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Vol. 10(36), pp. 1513-1523, 28 September, 2016
DOI: 10.5897/AJMR2016.8132
Article Number: E9EB99360739
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation, screening and statistical optimizing of L-methioninase production by *Chaetomium globosum*

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Received 28 May 2016, Accepted 16 August, 2016

Egyptian soil sample was screened for isolation methioninlytic fungi by a rapid plate assay procedure. Eighteen strains of different isolated fungi were screened quantitavely for their L-methioninase activity. *Chaetomium globosum* was the most efficacious isolate and a dematiaceous filamentous fungi, it was identified at the molecular level by ribotyping 18S rRNA along with the biochemical characterization, and lastly completed by BLAST analysis by structure of a phylogenetic tree. Results showed that, the optimum levels of the incubation period, temperature, pH, methionine, sucrose and sodium nitrate concentrations were 3 days, 30°C, 7, 0%, 30 g/l and 1g/l, respectively. According to the produced model, at these levels, *C. globosum* produce L-methioninase with predicted specific activity of (≈2225 U/mg); so L-methioninase could be a good source for clinical therapeutic application. The rRNA sequence of *C. globosum* was deposited to gene bank under accession number KXO24450

Key words: L-methioninase, *Chaetomium globosum*, 18S rRNA, enzyme optimization, soil.

INTRODUCTION

L-Methioninase (E.C 4.4.1.11) is a pyridoxal phosphate-dependent enzyme and is a fulfilling several functions enzyme system because it stimulates the, γ- and α, β-removal reactions of methionine and its derivatives. Physiologically, normal cells have the capability to grow on homocysteine, instate of methionine, due to their efficient methionine synthase (Mecham et al., 1983). Unlike normal cells, tumor cells freed from efficient methionine synthase thus rely on external methionine supplementation from the diet (Hoffman, 1984). So, L-methioninase has extradited reasonable heed as a therapeutic agent against different kind of methionine dependent tumors (Weisendanger and Nisman, 1953; Kokkinakis et al., 1997). Methionine reduction has a broad spectrum of antitumor activities (Kokkinakis, 2006). Under methionine reduction, tumor cells were blocked in the late S-G2 phase because the pleiotropic influences and suffered apoptosis. Thus, therapeutic making use of L-Methionine γ-lyase to reduction plasma methionine has been widely examined (Yoshioka et al., 1998; Hoffman., 2015). L-Methioninase is present in most of organisms, as bacteria, fungi, protozoa, and plants, except mammals. L-Methioninase was at first described from the rumen bacteria (Weisendanger and Nisman, 1953; Miwatani et al., 1954). A few studies were concentrated on the enzyme from eukaryotes (especially fungi), comparing it to the bacterial sources, even though the therapeutic response of the bacterial enzyme is commonly related

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with high immunogenicity, low substrate specificity, and risky influences to the kidney and liver (Sun et al., 2003). Like many bacterial and fungal species, the enzyme was detected in the cell-free extract (Lockwood and Coombs, 1991; Tokoro et al., 2003). L-methioninase was detected from different species of bacterial such as intracellular enzyme (Tanaka et al., 1976), the intracellular nature of bacterial L-methioninlytic enzyme may have extra limiting step during the scale production and also from fungi as intracellular and extracellular enzyme (Ruiz-Herrera and Starkey, 1969; El-Sayed, 2009). Due to the recurrent classification of L-methioninase as extracellular enzyme in the fungal extract, fungi could be considered as robust resources to this enzyme. As it was notified for other fungal enzymes, the extracellular output is more the intracellular one by four fold (Pandey et al., 1999; El-Sayed., 2008). *C. globosum* Kunze is a saprophytic fungus which is member of a genus whose species are described as cellulytic organism (Lakshmikant and Mathur., 1990). Due to their bio-deterioration ability, several strains are used in testing materials for mould growth resistance. *C. globosum* genes encoding putative proteins lead to the identification of 30 genes, 27 of which includes the degradation of various cell wall polymers (Longoni et al., 2012). According to the former researches, the production and purification of L-methioninase were fundamentally proceeds under submerged conditions (Bonnarme et al., 2000; Amarita et al., 2004; Martinez-Cuesta et al., 2006). L-methionine as many amino acids can be formed Amadori compounds through Millard reactions (Delgado-Andrade et al., 2007) that decrease their bioavailability as carbon and nitrogen for the organism. Screening for a new producers and new kinds of the growth medium for the bulk production of this enzyme with additional therapeutic characters to fulfill their requirements will be a challenge. Our work concentrated on screening for L-methioninase-producing fungi isolated from Egyptian soil and using statistical (response surface) methodology in a trial to reduce or eliminate L-methionine from the formulated medium in parallel with optimization of the production conditions.

