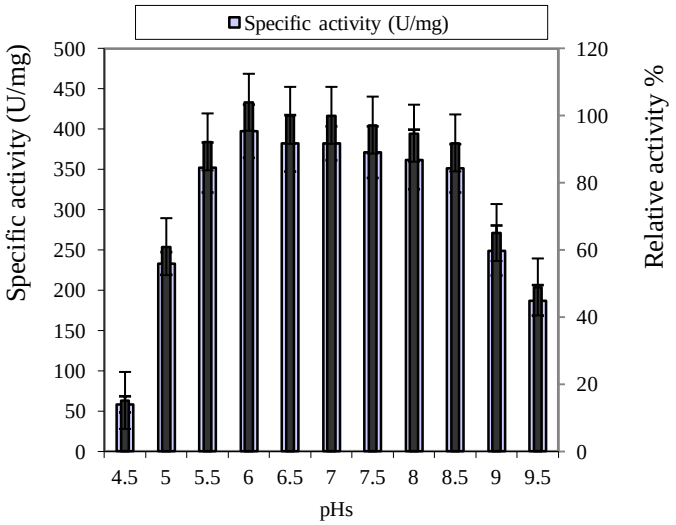

Fig. 3: Effect of pH on enzyme activity

In addition, when pH 6 adjusted using acetate buffer with different molarities from (0.025-0.20M), the response of enzyme activity was increased with the increase of buffer molarities reaching its maximum value when 0.05M was applied Fig. (4).

Furthermore, the enzyme was found to be stable in the pH range 6-8 and was more labial in the acidic region than in the alkaline region as shown in Table (2).

The optimum pHs for enzyme activity will be studied by many authors, for *Aspergillus flavipes* neutral to slightly alkaline pH (7-8 pH) was optima for its pure enzyme activity (El-sayed, 2011). While pH 6.5 was the most suitable for *Candida tropicalis* L-methioninase activity (Selim *et al*., 2015a). On the other hand, the alkaline range of pH correlated with maximum enzyme activity for bacteria, *Pseudomonas putida* (Nakayama *et al.*, 1984) & *Citrobacter freundii* (Mankhov *et al*., 2005).

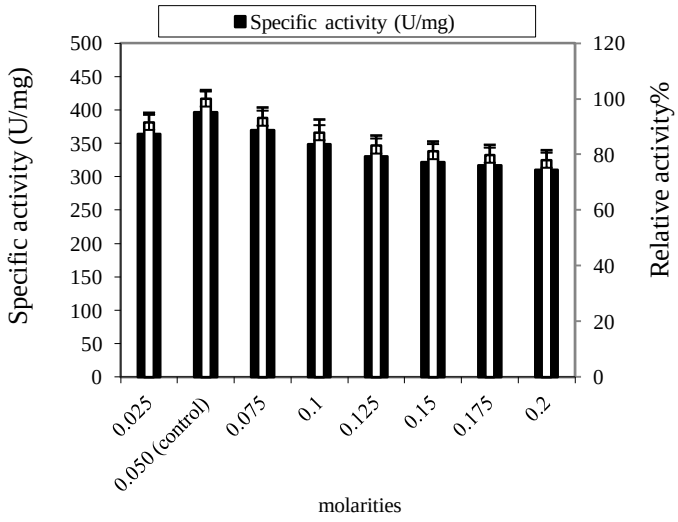

Fig. 4: Effect of different molarities on enzyme activity

Table 2: Effect of pH stability on the pure L-methioninase activity.

Buffer	pH	Relative activity %	Buffer	pH	Relative activity %
A	4	51.8	B	8	99.4
A	4.5	76.9	C	8.5	94.2
A	5.0	89.2	C	9	84.8
A	5.5	96	C	9.5	77.6
A	6	100	C	10	72.7
B	6.5	100			
B(control)	7	100			
B	7.5	100			

Where: A=Acetate Buffer, B= K. Phosphate Buffer, C= Glycine. NaOH Buffer.

On the other hand, the stability of the enzyme at different pHs higher than pH 8.5 and lower than pH 5 resulted in a decrease of enzyme activity, (El-sayed, 2011) suggesting that the lower stability of the enzyme at a higher and lower pHs may be attributed to the dissociation of pyridoxal-5-phosphate or unfolding of the enzyme active site. In addition, our data in agreement with (El-sayed, 2011) who mentioned that the isoelectric point of the enzyme was pH 4.8 and the lower enzyme stability in acidic conditions may be due to the closeness of his enzyme to the isoelectric point. The pH stability of the enzyme from other sources was over range from 7 to 8 (Dias and Weimmer, 1998; Tanaka *et al*,. 1976).

