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A N   I N T E R N A T I O N A L   J O U R N A L

Comparative study of a new alkaline L-methioninase production by *Aspergillus ustus* AUMC 10151 in submerged and solid-state fermentation

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ABSTRACT

Twenty four fungal species were screened for their ability to produce alkaline L-methioninase on methionine-glucose liquid medium. Aspergillus ustus *AUMC 10151 displayed the highest yield of enzyme (10.8 U/mg protein), followed by* A. ochraceus *and* Fusarium proliferatum. *Upon optimization of the submerged fermentation (SmF)conditions, the maximum enzyme yield (18.23 U/mg protein) was obtained on a medium containing L-methionine (0.5%), sucrose (0.95%), KH_2PO_4 (0.1%) and 175 rpm. Seven agro-industrial by-products were screened as substrates for L-methioninase production under solid-state fermentation (SSF). Wheat bran resulted 38.1 U/mg protein, followed by rice bran (27.6 U/mg protein) and soya bean meal (26.6 U/mg protein). Maximum alkaline L-methioninase (99.56U/mg protein) was achieved at initial moisture content of 71.5%, inoculum size of 2.0 mL of spore suspension, initial pH 8.5, incubation period eight days at 30°C and supplementation of the salt basal medium with pyridoxine(100 µg/mL) and beet molasses (20% v/v). The productivity of L-methioninase by A. ustus under SSF was higher than that of SmF about 5.45 fold under optimum conditions.*

Key words: L-methioninase, *Aspergillus ustus*, Submerged fermentation, Solid state fermentation, Wheat bran.

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INTROCUCTION

L-methioninase (E.C.4.4.1.11) is a pyridoxal L-phosphate dependent enzyme that catalyzes the deamination and demethiolation of L-methionine to α-ketobutyrate, methanethiol and ammonia (Ruiz-Herrera and Starkey 1969a). Microbial L-metioninase has received much attention since it shows anti-proliferative activity towards various types of malignant cells (Cellarier et al. 2003). The antitumor activity of L-methioninase elaborates from the absence of tumor cells to methionine-synthase (Halpern et al. 1974). Consequently, these cells are auxotrophic for L-methionine, depending absolutely on the exogenous supply of L-methionine for their survival and proliferation (Kokkinakis et al. 1997), whereas normal cells are relatively resistant to exogenous L-methionine restriction because it contains active methionine synthase (Bergstorm et al. 1987).

Therapeutic efficiency of bacterial L-methioninase has rarely occurred without some evidence of toxicity and immunogenic reactions, especially with regard to multiple doses (Tan et al. 1997). Therapeutic efficiency of fungal L-methioninase occurs with fewer immunogenic and allergic reactions, which may be attributed to the higher specificity of their substrates compared with the substrate analogues, displaying less problems during the course of tumor therapy (Hawkins et al. 2004). L-methioninase production by fungi under submerged fermentation (SmF) by *Aspergillus* sp. (Ruiz-Herrera and Starkey 1969b), *Debaromycs hanseni* (Bonnarme et al. 2001), *A. flavipes* (Khalaf and El-Sayed 2009) has been reported. SSF has also been reported for its production by *A. flavus* (El-Sayed 2009). Physiologically L-methionine can be rapidly oxidized through Millard reactions forming amadori compounds that consequently reduce their bioavailability as carbon and nitrogen for the organism (Delgado-Andrade et al. 2007). Thus, using L-methionine as substrate for enzyme induction could be, at least technically, not the superior substrate, in addition to the economic expense of these medium components (El-Sayed 2010). Consequently, the search for a novel producers and new forms of the growth medium for large scale production for this enzyme will be a challenge. SSF on agro-industrial residues promises a cost-effective bioprocess as it requires small vessels and gives a higher yield (El-Sayed 2009). SSF for the enzyme production employing methionine-containing solid substrates displayed a more stable physical form of bounded methionine that could be a new strategy from the technical and economical points of views (El-Sayed 2009).