MATERIALS AND METHODS

L-Methionine, sodium nitrate, sucrose, sodium nitroprusside, Nessler and Bradford reagent were of analytical grade.

Isolation and purification of methioninolytic fungi

The dilution-plate method was used for isolation of Egyptian soil fungi capable L-methioninase production as described by Johnson et al. (1959). Using modified medium, contains methionine (5 g/l), glucose (20 g/l), $NaNO_3$ (2 g/l), KCl (0.5 g/l), K_2HPO_4 (1 g/l), $MgSO_4$. $7H_2O$ (0.5 g/l), all dissolved in 1 L of distilled water. The final pH of the medium was adjusted to 6.0. The final plates were incubated at 28°C for 7 days, and the developed fungal isolates were purified on the same medium.

Screening for L-methioninase producer strains

The fungal isolates were screened for their L-methioninase productivities using qualitative rapid plate assay using the above medium and phenol red was added to the medium as indicator at final concentration of 0.007% just before pouring the plate and it incubated at 28°C for 7 days (Sundar and Nellaiah, 2013).

Morphological identification of methioninolytic fungi

Identification of the isolated fungi during our investigation was carried out using the morphological characteristics as colony diameter, the color of condia, extracellular exudates, pigmentation and the color of reverse mycelium and microscopic features were examined also as conidial heads, fruiting bodies, degree of sporulation and the homogeneity characters of conidiogenous cells by optical light microscope (10×90) Olympus CH40 according to the following studies: Barron (1968), Booth (1971, 1977), Ainsworth (1971), Ellis (1971, 1976) and Pitt (1985). Fungal isolates were grown onto malt extract-agar (MA) medium at 28°C for several days (7-10). The cultures were then kept in 4°C.

Molecular identification of methioninase producer fungi

Preparation of the fungal culture

The spores of 4 days old culture of tested fungi were collected by addition of sterile saline (5 ml) to slant and the suspension was inoculated to 100 ml of Czapek Dox's medium in 250 ml Erlenmeyer flask. After incubation for 4 days, the cultures were filtered and the mats were collected and washed with distilled water.

Genomic DNA extraction

Genomic DNA of fungi was extracted according to Sharma et al. (2007) as the following; 50 to 100 mg fungal mycelia were homogenized. Five hundred microliter of DNA extraction buffer (200 mM Tris-HCl pH 8, 240 mM NaCl; 25 mM EDTA, and 1% SDS) were then added to the homogenized fungal materials. One volume of phenol/$CHCl_3$, in the ratio of 1:1 (v/v), was added and mixed gently for 10 min on a shaker followed by centrifugation at 15000 x g for 10 min. The upper phase was transferred to a new tube and 0.1 vol of 3 M Na-acetate (pH 5.2) and 2 volume of ethanol (96%) were added and mixed well, incubated for 30 min at -20°C followed by centrifugation (15000 x g/4°C/20 min). The resulting pellet was washed with 700 µl of 70% ethanol, air dried and re-suspended in 100 µl of sterile bi-distilled water.

PCR amplification

The primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µl reaction mixture by using a *EF-Taq* (Sol Gent, Korea) as follows: Activation of Taq polymerase at 95°C for 2 min, 35 cycles of 95°C for 1 min, 55°C, and 72°C for 1 minutes each were performed, finishing with a 10- minute step at 72°C. The

Table 1. Experimental design matrix prepared using Taguchi orthogonal array (OA) for L-methioninase production.

Run order	Incubation time (days)	Temperature (ºC)	pH	Methionine Conc. (g/l)	Sucrose (g/l)	Sodium nitrate (g/l)
1	7	35	4	5	30	1.5
2	3	40	8	10	30	3
3	3	20	4	0	10	1
4	11	30	5	0	30	2.5
5	5	20	5	5	25	3
6	3	25	5	2.5	15	1.5
7	7	30	8	2.5	25	1
8	7	25	7	0	20	3
9	5	40	4	2.5	20	2.5
10	11	35	6	2.5	10	3
11	9	40	6	0	25	1.5
12	9	30	4	7.5	15	3
13	9	20	7	2.5	30	2
14	3	35	7	7.5	25	2.5
15	11	40	7	5	15	1
16	11	25	4	10	25	2
17	7	20	6	10	15	2.5
18	9	35	5	10	20	1
19	11	20	8	7.5	20	1.5
20	7	40	5	7.5	10	2
21	5	35	8	0	15	2
22	9	25	8	5	10	2.5
23	3	30	6	5	20	2
24	5	25	6	7.5	30	1
25	5	30	7	10	10	1.5

amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM Big Dye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