Effect of temperature on the enzyme activity and stability

Data presented in Fig. (5) demonstrated the optimum temperature for the enzyme activity was found to be 70^{O}C and it has significant activity over rang 60-75^{O}C. A slight decrease is obtained at higher temperatures (80-90^{O}C).

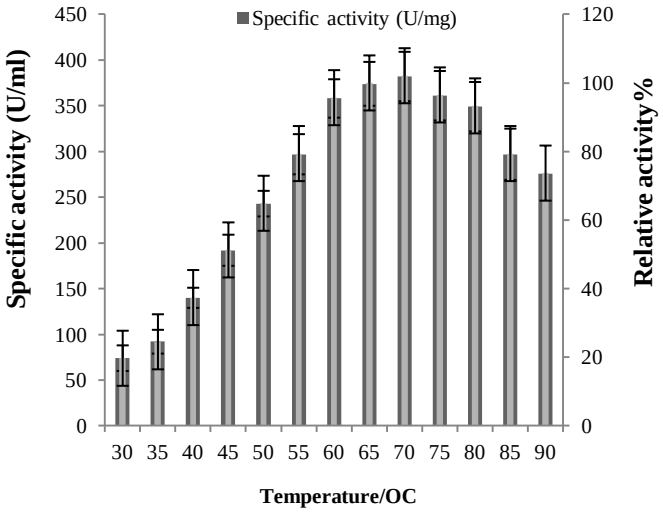

Fig. 5: Effect of temperature on enzyme activity

The progress of the enzymatic reaction with time was studying at the optimum temperature 70°C. The rate of L-methionine hydrolysis with time up to 10 min. Thereafter, no more hydrolysis occurred (data not shown). On the other hand, From the results are shown in Fig. (6), it could be noticed that, the pure enzyme was more stable up to 50°C for 100 min and not loss of activity followed by a slight decrease of the enzyme stability after 1 hr at 60°C was occurring. In addition, a severe inhibition of enzyme activity (15%) was noticed when the enzyme was incubated at 90°C for 100 min. In comparison with enzyme produced by other sources, our enzyme could be consider as a promising agent for its high stability (thermo stable).

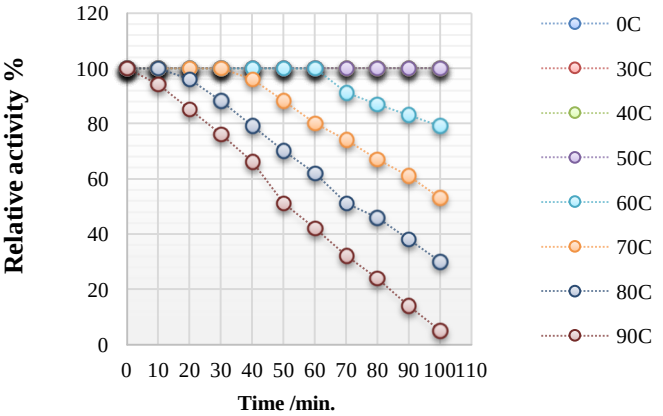

Fig. 6: Thermal stability of L-methioninase

Pseudomonas putida had optimal activity at 35°C for different times reported by (Nakayama *et al.*, 1984). Similar ranges were reported for several bacteria such as *Brevibacterium Linens* (Pinnamaneni *et al.*, 2012), and *Citrobacter intermedius* (Faleev *et al.*, 1996). In addition, (El-sayed, 2011) reported that, the optimum temperature for L-methioninase activity obtained from *Asperigillus flavipes* was observed 35°C. Moreover, maximum activity of L-methioninase from *Candida trobicalis* was 45°C and the reaction time increased up to 20 min (Selim *et al.*, 2015a). Thermal stability of the enzyme from different sources was investigated by (Dais and Weimmer, 1998) they showed that L-methioninase from *Brevibacterium linins* had a thermal stability below 40°C and also (El-sayed, 2011) finding the enzyme from *Aspirgillus Flavipes* displayed a relative catalytic stability below a temperature of 40°C.