Practically no comprehensive studies on the potential of filamentous fungi for the L-methioninase production (Khalaf and El-Sayed 2009). Therefore, in this study, the potential of *A. ustus* AUMC 10151 for production of alkaline L-methioninase was optimized under SmF and SSF. To our knowledge, this is the first paper on the production of extracellular alkaline L-methioninase from *A. ustus* under SmF and SSF conditions.

MATERIAL AND METHODS

Microorganism
Fungi used in this work were previously isolated from Mangrove soil of Ras Mohammed protected area at Sharm El-Sheikh, Egypt and different soil samples from Kingdom Saudi Arabia and identified by Assiut University Mycological Centre (AUMC), Egypt. All the cultures were deposited in AUMC with their accession numbers. These fungi were grown on Czapek's agar medium at 30°C for seven days and subcultured whenever required.

Material
Nessler's reagent, Folin reagent, pyridoxine, soya bean meal and corn steep liquor were obtained from Sigma-Aldrich (St. Louis, Mo). Cane and beet molasses were obtained from Hawamdiah and Kafr El-Sheikh sugar factories, respectively. Crude whey was collected from the rural area of Kalyoubia governorate, Egypt and agro-industrial byproducts, such as wheat bran, wheat straw, rice bran, rice husk, sugar cane bagasse, and corn cobs were obtained locally. All other chemicals were of analytical grade.

Treatment of Beet and Cane molasses
Two hundred milliliter of either cane, or beet molasses was diluted to one liter by tap water, autoclaved, and then filtered to remove the precipitated mud. The filtrate was stored in dark bottles in refrigerator until use. Molasses and whey were analyzed for nitrogen content, carbohydrates and minerals by the Services

Central Lab, National Research Centre Cairo-Egypt.

Screening of fungal methioninolytic potentialities under SmF
The fungal isolates were screened for their L-methioninase productivity using modified methionine-glucose liquid medium as described by Ruiz-Herrera and Starkery (1969a). Triplicate sets of 250 mL Erlenmeyer flasks containing 50 mL of sterilized medium containing (g/L): methionine 5.0; glucose 10; KH_2PO_4 1.0; $MgCl_2.6H_2O$ 0.5; $CaCl_2.2H_2O$ 0.1; $FeCl_3.6H_2O$ 0.02 and $ZnCl_2$ 0.02 were used. The medium was adjusted to pH 8.0 using 0.1 N NaOH, inoculated with 1.0 mL of fresh spore suspension obtained from 7-days old cultures of the fungi and incubated at 30°C for six days in at 1.7 x g. The cultures were then filtered through Whatman No. 1 filter paper and the supernatant was centrifuged at 4000 rpm for 5 min, then the enzyme activity and total protein were measured as described below.

Solid-State Fermentation (SSF)
Wheat bran, wheat straw, rice bran, rice husk, sugar cane bagasse, soya bean meal and corn cobs were screened for alkaline L-methioninase production under SSF by A. *ustus*. Five gram of each dried substrate in 250 mL Erlenmeyer conical flasks was moistened with 5.0 mL of the optimized SmF medium without methionine. After autoclaving, the medium was inoculated with 1 mL of fungal spore suspension of 7-days old culture of *A. ustus* and incubated at 30°C for eight days. The fermented substrate was thoroughly mixed with 50 mL of 0.2 M Glycine-NaOH buffer (pH 8.0) using a rotary shaker at 3 x g for 30 min. The whole content of each flask was filtered through muslin cloth and the pooled filtrate was centrifuged at 1252 x g at 4°C for 15 min. The clear supernatant was used as the crude enzyme preparation.

