Egypt. J. Chem. **Vol. 65**, No. 12 pp. 275 - 281 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

L-methioninase enzyme production by E. coli WSM2 using some organic by product residues

Afra M. Baghdadi[1] and Enas N. Danial[2]

[1]*University of Jeddah, College of Sciences, Department of Biology , Jeddah, Saudi Arabia*

[2]*Department of Chemistry of Natural and Microbial Products, National Research Centre, Egypt.*

Abstract

Cancer is a growing reason for death and morbidity worldwide. L-methioninase (EC 4.4.1.11) has a possible application against many types of cancers like glioblastoma, kidney, breast, lung, and colon cancer. The aim of the present study screening of L-methioninase production by bacteria isolated from sewage samples obtained from Makkah city in Saudi Arabia. Twelve bacterial were isolated from sewage samples. The bacterial isolate's ability for L-methioninase production was tested on a modified mineral salt M9 L-methionine agar medium using phenol red as the pH indicator. Selected colonies with pink-red and yellow zone around as L-methionine degrading bacteria. The highest L-methioninase produced isolate identified based on16S rDNA sequencing, along with the biochemical characterization, and lastly completed by BLAST analysis by the structure of a phylogenetic tree. It was *Escherichia coli* WSM2 strain. The rRNA sequence of *Escherichia coli* WW11 was deposited to the gene bank (NCBI accession number MK072729). Results revealed using of organic byproduct residues, the high amount of the enzyme produced 0.128U/min/ml in cultures supplemented with pomegranate juice wastes with L-methionine and yeast extract after 48h of incubation, while the maximum amount of L-methioninase 0.237U/min/ml was produced by *E. coli* when 75% Whey and 25% and was used as fermentation media, incubated in a shaking incubator (150rpm).

Keywords: L-methionine, date, whey, pomegranate and bacterial identification

1. Introduction

L-methioninase (E.C 4.4.1.11), a pyridoxal 5′-phosphate-dependent enzyme, catalyzes the deamination and demethiolation of L-methionine to α-ketobutyrate, methanethiol and ammonia. L-methioninase was reported widely as an antitumor factor against various malignant cell lines, breast, lung, colon, kidney, and glioblastoma [1]. It is absent in mammalian system and intracellularly existent in bacteria and extracellularly in fungi [2]. The normal cells have active methionine synthase; they could grow on a medium supplemented with homocysteine, vitamin B12 [3], and vitamin Bc rather than methionine [4]. Unlike normal cells, tumour cells liberated from efficient methionine synthase, thus rely on external methionine supplementation from the diet [5]. The majority of tumour cells are auxotrophic for L-methionine, depend on the exogenous supply of L- methionine for their survival and proliferation [1].

L-methioninase locates application inside the pharmaceutical industry since it has an antioxidant activity that assists in downregulation of polyamines including putrescine, spermidine and spermine. [6] similarly as in the food industry by improving odour by improving Discharge of volatile sulfur compounds [7]. L-methioninase was purified and characterized by many bacterial species, including *Pseudomonas putida, Aeromonas* sp., *Citrobacter freundii, Brevibacterium linens, Lactococcus lactis, Clostridium sporogenes* [8]. Reports depict L-methioninase at the culture filtrates of yeast, including *Debaromyces hansenii, Geotrichum candidum* and *Saccharomyces cerevisiae* [7]. Employment of natural agro-byproduct residues as substrates for enzyme production is favoured environmentally and economically.

Screened Abu-Tahon and Isaac [9] seven agro-byproduct by-products as substrates for L-methioninase production under solid-state fermentation (SSF). Wheat bran was the best, followed by rice bran and soya bean meal.

The objective of the present study was to evaluate the potential of bacterial isolate for

*Corresponding author e-mail: amboghdadi@uj.edu.sa

Receive Date: 22 February 2022, Revise Date: 19 March 2022, Accept Date: 23 March 2022

DOI: 10.21608/EJCHEM.2022.123441.5517

production L- methioninase using different organic byproduct residues as substrates under SMF conditions.

2. MATERIALS AND METHODS

Collection of Samples

The objective of the present study was to straighten the potential of bacterial isolate for production L- methioninase using different organic byproduct residues as substrates under SMF conditions

Isolation of L-methioninolytic bacteria by rapid plate method:

Utilized the dilution-plate method for isolation of L-methioninase producer bacteria. Modified M9 medium, contains methionine (1g/l) was used: L-methionine 1, Glucose 2, KH_2PO_4 3.0, NaCl 0.5, $MgSO4.7H_2O$ 0.25, $CaCl_2$ 0.014, for solid medium, 20 g/L agar was added. The final pH of the medium was the adjustment to pH7±0.2. The final pH of the medium was an adjustment to pH7±0.2. Additionphenol red tothe medium as pH index at a final concentration of 0.009% just prior pouring the platesPhenol red was added to the medium as pH index at a final concentration of 0.009% just before pouring the plates.