Substrate Specificity of Purified L-Methioninase

The ability of enzyme to catalyze the γ-elimination of various Sulfur containing amino acids is presented in Fig. (7). These amino acids were added with equal amounts (20mM) separately to the reaction mixture and incubated under optimum assay conditions. Data obtained from Fig. (7) indicate relative activities of the enzyme on these substrates. L-methioninase was found to degrade DL-ethionine with 103.7% more than L-methionine, followed by cystine and cysteine with 96.4% and 83.2% respectively. The enzyme exhibited high specificity for DL-ethionine then L-methionine and L-cystine.

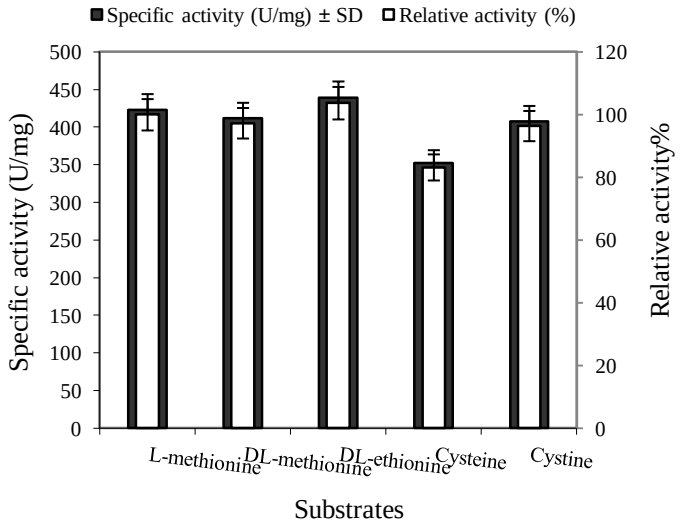

Fig. 7: Substrate specificity of purified L-methioninase.

The relative activity of L-methioninase towards the sulfur containing amino acids may be due to the similar molecular configuration of α &ß carbons (El-sayed, 2011). (Tanaka *et al.*, 1976) mentioned that L-methionine is the preferred substrate for L-methioninase purified from *Pseudomonas putida*. Furthermore, several derivatives of L-methionine and L-cysteine serve as effective substrate. Studies on the substrate specificity of *Candida tropicalis* L-methioninase revealed that the enzyme had a relative activity towards various sulfur containing amino acids (Selim *et al.*, 2015a).

Determination of Km and *Vmax*

L-methionine, DL-ethionine and L-cystine were serve as effective substrates thus we are calculating Km and Vmax for each from a Lineweaver-Burk plot. The Km and Vmax values was found to be (0.7; 0.15 & 0.25 mM) and (441; 452 & 504 U/mg/min.) for L-methionine, DL-ethionine and L-cystine

respectively as shown in Table (3). The enzyme showed maximal activity at a substrate level of 40, 60 and 50 mM for L-methionine, DL-ethionine and L-cystine respectively; this indicates that the active center of the enzyme became saturated with each substrate at concentrations above 40, 60 and 50 mM. Km and V_{max} were showing a high affinity of enzyme to its substrates exhibiting a low value of Km and confirmed its high therapeutic value of the enzyme. In this respect, L-methioninase from *Pseudomonas putida* (Esaki and Soda, 1987) was reported to exhibit a Km of 1mM. In addition, The purified enzyme exhibited a Km of 0.7mM from *Citrobacter freundii* (Munkhov *et al*., 2005).

Table 3: Kinetics of L-methioninase for different substrates.

Substrate	Vmax (U/mg/min.)	Km(mM)
L-methionine	441	0.7
DL-ethionine	452	0.15
D-cystine	504	0.25

Effect of Different Metal Ions and Inhibitors on L-methioninase Activity

The enzyme activity in the presence of metal ions and some chemical reagents was also determined. The results presented in Table (4) demonstrated that L-methioninase activity was inhibited and loss more than 50% of its activity when preincubating with Ni, Cu, Cr, Cd, SDS and iodoacetate at a final concentration of 10 mM. Whereas an increase and the stimulatory effect on enzyme activity found to be occurring with Mg and EDTA at both concentrations (1&10mM) by the relative enzyme activities (102& 100.2% and 101.8& 100.4%, respectively).