Production of L- methioninase

The higher producing isolates for L-methioninase were taken up for studies on enzymes production in the same medium. The conidial suspension was prepared by injecting 10 ml of sterilized saline solution (0.85%) into a 7 day old slant of selected fungus. 1 ml of spore suspension was inoculated in 30 ml of modified liquid Czapek-Dox medium containing methionine in 100-ml Erlenmeyer conical flasks. The submerged cultures were incubated at 28°C for 7 days at 120 rpm.

Experimental design

Response surface method; Taguchi orthogonal array (OA) model (Table 1) based on six factors, five levels each, was used to study the effect and interactions between different factors; incubation time (days), temperature (ºC), pH, methionine concentration (%), sucrose concentration (%) and sodium nitrate concentration (%) for maximum production of L-methioninase enzyme (U/mg protein). Experimental designs were performed using Design-Expert software (Stat-Ease Inc., Minneapolis, MN, USA, ver 7.0.0). Experimental significance of the obtained model was checked by F-test (calculated p-value) and goodness of fit by multiple correlation R as well as determination R^2 coefficients. L-Methioninase specific activity (U/mg protein) was measured as an experimental response.

Statistical analysis

Analysis of variance (ANOVA) was used to estimate the statistical parameters for maximum productivity of L-methioninase. A probability value of p value <0.05 was used as the criterion for statistical significance.

Methioninase assay

L-Methioninase activity was assayed by direct Nesslerization according to the method of Thompson and Morrison (1951) with some modifications. The standard reaction system contains 1 ml of 1% L-methionine in phosphate buffer (pH 7.0), 0.1 ml of pyridoxal

Table 2. Screening for L-methioninase production by different fungal strains.

Scientific name	L-methioninase activity
Penicillium sp. Pitt	-ve
Penicillium janthinellium Biourge	-ve
Aspergillus niger Van Tieghem	-ve
Aspergillus joponicus Saito	-ve
Aspergillus aculeatus Iizuka	-ve
Aspergillus sp. link	-ve
Penicillium nigricans (Bainier) Thom	-ve
Fusarium sp. Link	-ve
Collectrichum sp. (Sacc. & Magnus) Briosi & Cavara.	++
Penicillium digitatum (Persoon Ex Fr.) Saccardo	-ve
Pythium sp. Pringsh.	++
Fusarium oxysporium Snyder & Hansen	+
Penicillium glabrum (Wehmer) Westling	-ve
Aspergillus ochraceus Wihelm	-ve
Fusarium poae (Peck) Wollenw	-ve
Chaetomium globosum Kunzen, Fries.	+++
Aspergillus flavus Link	-ve
Aspergillus cervinus Massee	++

phosphate, and 1 ml of crude enzyme. The reaction system was incubated at 30°C for 1 h. The enzymatic activity was stopped by adding 0.5 ml of 1.5 mol/l trichloroacetic acid or by boiling for 5 min. The system was centrifuged at 5,000 rpm for 5 min to remove the precipitated protein. 0.1 ml of above mixture was added to 3.7 ml of distilled water and the released ammonia was determined using 0.2 ml of Nessler reagent, and the developed colored compound was measured at 480 nm using UV/VIS-2401 PC visible spectrophotometer (Shimadzu, Kyoto, Japan). Enzyme and substrate blanks were used as controls. One unit of L-methioninase was defined as the amount of enzyme that liberates ammonia at 1 µmol/h under optimal assay conditions. The specific activity of L-methioninase was expressed as the activity of enzyme in terms of units per milligram of protein.

Determination of extracellular protein

The protein concentration of the prepared crude was estimated by Bradford reagent according to Bradford (1976).