Optimization of alkaline L-methioninase production parameters
The L-methioninase production parameters were optimized under SmF and SSF conditions. In SmF, the L-methionine-free medium was supplemented with various nitrogen sources, namely, L-asparagine, L-glutamine, glysine, tyrosine, urea, beef extract, yeast extract, ammonium chloride, ammonium sulphate and sodium nitrate. The added nitrogen sources were in equimolecular weight to nitrogen base of methionine in fermentation medium. Several carbon sources, namely, arabinose, mannitol, sorbitol, sucrose, lactose, maltose, cellulose and starch were supplemented to the fermentation medium in equimolecular weight to carbon in glucose. The effect of shaking velocity (100, 125, 150, 175 and 200 rpm) was investigated. In addition, the SSF was also optimized through various parameters including moisture content of substrate (58, 71.5, 78.3, 82 and 85%), size of inoculum (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL of spores suspension), initial pH of basal medium (7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0). The effect of supplementation of the basal medium with some vitamins as ascorbic acid, biotin, folic acid, pantothenic acid, pyridoxine, and riboflavin (100 µg/mL) and different by-products (whey, cane molasses, beet molasses and corn steep liquor) as additives (20%, v/v) was also ascertained.

L-methioninase assay
The activity of alkaline L-methioninase was determined using Nessler's reagent (Thompson and Morrison 1951) with some modification as follow: 1.0 mL of the enzyme preparation was incubated with 1.0 mL of 1% L-methionine in 0.2 M Glycine-NaOH buffer (pH 8.0) and 0.1 mL of pyridoxal phosphate at 30°C for 1h. The crude enzyme was extracted from the solid fermented substrate by a simple contact method with minor modification (Horvath 1972). The enzyme activity was stopped by adding 0.5 mL of 1.5 N trichloroacetic acid, followed by centrifugation at 1957xg for 5 min. The released ammonia was determined using 0.5 mL Nessler's reagent; the developed color was measured at 480 nm. Enzyme and substrate blanks were used. One unit of L-methioninase was defined as the amount of enzyme that liberated one µmole of ammonia/h under optimal assay conditions. Specific activity of L-methioninase was expressed as the activity of enzyme in term of units per milligram of protein.

Protein Determination
Protein was determined by Lowry et al. (1951) method using bovine serum albumin as the standard.

Isaac, GS et al.

Statistical Analysis

The data were statistically analyzed with SPSS (Scientific Package for Scientific Social Studies, version 20), in which the equations of the hypothesis tests, including the mean, standard deviation, T-statistics value and probabilities (p) were used. Results were considered highly significant, significant, or non-significant, where $p \leq 0.01$, $p \leq 0.05$ and $p > 0.05$, and represented by ***, ** and *, respectively. The data were statistically compared to the highest value obtained, which was marked as ●.

RESULTS

Screening for fungi producing alkaline L-methioninase under SmF

Potentialities of twenty-four fungal species were investigated for their ability to produce alkaline L-methioninase on methionine-glucose liquid medium (pH 8.0). These species belonged to nine genera, namely, *Aspergillus*, *Cunninghamella*, *Emericella*, *Fusarium*, *Humicola*, *Paecilomyces*, *Penicillium*, *Rhizopus* and *Trichoderma*. The results in Table 1 showed that the maximum alkaline L-methioninase productivity (10.8 U/mg protein) was obtained by *A. ustus*, followed by *A. ochraceus*, *Fusarium proliferatum*, *Penicillium crustosun* and *Trichoderma longibrachiatum*, which produced 9.1, 8.9, 8.5 and 8.0 U/mg protein, respectively. The lowest enzyme productivity was obtained by *Paecilomyces lilacinus* (5.8 U/mg protein), followed by *P. citrinum*, *H. grisea*, and *P. corylophilum*, they produced 6.1, 6.3 and 6.8 U/mg protein, respectively.