Furthermore, insensitivity of the enzyme activity to EDTA (a metallic protease inhibitor) indicated that the functioning of enzyme did not have the absolute requirement of metal ions and ensuring the non-metallic nature of this enzyme. All thiol compounds, DTT, glutathione, and β-mercabtoethanol had an inhibitory effect on enzyme activity by 68.2, 88.8 and 65%, respectively while the enzyme was completely inactivated by a thiol reducing agent iodoacetate, the complete inhibition of enzyme with this agent provides evidence for the presence of –SH group in the active sites of enzyme. Triton X-100 had a significant slight effect on enzyme activity compared to tween (80; 20) as reflected by the relative enzyme activities (92.7 and 100%, respectively). While the effect of sodium dodecyl sulfate as a strong surfactant on the enzyme was remaining 40.5% of its activity at 10 mM. Additionally, enzyme was strongly inhibited by PMSF, β-mercaptoethanol, SDS, and DTT suggests the presence of a cysteine/disulfide bond for maintaining the molecular catalytic folding state of the enzyme, consistent with the results obtained by (Thong *et al*., 1987) and (Lockwood and Coombs, 1991)**.** (Ferchechi *et al*., 1986) showed that, the enzyme activity of *Brevibacterium linens* was stimulated by Na^+ and K^+ and strongly inhibited by Zn^{+2}, Mn^{+2}, and Cu^{+2}. (Dais and Weimmer, 1998) founded that iodoacetate inhibited enzyme activity at 10mM while metal chelating agents did not influence enzyme activity. On the other hand, enzyme purified from *Aspergillus flavipes* was strongly inhibited by DL-propargylglycine, hydroxylamine, PMSF, β-mercaptoethanol, Hg^+, Cu^{2+}, and Fe^{2+} and slight inhibition by Triton X-100 (El-Sayed, 2011).

Table 4: Effect of some Metal Ions and chemical reagents.

Metal Ions and chemical reagents	Concentrations	
	1mM	10mM
	Relative activity (%)	Relative activity (%)
Control	100	100
K^+	98.8	96.8
Na^+	97.7	95.2
Ca^{2+}	98.6	98.8
Mg^{2+}	102	100.2
Ba^+	95.9	66.4
Zn^{2+}	96.5	77.3
Ni^{2+}	51.4	35.1
Cu^{2+}	77.3	2.26
Mn^{2+}	61.8	55
Hg^{2+}	11.7	0
Cd^{2+}	72.7	15.4
Co^{2+}	99	97.9
Fe^{2+}	55.3	50.4
Cr^{2+}	24.2	13.3
Li^{2+}	98.8	92.7
Dithiotheritol	87.7	68.2
Glutathione	89.3	88.8
Mercaptoethanol	87.9	65
Tween 80	100	100
Tween 20	100	97.7
Triton x-100	97.7	92.7
SDS	91.1	40.5
DMSO	94.7	86.6
EDTA	101.8	100.4
PMSF	92.7	60.7
Iodoacetate	39	0

***In-vitro* anticancer activity of L-methioninase**

The results Table (5) revealed that a good remarkable anticancer activity against cancer cells. L-methioninase was found to be potent anticancer agents had IC_{50}values near to the standard drug doxorubicin in various cancer cells with IC_{50} values 4.29±0.44 µg/ml (0.127 U/ml) in case of lung A549; 7.11±0.82 µg/ml (0.21 U/ml) in case of colon HCT116; 6.39±0.71 µg/ml (0.19 U/ml) in case of prostate PC3; 5.71 ±0.64µg/ml (0.17 U/ml) in case of breast MCF-7; and 4.33 ±0.46µg/ml (0.13 U/ml) in case of liver HepG-2 versus the standard drug doxorubicin which had IC_{50}values 4.11±0.50, 5.80±0.65 ,5.60±0.63, 2.96 ±0.34 and 3.97±0.45 µg/ml respectively, in A549, HCT116 , PC3, MCF-7 and HepG2 cells. It is clear that cancer cell lines were tested are more sensitive to L-methioninase. Additionally, the results revealed that L-methioninase has no toxicity against the growth of normal melanocytes HFB4 cells.

The strong inhibition of these cancer growth by action of L-methioninase, ensure its methionine auxotrophic identity for cancer cells. The efficiency of L-methioninase against various cell lines was reported by many authors (Tan *et al*., 2010; Sundar and Nellaiah, 2013 & Kui-Ying *et al.,* 2015). The sensitivity of cancer cell lines to L-methioninase was investigated by (Hori *et al*., 1996) they tested L-methioninase produced by *Pseudomonas putida* against various cancer cell lines and leukemia cell lines are more sensitive to L-methioninase than solid tumor cell lines. (Tan *et al*., 2010) showed that the rMETase was efficacy in broad series of cancer cell lines.

Table 5: In-vitro cytotoxic activity of L-methioninaseas expressed as IC_{50} values on different cell lines.