Determination of methionine uptake

The residual methionine of culture filtrate was determined on the basis of the thioether group according to the method of Hess and Sullivan (1943) with some modifications. Using this method, 1 ml of the supernatant was mixed with 0.5 ml of 3% glycine, 1 ml of 2% sodium nitroprusside, and 0.5 ml of 1 N NaOH. The mixture was incubated in a water bath at 40°C for 15 min., then chilled in an ice bath for 5 min. Next, 1 ml of a 1:9 (v/v) mixture of H_2SO_4:H_3PO_4 was added with vigorous shaking for 5 min. The developed color was measured spectrophotometrically at 530 nm. The methionine concentration was determined from the standard curve of methionine prepared under the same conditions. The rate of methionine uptake was expressed as the amount of consumed methionine/initial methionine concentration $\times$100.

Biomass determination

After the fermentation process, the cultures were centrifuged at 5,000 rpm for 10 min at 4°C followed by filtration through Whatman no. 1 filter paper. The cell pellets were washed with distilled water and dried at 80°C until a constant weight was achieved. The dry biomass was expressed as grams per liter of fermentation medium.

RESULTS AND DISCUSSION

Screening for L-methioninase production by soil fungi

The screening profile (Table 2) shows the ability of different fungal strains to produce L-methioninase

From 18 terrestrial fungi screened, only five isolates were identified as methioninlytic fungi as manifested by the pink color of the colonies, generating from the manufacture of ammonia by the action of L-methioninase on L-methionine (Table 2). Production of L-methioninase by *Fusarium oxysporium* was previously recorded (Bahl et al., 2012) but from endophytic isolate not terrestrial isolate like in our study. *Aspergillus* species are used in commercial enzyme production, more than 80 recombinant enzymes of fungal origins are universally used, and 55 of these proteins were produced from *Aspergillus* species (Yoder and Lehmbeck, 2004). According to our data, it is first record for production L-methioninase by *Aspergillus cervinus* and also it is first

Table 3. L-Methioninase productivity, methionine uptake and dry weight by *C. globosum* based on actual, predicted and residual values according to Taguchi OA:

Run order	Specific activity (U/mg protein)			Methionine uptake (%)	Dry weight (mg/ml)
	Actual value	Predicted value	Residual		
1	92	91.93	0.07	94.6	1.612
2	9.67	9.14	0.53	35.07	0.514
3	195.45	192.42	3.03	-	0.588
4	55.24	57.17	-1.93	-	1.208
5	9.31	7.61	1.70	15.98	0.94
6	33	41.31	-8.31	11.20	1.22
7	8.66	7.84	0.82	30.52	1.01
8	12.53	12.46	0.07	-	0.384
9	6.25	4.55	1.70	11.68	1.768
10	258.9	258.57	0.33	26.18	0.674
11	6.4	5.19	1.21	_	0.51
12	167.05	167.35	-0.30	15.88	0.998
13	31.23	30.02	1.21	36.68	1.202
14	15.26	18.57	-3.31	20.80	1.328
15	14.58	15.76	-1.18	24.32	0.462
16	23.125	22.80	0.33	42.18	0.44
17	53.75	54.43	-0.68	57.81	3.252
18	19.25	18.79	0.46	35.78	1.238
19	147.34	148.52	-1.18	61.72	2.388
20	10.53	11.21	-0.68	13.5	0.988
21	8.13	7.93	0.20	_	0.502
22	10.37	9.91	0.46	97.23	0.21
23	9.15	6.32	2.83	25.67	0.22
24	6.4	6.20	0.20	15.88	0.26
25	7.39	4.94	2.45	36.02	0.21

record for production L-methioninase by *Pythium* sp. and *Collectrichum* sp. While *C. globosum* has the best methioninase activity. Our results in this research directed to the identification of methioninlytic enzymes of the terrestrial fungus *C. globosum* as the first record. Thus, *C. globosum.* was selected as superior isolate for further experiment.

Morphological identification of *C. globosum*

C. globosum is a dematiaceous filamentous fungus isolated from soil, air and debris of plant. *C. globosum* is considered as causative agents of infections in humans. *C. globosum* have highly growth rate with texture is cottony; and color of surface colony is white and with colonies mature, color transform to olive while the color of reverse change from tan to red or brown to black. The fungal hyphae are septate, hyaline to brownish. Perithecia, asci and ascospores are present, the color of perithecia are brown to black, huge, brittle, and globose to flask-shaped and enclitic by tall spine. Perithecia have teeny rounded apertures named ostioles which include asci and ascospores inside ascospores are clavate to cylindrical in shape and are unicellular, brown in color (Ellis., 1971, 1976; Prokhorov and Linnik, 2011).