Table 1- Screening for alkaline L-methioninase production by investigated fungi

	Fugal species	AUMC No.	L-methioninase activity (U/mL)	Protein content (mg/mL)	Specific activity (U/mg)	Biomass (g/L)
Kingdom Saudi Arabia	*Aspergillus flavus* Link	8653	115	16	7.2 ±0.77***	2.46±0.09
	A. fumigatus Fresenius	8594	163	22	7.4 ±1.2***	3.10±0.05
	A. niger Van Tieghem	8593	210	29	7.2 ±0.45***	4.60±0.17
	A. ochraceus Wilhelm	8670	210	23	9.1 ±0.55**	4.12±0.10
	A. sydowii (Bainier and Sartory) Thom and Church	8660	160	22.8	7.0 ±0.99***	4.56±0.12
	A. terreus Thom	8605	170	22.7	7.5 ±0.76***	5.12±0.08
	Cunninghamella phaeospora Boedijn	8662	130	19.12	6.79 ±1.03 ***	2.89±0.06
	Emericella nidulans (Eidam)Vuillemin	8640	-	-	-	-
	E. quadrilineata (Thom and Raper) Benjamin	8636	-	-	-	-
	Fusarium proliferatum (Matsush.)Nirenberg	8617	210	23.5	8.9 ±1.00**	4.87±0.15
	F. solani (Martius) Saccardo	8615	166	22.8	7.28 ±1.06***	3.92±0.11
	Humicola grisea Traaen	8598	120	19	6.3 ±1.04***	2.12±0.06
	H. insolens Cooney and Emerson	8607	-	-	-	-
	Penicillium chrysogenum Thom	8656	200	26	7.7 ±0.76***	5.23±0.08
	P. corylophilum Diercks	8601	170	25.1	6.8	4.83±0.13

					±0.43***	
	P. duclauxii Delacroix	8667	198	26.9	7.4 ±0.34***	3.60±0.11
	Rhizopus stolonifera (Ehrenberg) Vuillemin	8671	215	27.45	7.8 ±0.68***	2.98±0.09
	A. flavus Link	10150	109	14.8	7.4 ±0.94***	2.23±0.15
Ras Mohammed (Egypt)	*A. niger Van Tieghem*	10146	120	16.9	7.1 ±0.33***	3.23±0.11
	A. ustus (Brinier) Thom and Church	10151	245.5	22.8	10.8± 0.20 •	5.40±0.22
	P. citrinum Thom	10147	169	27.6	6.1 ±0.88***	4.33±0.21
	P. crustosun Thom	10148	187	22	8.5 ±0.13***	3.56±0.26
	Paecilomyces lilacinus (Thom) Samson	10149	160	27.8	5.8 ±0.16***	3.54±0.07
	Trichoderma longibrachiatum Rifai	10155	220	27.45	8.0 ±0.11***	5.45±0.04

AUMC= Assiut University Mycological centre; Data represent the mean of three different readings approximated to two decimal ± standard deviation. *** Highly significant, p ≤ 0.01. Data was statistically compared using t-test (N = 3).

The obtained data were statistically compared to the highest value obtained, which is marked as ●.

Effect of nitrogen sources on alkaline L-methioninase production by *A. ustus*

A set of synthetic or natural nitrogen sources was separately added as a sole nitrogen source to the fermentation medium in equimolecular weight to nitrogen base of methionine (0.5%). As shown in Figure 1, maximum L-methioninase produced 10.74 ± 0.82 U/mg protein by *A. ustus* with the addition of L-methionine. Other nitrogenous sources showed an inhibitory effect on production of alkaline L-methioninase. The results showed that the maximum growth rate was recorded with L-glutamine (7.0 ± 0.04 g/L), followed by yeast extract (6.50 ± 0.09 g/L) as nitrogen source.
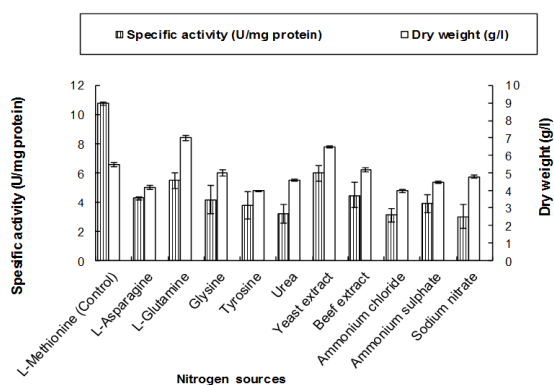
Effect of carbon sources on alkaline L-methioninase production by A. ustus