Compounds	IC_{50} (µg/ml)					
	MCF-7	HepG2	A549	HCT116	PC3	HFB4
Doxorubicin (Standard drug)	2.96 ±0.34	3.97 ±0.45	4.11 ±0.50	5.80 ±0.65	5.60 ±0.63	87.71 ±9.83
L-methioninase (U/ml)	5.71 ±0.64 (0.170 U/ml)	4.33 ±0.46 (0.131 U/ml)	4.29 ±0.44 (0.127 U/ml)	7.11 ±0.82 (0.216 U/ml)	6.39 ±0.71 (0.190 U/ml)	N.A

They reported that rMETase had a mean IC_{50}(units/ml) for the following cancer cell types: renal, 0.07; colon, 0.04; lung, 0.12; prostate, 0.01; melanoma, 0.19 and CNS, 0.195. In addition, methionine concentration lower than 10µM is necessary for inhibition of tumor-cell growth accompanied by cell death, this level of methionine depletion can be rapidly attained at 2 units/ml rMETase in vitro (Yoshioka *et al*., 1998)**.** (El-Sayed *et al*., 2012) mentioned that the enzyme showed a remarkable activity against prostate **(**PC3), liver (HEPG2), and breast (MCF7) cancers, with IC_{50} 0.001U/mL, 0.26 U/ml, and 0.37 U/ml, respectively. Toxicity of cancer cells to L-methioninase by *Candida trobicalis* against differed among cell lines studied by (Selim *et al*., 2015a); and they showed the breast cancer cell line was more sensitive ($IC_{50}$of 0.13 U/mL) than liver cancer cell line (IC_{50} 0.2U/mL).

CONCLUSION

The bacterium *Streptomyces* has so far never been investigated for L-methioninase purification. The studies presented in this report show that this bacterium may be a future source for larger production of a pure thermo stable L-methioninase. Catalytic parameters of L-methioninase from *Streptomyces* showed new biochemical prosperities about another L-methioninase producer. Our data observed that significantly inhibited of the different cancer cell lines and the enzyme did not display toxicity signs as shown using normal melanocytes cell line(HFB4),we can conclude that purified L-methioninase from a new source *Streptomyces* Sp might be a promising drug for the treatment of different cancer cells.

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REFERENCES

Anderson ME. Glutathion: an overview of biosynthesis and modulation. Chemico-Biological Interaction, 1998; 112:1-14.

Arfi K, Amarita F, Spinnler H, Bonnarme P. Catabolism of volatile sulfur compounds precursors by *Brevibacterium linens* and *Geotrichum candidum*, two microorganisms of the cheese ecosystem. Journal of Biotechnology, 2003; 105:245-253.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976; 72:248-254 .

Cellarier E, Durando X, Vasson MP, Farges MC, Demiden A, Maurizis JC, Madelmont JC, Chollet P. Methionine dependency and cancer treatment. Cancer Treatment Reviews, 2003; 29(6):489-499.

Dias B, Weimer B. Purification and characterization of L-methionine γ-lyase from *Brevibacterium linens* BL2. Applied and Environmental microbiology, 1998; 64:3327-3331.

El-Sayed AS. Purification and characterization of a new L-methioninase from solid cultures of *Aspergillus flavipes*. Journal of Microbiology, 2011; 49(1):130-140.

El-Sayed AS,. Shouman SA, Nassrat HM. Pharmacokinetics, immunogenicity and anticancer efficiency of *Aspergillus flavipes* L-methioninase. Enzyme and Microbial Technology, 2012; 51(4):200-210.

Esaki N, Soda K. L-methionine γ-lyase from *Pseudomonas putida* and *Aeromonas*. Methods Enzymology, 1987; 143:459-465.

Faleev N, Troitskaya M, Paskonova M, 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.

Ferchichi M, Hemme D, Nardi M. Induction of methanethiol production by *Brevibacterium linens* CNRZ 918. Journal of general microbiology, 1986; 131(4):715-723.

Hori H, Takabayashi K, Orvis L, Carson DA, Nobori T. Gene cloning and characterization of *Pseudomonas putida* L-methionine-*α*-deamino-*γ*-mercaptomethane-lyase. Cancer Research, 1996; 56(9):2116–2122.

Ito S, Nakmura T, Eguchi Y. Purification and characterization of methioninase from *Pseudomonas putida*. Journal of Biochemistery, 1976; 79:1263-1272.

Kahraman H, Aytan E, Kurt AG. Production of methionine γ-lyase in recombinant *Citrobacter freundii* bearing the hemoglobin gene. BMB Report, 2011; 44(9):590-594.