Sequencing of the 18S rRNA gene of *C. globosum*

Therefore, to portray the strain, the nucleotide sequences of the 18S rRNA of the strain were detected. Phylogenetic tree was structure by the method based on the 18S rRNA sequences. The 18S rRNA gene from the genomic DNA of the *C. globosum* (based on the Biochemical and staining characters) was enzymatically amplified by Taq DNA polymerase by using a universal fungal primer. From the phylogenetic analysis of sequence of *C. globosum* (Figure 1) with the watch closely related strains from the database. It appears a distinguished identity with *C. globosum.* The rRNA sequence of *C. globosum* was deposited to gene bank under accession number KXO24450 (http://www.ncbi.nlm.nih.gov/nuccore/KXO24450).

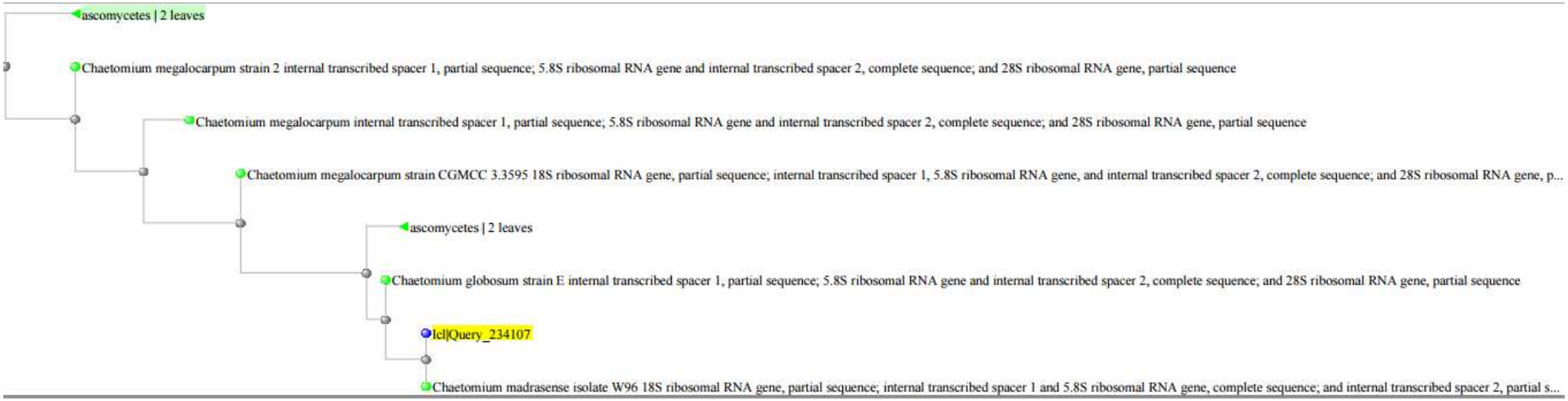


Figure 1. Phylogenetic analysis of *Chaetomium globosum.*

Taguchi orthogonal array (OA) experiments, statistical model and analysis

In Table 4, the ANOVA of L-methioninase production by *C. globosum* demonstrates that the model is significant due to a very high model F-value of 135.64 compared with a very low *p*-value of 0.0009. Values of "Prob > F" less than 0.05 point model terms are significant. For L-methioninase production model, B, C, D, E, F, AB, AC, AE, BC, BD, BE, BF, CD, CE, ABC, ABD, ABE, BCD are significant model terms. The model "Pred R-Squared" of 0.8126 is credible agreement with the model "Adj R-Squared" of 0.9916 with values close to 1 which shows good fit of the data to the regression model which also can be concluded from Table 2 due to small residuals between actual and predicted values.

The model "Adeq Precision"- measures the signal to noise ratio - of 42.646 shows an adequate signal and this model can be used to navigate the design space.

Last model equation for L-methioninase production by *C. globosum* in terms of coded factors:

L-Methioninase specific activity (U/mg protein) = -12.95 - 19.28*A - 594.02*B - 555.66*C -866.06*D - 587.41*E - 813.47*F - 1015.93*A*B - 1366.78*A*C + 42.97*A*D - 1077.11*A*E - 224.81*B*C - 128.49*B*D - 257.23*B*E - 905.15*B*F + 935.84*C*D + 183.16*C*E - 125.18*A*B*C - 82.41*A*B*D - 246.19*A*B*E - 37.35*A*B*F - 91.86*B*C*D

Where, A = Incubation time (days); B = Temperature (ºC); C = pH; D = Methionine concentration (%); E = Sucrose (%); F = Sodium nitrate (%).