Figure 2 showed that the maximum alkaline L-methioninase production and growth yield by *A.ustus* were recorded with sucrose (12.82 ± 0.18 U/mg protein and 5.8 ± 0.09 g/L, respectively), followed by mannitol (12.0 ± 0.11 U/mg protein and 5.3 ± 0.06 g/L, respectively). Without any carbon source, the specific activity of L-methioninase and growth rate showed lowest value (2.6 ± 0.35 U/mg protein and 2.43 ± 0.10 g/L, respectively).

Figure 1- Effect of different nitrogen sources on alkaline L-methioninase production by *A. ustus* under Submerged Fermentation.
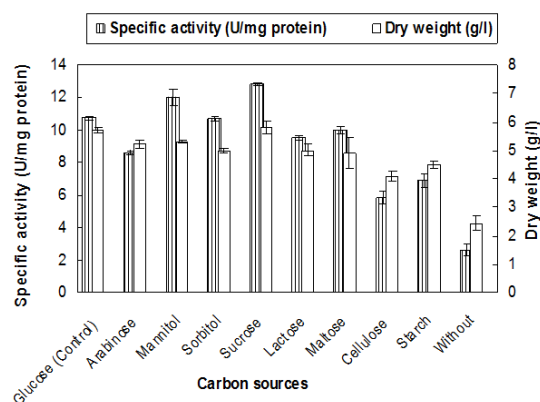

Figure 2- Effect of different carbon sources on alkaline L-methioninase production by *A. ustus* under SmF.

Effect of shaking velocity on the production of alkaline L-methioninase by *A. ustus*

Figure 3 showed that 175 rpm was the optimum shaking velocity for the maximum accumulation of alkaline L-methioninase and growth yield by *A. ustus* (18.23 ± 1.1 U/mg protein and 6.3 ± 0.1 g/L).

Screening for alkaline L-methioninase production by *A. ustus* using different agro-industrial by-products under SSF

Table 2 showed that the maximum yield of alkaline L-methioninase by *A. ustus* was by wheat bran (38.1 ± 0.99 U/mg protein), followed by rice bran (27.6 ± 1.31 U/mg protein), soya bean meal (26.6 ± 0.55 U/mg protein), corn cobs (23.0 ± 1.52U/mg) and sugar cane bagasse (22.6 ± 0.67 U/mg protein).

Figure 3- Effect of shaking velocity on alkaline L-methioninase production by *A. ustus* under SmF.

Table 2- Screening for alkaline L-methioninase production by *A. ustus* using different agro-industrial by-products
Data represent the mean of three different readings approximated to two decimal ± standard deviation.

Substrate	L-methioninase activity (U/ml)	protein (mg/ml)	L-methioninase Specific activity (U/mg protein)
Wheat bran	15.22	0.40	38.1 ±0.99
Wheat straw	11.2	0.66	17.0 ±0.53
Rice bran	15.2	0.55	27.6 ±1.31
Rice husk	12.5	0.68	18.4 ±0.87
Sugar cane bagasse	12	0.53	22.6 ±0.67
Soya bean meal	16.2	0.61	26.6 ±0.55
Corn cobs	12.2	0.53	23.0 ±1.52

Effect of initial moisture content on alkaline L-methioninase production by A. ustus under SSF