Kokkinakis DM, Hoffman RM, Frenkel EP, Wick JB, Han Q, Xu M, Tan Y, Schold, SC. Synergy between methionine stress and chemotherapy in the treatment of brain tumor xenografts in athymic mice. Cancer Research, 2001; 61(10):4017- 4023.

Kreis W, Hession C. Isolation and purification of L-methionine-alpha-deamino-gamma-mercaptomethane-lyase (L-methioninase) from *Clostridium sporogenes*. Cancer Research, 1973; 33(8):1862-1865.

Kui-Ying H, Hai-Yan H, Yan-Lai T, Feng-Geng X, Xue-Qun L, Jian-Zhong L. High-Level Expression, Purification and Large-Scale Production of L-Methionine γ-Lyase from *Idiomarina* as a Novel Anti-Leukemic Drug. Marian Drugs, 2015; 13:5492-5507.

Laakso S, Nurmikko V. A spectroscopic assay for demethiolating activity. Anal Biochem, 1976; 72:600-605.

Laemmli UK. Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature, 1970; 227:680-685.

Lockwood BC, Coombs GH. Purification and characterization of methionine *γ*-lyase from *Trichomonas vaginalis*. Biochemical Journal, 1991; 279(3):675-682.

Manukhov IV, Mamaeva DV, Rastorguev SM, Faleev NG, Morozova A, Demidkina TV, Zavilgelskyroil GB. A gene encoding L-methionine γ-lyase is present in Enterobacteriaceae family genomes: identification and characterization of *Citrobacter freundii* L-methionine γ-lyase. J Bacteriol, 2005; 187(11):3889-3893.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983; 65:55-63

Nakayama T, Esaki N, Sugie K, Beresov TT, Tanaka H, Soda K. Purification of bacterial L-methionine *γ*-lyase. Anal Biochem, 1984; 138, (2):421-424.

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.

Schleif RF, Wensink PC. Practical methods in molecular biology. Springer-Verlag, New York, Springer- Verlag, 1981; 74.

Selim MH, Karm Eldin E, Saad MM, Mostafa EE, Shetia YH, Hassabo AA. Purification, characterization of L-methioninase from *Candida tropicalis*, and its application as an anticancer. BioMed Res Int J, 2015a; 173140:1-10.

Selim MH, Elshikh HH, El-Hadedy DE, Saad MM, 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 & Biotechnology, 2015b; 13(2):129-137.

Sharma B, Sukhdev SS, Kanwar SS. L-methionase: A therapeutic enzyme to treat malignancies. BioMed Res Int J, 2014; 506287:1-13.

Spinnler H, Berger C, Lapadatescu C, Bonnarme P. Production of sulfur compounds by several yeasts of technological interest for cheese ripening. International Diary J, 2001; 11: 245-252.

Sundar W, Nellaiah H. Production of methioninase from *Serratia marcescens* isolated from soil and its anti-cancer activity against Dalton's Lymphoma Ascitic (DLA) and Ehrlich Ascitic Carcinoma (EAC) in Swiss Albino Mice. Tropical J Pharma Res, 2013; 12(5):699-704.

Tan Y,Sun X, Xu M, An Z, Tan X, Tan X, Han Q, Miljkovic DA, Yang M, Hoffaman RM. Polyethelyene glycol conjugation of recombinant methioninase for cancer therapy. Protein Expression and Purification, 1998; 12:45-52.

Tan, Y, Xu, M, and Hoffman, RM. Broad selectivity efficacy of recombinant methioninase and polyethelene glycol-modified recombinant methioninase on cancer cells in vitro. Anticancer Research, 2010; 30(4):1041-1046.

Tanaka H, Esaki N, Yamamoto T, Soda K. Purification and properties of methioninase from *Pseudomonas ovalis*. FEBS Letters, 1976; 66(2):307-311.

Tanaka H, Esaki N, Soda K. Bacterial L-Methionine gamma-lyase: Characterization and application. Progress in Clinical and Biological Res., 1983; 125:365-377.

Thong KW, Coombs GH, Sanderson BE. L-Methionine catabolism in Trichomnads. Mol. Biochem. Parasitol., 1987; 23(3):223-231.

Yoshioka T, Wada T, Uchida N, Maki H, Yoshida H, Ide N, Kasai H, Hojo K, Shono K, Maekawa R, Yagi S, Hoffman RM, Sugita K. Anticancer efficiency in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Research, 1998; 58(12): 2583-2587.

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