The three dimensional (3D) response surface plots-generated by Design-Expert software shown in Figure 2 represent the relationships and effects of different experimental variables (factors) on L-methioninase productivity produced by *C. globosum*. Best experimental variables levels for maximizing L-methioninase production were predicted through analysis of these plots in combination with numerical optimization for each variable and desirability analysis.

Optimum conditions for L-methioninase production

According to desirability analysis of the model variables and numerical optimization, the optimum levels of the incubation period, temperature, pH, methionine, sucrose and sodium nitrate concentrations were 3 days, 30°C, 7, 0%, 30 g/l and 1 g/l, respectively. According to the produced model, at these levels, *C. globosum* produce L-methioninase with predicted specific activity of

Table 4. Analysis of variance (ANOVA) for response surface to reduce cubic model for L-methioninase production by *Chaetomium globosum.*

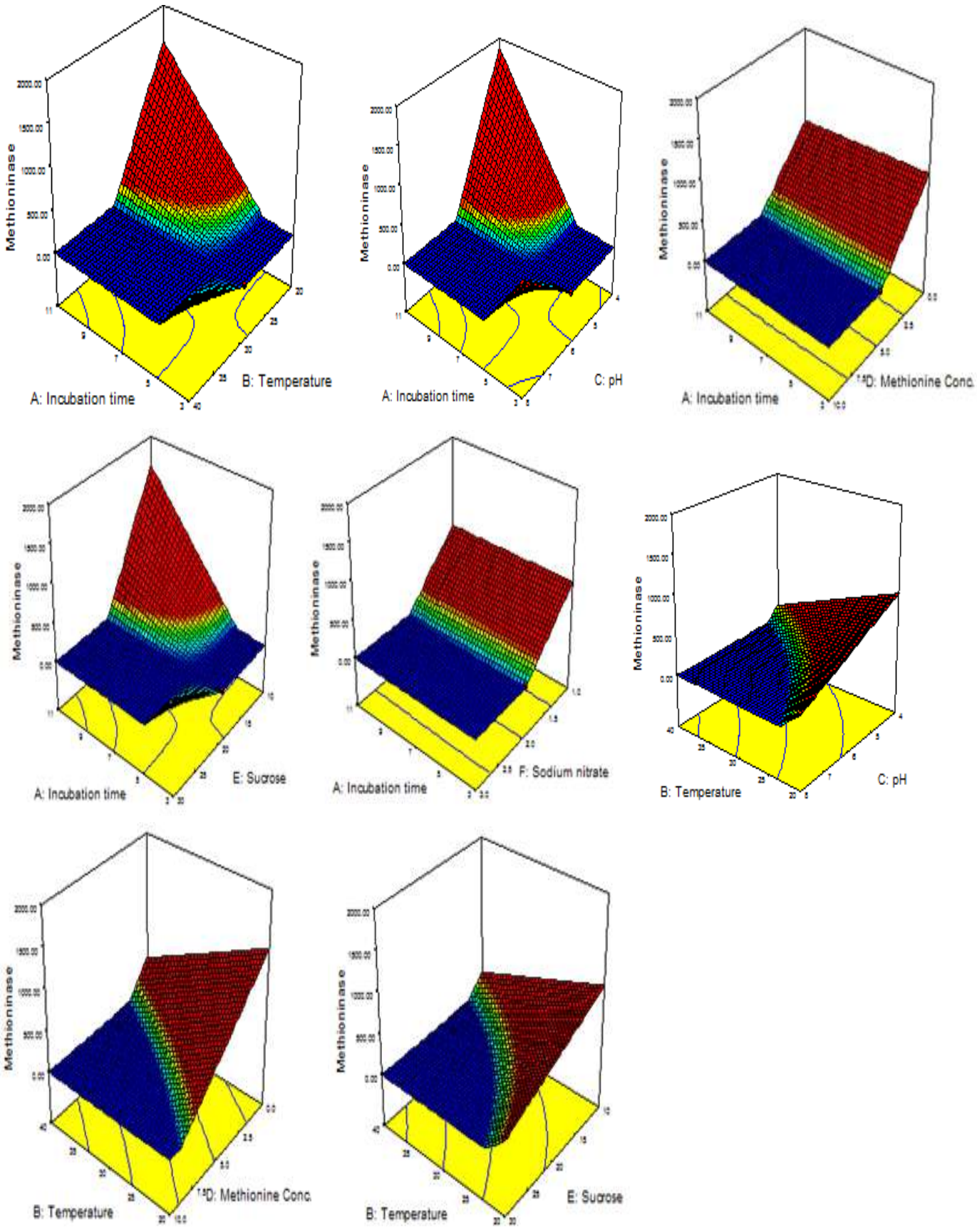
Source	Sum of squares	df	Mean square	F-Value	*p*-Value Prob > F*
Model	114841.90	21	5468.66	135.64	0.0009
A-Incubation time	402.18	1	402.18	9.98	0.0509
B-Temperature	5804.92	1	5804.92	143.98	0.0012
C-pH	6038.95	1	6038.95	149.78	0.0012
D-Methionine concentration	5658.72	1	5658.72	140.35	0.0013
E-Sucrose	5565.11	1	5565.11	138.03	0.0013
F-Sodium nitrate	5412.53	1	5412.53	134.24	0.0014
AB	5143.26	1	5143.26	127.57	0.0015
AC	5581.20	1	5581.20	138.43	0.0013
AD	346.03	1	346.03	8.58	0.0610
AE	6152.06	1	6152.06	152.59	0.0011
BC	3638.35	1	3638.35	90.24	0.0025
BD	5450.18	1	5450.18	135.18	0.0014
BE	3750.80	1	3750.80	93.03	0.0024
BF	5036.00	1	5036.00	124.91	0.0015
CD	5460.29	1	5460.29	135.43	0.0014
CE	5606.13	1	5606.13	139.05	0.0013
ABC	2381.47	1	2381.47	59.07	0.0046
ABD	1480.04	1	1480.04	36.71	0.0090
ABE	13740.96	1	13740.96	340.81	0.0003
ABF	315.07	1	315.07	7.81	0.0681
BCD	2830.56	1	2830.56	70.21	0.0036
Residual	120.95	3	40.32		
Cor Total	114962.86	24			