Figure 4 demonstrated the effect of different ratios of salt basal medium at fixed weight of wheat bran (0.5 g). The maximal production of alkaline L-methioninase was obtained at the ratio of 1:2 (44.8 ± 1.38 U/mg protein) with initial moisture content of 71.5%. An increase in the moisture content of the substrate than 78.3% resulted in a great decline in alkaline L-methioninase yield.
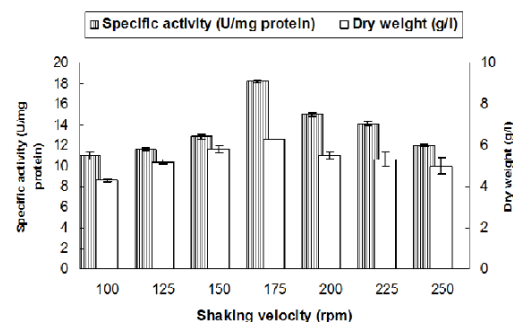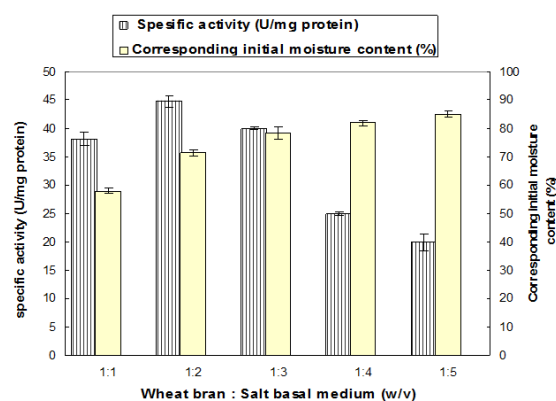

Figure 4- Effect of initial moisture content on alkaline L-methioninase production by *A. ustus* under Solid State Fermentation

Effect of inoculum size on alkaline L-methioninase production by *A. ustus*

With the increase in the inoculum size, the enzyme productivity increased, reaching the maximum at 2.0 mL of spore suspension (55.21 ± 2.10 U/mg protein). With further

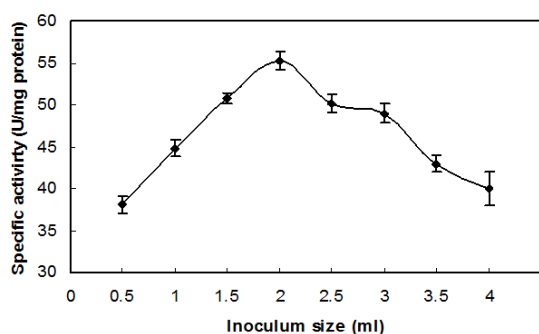increase in inoculum size, the enzyme productivity decreased (Fig. 5).

Figure 5- Effect of inoculum size on alkaline L-methioninase production by *A. ustus* under SSF.

Effect of initial pH on alkaline L-methioninase production by *A. ustus*
Figure 6 revealed that the productivity of L-methioninase increased with increasing the alkalinity of the medium to reach maximum yield at pH 8.5 (63.2 ± 1.41 U/mg protein).
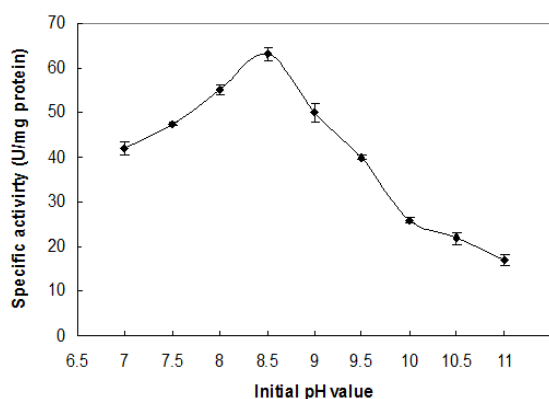

Figure 6- Effect of initial pH on alkaline L-methioninase production by *A. ustus* under SSF.