*Values of "Prob > F" less than 0.05 indicate model terms are significant. Reduced cubic model.

(≈2225 U/mg). The L-methioninase production by *Antechinus flavipes* is similar to the L-methioninase produced by *Achromobacter starkeyi, Aspergillus* sp. RS-1a, *Phoronis ovalis* and *Yarrwia lipolytica* (Ruiz-Herrera and Starkey, 1970; Rifai, 1969; Tanaka et al., 1976; Bondar et al., 2005) found to be L-methionine dependent. In contrast, L-methioninase biosynthesis by *Geotrichum candidum* and *Pseudomonas putida* were found to be L-methionine freelance (Bonnarme et al., 2001; Tan et al., 1997). The effect of various L-methionine concentrations on enzyme productivity by *A. flavipes* was investigated. The initial concentration of fermentation medium L-methionine does a significant influence on the uptake of L-methionine and so, on enzyme productivity by *A. flavipes*. The highest yield of L-methioninase and methionine uptake (94%) by *A. flavipes* was registered using 0.8% L-methionine. Higher levels of L-methionine (3.2%) suppress the enzyme yield by about 42.5% on a par with the control. It could be finished that the productivity of L-methioninase by the fungal isolate is L-methionine concentration subordinate. Moreover, the growth rate of *A. flavipes* was gradually raised with the level of L-methionine, arriving to its highest value (6 g/L) at 0.8% L-methionine, pursued by a gradual reduced to about 41.7 at 3.2% L-methionine. The lower enzyme yield with higher concentrations of L-methionine may be imputed to the down regulation of GATA gene transcription that blocked the gene expression of methioninase (Caddick et al., 1994, Mitchell and Magasanik, 1984), methionine catabolic suppression, or the transinhibition phenomenon (Pall, 1971).

The production of L-methioninase in any case the presence of its inducer methionine in the culture medium suggestes that, L-methioninase was found to be L-methionine freelance. Similar results were denoted for L-methioninase production by *G. candidum* and *P. putida* ((Bonnarme et al., 2001; Tan et al., 1997).

In contrast, Khalaf and El-Sayed (2009) indicated that the fashioning of L-methioninase production depended on L-methionine on containing medium (Bonnarme et al., 2001) on *Yarrwia lipolytica*. In addition to, the use of yeast extract and peptone in medium for the production of L- methioninase was registered by Arfi et al. (2006) from *G. candidum*.