Effect of different vitamins on alkaline L-methioninase production by *A. ustus*
As shown in Figure7, the addition of pyridoxine led to an increase in enzyme production compared with control (79.54 ± 2.05 U/mg protein).
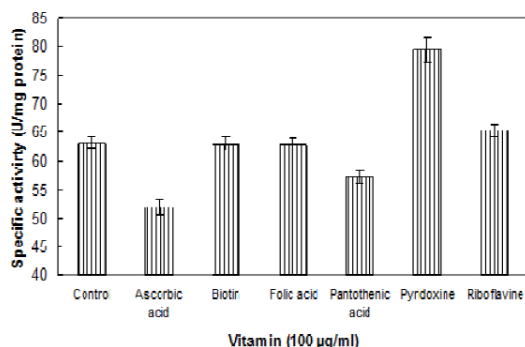

Figure 7- Effect of different vitamins on alkaline L-methioninase production by *A. ustus* under SSF.

Effect of different additives on alkaline L-methioninase production by *A. ustus*
As shown in Figure 8, the addition of beet molasses to optimized salt basal medium by (20%, v/v) resulted high increase in L-methioninase production by *A. ustus* compared with the control (99.56 ±1.63 U/mg protein) followed by cane molasses (85.32 ± 0.89 U/mg protein) and corn steep liquor (82.56 ±1.02 U/mg protein).
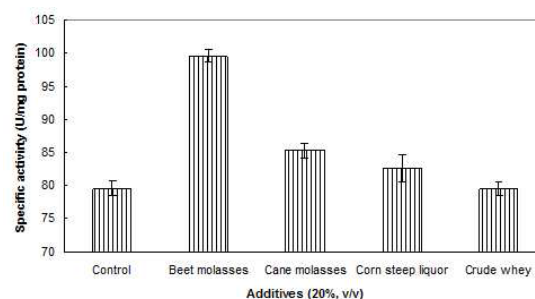

Figure 8- Effect of differentadditives on alkaline L-methioninase production by *A. ustus* under SSF.

DISCUSSION

The production of L-methioninase under submerged conditions has been reported by Raimbault and Alazard (1980), Khalaf and El-Sayed (2009). Many microorganisms including fungi attack methionine but do not grow on it, perhaps because of their inability to methabolize the deaminated (α-ketomethionine) and demethiolated (α-keto-butric acid and methaniol) residues of L-methionine. The inability of filamentous fungi to grow on L-methionine may be partially overcome by the use of a growth-supporting organic compound such as glucose, or an equivalent carbohydrates desinated as a co-dissimilater (Lockwood and Coombs 1999). From the screening experiment, *A. ustus* was the most potent fungal isolate for alkaline L-methioninase production. Therefore, it was selected for further study. These results were in accordance with Ruiz-Herrera and Starkey (1969b) and El-sayed (2009) who recorded the production of L-methioninase by different species of *Aspergillus*. On the other hand, *E. nidulans, E. quadrilineata* and *H. insolens* did not show any enzyme activity.
Supplementation of the SmF medium of *A. ustus* with various nitrogen sources showed that none of the incorporated nitrogenous compound had a stimulatory effect on alkaline

L-methioninase 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. (Ruiz-Herrera and Starkey 1969a), *Yarrwia lipolytica* (Bondar et al. 2005) and *A. flavipes* (Khalaf and El-Sayed, 2009). However, L-methioninase production by *Pseudomonas putina* (Tan et al.1998) and *Geotricum candidum* (Bonnarme et al. 2001) were L-methionine independent.

Among different carbon sources, Sucrose exerted the highest increase in the enzyme production as compared with other carbon sources. Similar results obtained for L-methioninase production by *Pseudomonas ovalis* (Tanaka et al. 1976) and *Trichoderma vaginalis* (Lockwood and Coombs 1999). The considerable decrease in the production of the enzyme in the absence of carbon source could be attributed the ability of *A. ustus* to utilize L-methionine to start the growth but it could not utilize its catabolized products to continue. This result was similar to reported by Khalaf and El-Sayed (2009). In contrast, Ruiz-Herrera and Stakey (1969b) and Khalaf and El-Said (2009) reported that glucose was the favored carbon source for L-methioninase production by filamentous fungi.