Methioninase
A: Incubation time
B: Temperature
C: pH
D: Methionine Conc.
E: Sucrose
F: Sodium nitrate

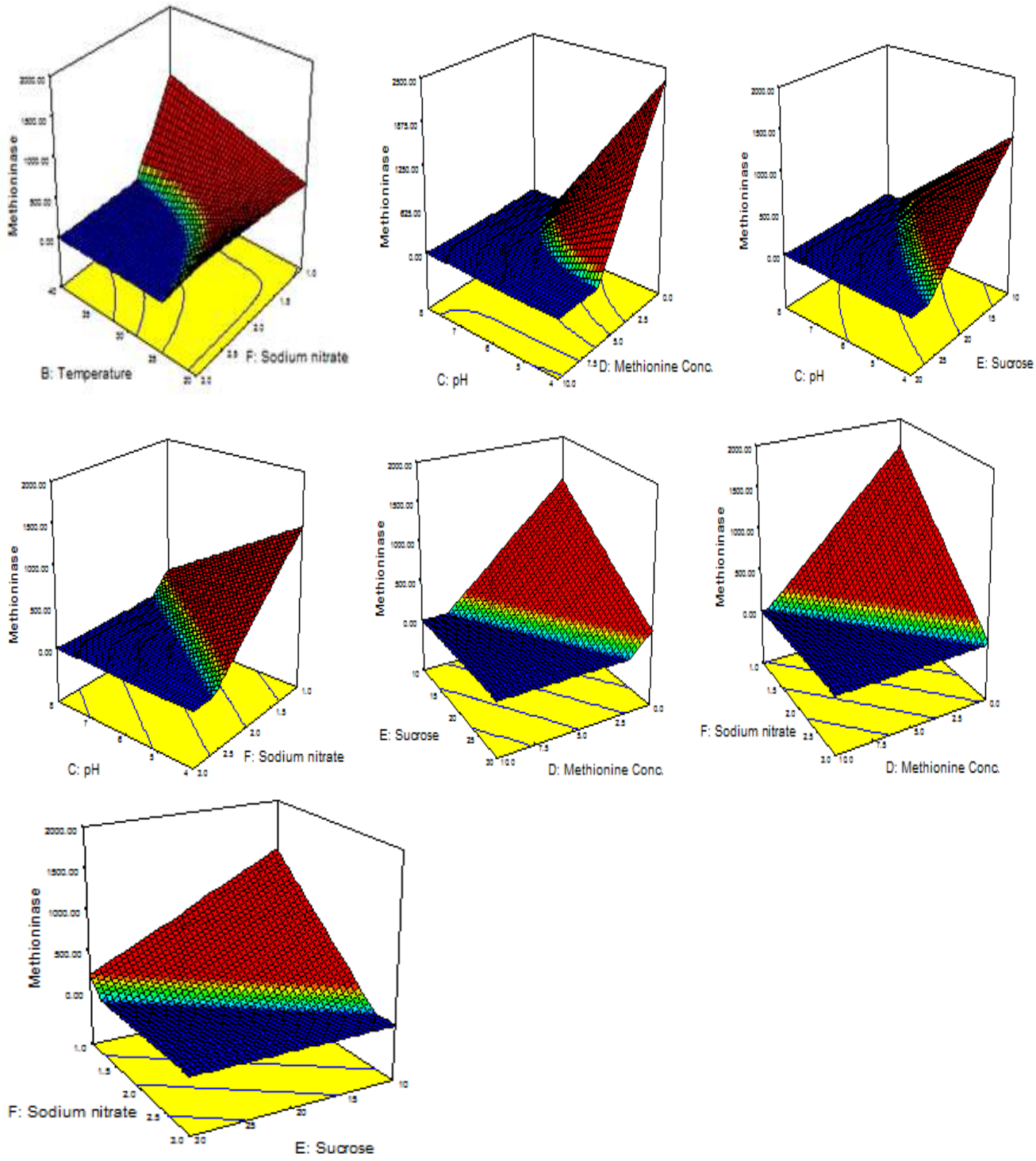


Figure 2. Response surface plots showing the effect of incubation time, temperature, pH, methionine concentration, sucrose concentration and sodium nitrate concentration for production of L-methioninase in terms of specific activity (U/mg).

Conclusion

In this study, the production of L-Methioninase (E.C 4.4.1.11) from a novel fungal source (*C. globosum*) from Egyptian soil as well as statistical modelling using AO, of the production process based on the production

Variables was successful. L-methioninase was also produced from fungal source at low methionine concentration and used in biotechnology and medical application.

Conflict of Interests

The authors have not declared any conflict of interests.

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