Shaking velocity is known as a possible parameter affecting enzyme productivity. This may be attributed to the mechanical forces that can lead to vacuolation of older hyphal compartments, which may lead to weakened hyphae and /or accelerating hyphal fragmentation (Paul et al. 1994).

Physiologically, SSF holds potential for the production of enzymes. This system offers numerous advantages, including high volumetric productivity, relatively higher concentration of the products, less effluent generation and requirement for simple fermentation equipment (Sharma et al. 1991). Moreover, bounded amino acids (solid substrates) are more stable chemically than free ones, therefore, the former are preferred for broad technological application as substrates for bulk production of economically valuable compounds (Delgado-Andrade et al. 2007; El-Sayed et al. 2012).

The variation on the inductivity on the production of alkaline L-methioninase *A. ustus* according to the type and nature of solid substrate may be closely related to fluctuations in the chemical composition of these compounds (El-Sayed 2009). The preferential use of wheat bran may be attributed to their high protein content (18%) and methionine (1.5%) relative to their ratios of ash and fiber in comparison to other substrates (Yamazaki *et al.* 1988). In addition to their unique mechanical properties as structure retention and lack of particle agglomeration (El-Sayed, 2009). Therefore, wheat bran was selected as the optimal solid substance for subsequent cultural optimization to enhance the enzyme productivity by *A. ustus*.

With respect to the effect of initial moisture content, increasing moisture level may cause decreasing in the porosity of substrate, thus limiting the oxygen transfer into the substrate (Raimbault and Alazard 1980). Similar result was obtained by El-Sayed (2009) who recorded a great decrease in L-methioninase productivity obtained from *A. flavipes* with increasing the moisture content of solid substance.

Inoculum size is also an important factor that influences the production of metabolites under SSF conditions. This may be attributed to nutrient limitation, accumulation of spore self-inhibiting and non-volatile substances (Pandey et al. 1999), or an increase in initial moisture contents causing substrate agglomeration and lower O_2 transfer (Hesseeltine 1972).

The importance characteristic of most alkalophilic microorganisms is their strong dependence on the extracellular pH of the medium, which has a great effect on the cell growth and enzyme production. The present results were in line with that for L-methioninase production by *Clostridium sporogenes* at pH 8.5 (Kreis and Hession 1973).

The supplementation of the solid cultures of *A. ustus* for L-methioninase production with different vitamins also investigated. The induction effect of addition pyridoxine is in complete accordance with that recorded for *Clostridium sporogenes* (Kreis and Hession 1973), followed by riboflavin. Biotin and folic acid showed no stimulatory effect on enzyme production; ascorbic acid and pantothenic acid resulted in significant decrease in the productivity of alkaline L-methioninase. Similar result was obtained by El-Sayed (2009) who recorded a great decrease in L-

methioninase production by *A. flavipes* by using ascorbic acid.

There were no reports on the influence of different natural additives such as beet and cane molasses, corn steep liquor and whey on alkaline L-methioninase production. The present study found that beet molasses showed a considerable stimulatory effect on enzyme production. Beet molasses is one of the cheapest sources of carbohydrates (12.87%), nitrogenous substances (0.227%) and minerals (%) Na 0.32, K 0.66, Ca 1.00, Mg 0.37, Fe 1.09, Mn (0.06) and Zn (0.248). In addition, several vitamins such as thiamine, riboflavin, pyridoxine, folic acid and biotin are present in beet molasses (Crueger and Crueger 2000).

CONCLUSIONS

A locally isolated fungal strain of *A. ustus* was used for the production of alkaline L-methioninase in submerged culture condition. However, better results were obtained in solid-state fermentation using wheat bran as substrate. The yield of L-methioninase in SSF was 5.45-fold higher than SmF.

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