Poured About 0.1ml of the serial dilution of the samples on M9 solid medium. Incubated the plates at 35±2 ºC for 72 hours. formation a pink-red and yellow zone around the colonies mean that the isolates produce L-methioninase. Colonies were purified and subcultured from each plate for further studies [10].

Estimation of L-methioninase in submerged culture of bacterial isolates by rapid plate method:

Used the modified M9 broth medium as the fermentation medium, inoculated the medium with the bacterial isolates were incubated in a constant incubator and other cultures were incubated in a shaking incubator (150rpm) at 35±2°C.

L-methioninase evaluated by agar well diffusion method of cell-free filtrate culture (CFF) on solid media in Petry dishes. Solidify media in the plates, loaded 7 mm wells were punching using sterilized cork borer, 100 µl of CFF in the wells, the plates kept in an upright position at 35±2 °C, after 48 hours of incubation the size of the area was measured [11].

Production of L-methioninase by the selected isolates using some local organic byproduct residues

L-methioninase was produced using local organic wastes as fermentation media by the two selected isolates. The first cultures contain juice waste that was supplemented with or without L-methionine, supplemented the second culture of the pomegranate juice wastes with 1g/l of yeast extract with or without L-methionine. Other media contain dates and whey. The first media: contained 100% of dates, the second medium contained 50 % Whey and 50% Dates, the third medium contained 75 % Whey and 25% dates, the fourth medium contained 100 Whey, 20gm of date and 1gm Yeast extract and the fifth medium contained 50 % Whey, 50% Dates and 1gm Yeast extract. All midia were sterilized using autoclave at 121Cº for 15 min. The sterilized media were inculated by 1 ml of selected isolates bacteria , which containe 10^6 CFU/ml The inculated cultures were incubated at 35±2Cº. The broth culture assayed for L-methioninase production after 24, 48 and 72 hours. Determined L- methioninase in cell free filtrate (CFF) of the submerged cultures of the bacterial isolates that produced L-methioninase by Nesslerization method [12], the developed coloured compound was measured at 480 nm using a spectrophotometer, the specific activity of L-methioninase was expressed as the activity of the enzyme in terms of units per milligram of protein [13].

Molecular characterization of the new bacterial strain

a. Genomic DNA extraction

Prepared genomic DNA in Luria-Bertani medium from an exponential phase. Centrifuged bacterial culture at 12,000 rpm for 15 min and aliquots of 10 ml were harvested and washed once in sterile distilled water. Isolation of genomic DNA was done using GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific, Massachusetts, USA).

b. Identification of the new bacterial isolate and the *methioninase* gene

The identification of the new bacterial isolate 16S rDNA universal oligonucleotide primers was utilized as described by Buonaurio *et al.* [14]. L-methioninase gene detection of forward (5` AGAGTTTGATCCTGGCTCAG3`) and reverse (5`GCAACGCGCATGATCTACTG3`) primers were designed and synthesized the recover full-length gene (1197 bp) based on the available sequence of the gene in *Escherichia coli* in the National Center for Biotechnology Information (NCBI) (accession no. LT629708.1). For amplifying the two genes, did PCR in a thermal cycler (Bibby Scientific, UK) and reactions (25 µl each) contained 2 µL DNA, 1 µL of each primer (10/moles), 12.5 µL master mix (2x) and 9.5 µL ddH2O. PCR conditions for amplifying the two genes involved initial denaturation for 5 min at 94°C, followed by a number of 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C for 16S rRNA

gene, while 60°C for methioninase gene and extension for 2 min at 72°C, then, a final extension cycle for 10 min at 72°C.

Carried out Sanger sequencing at Beijing Genomic Institute (BGI), Hong Kong, China, for the 16S rRNA gene. Compared recovered nucleotide sequence of the gene with those available in the NCBI database using the BLAST search tools.

Aligned the sequence for the new isolate along with those retrieved from the gene bank in Ugene [15] using T-Caffee algorithm (https://www.ebi.ac.uk/, EMBL-EBI,

Cambridgeshire, CB10 1SD, UK) and inferred phylogenetic cladograms. The generated trees from Ugene was displayed using iTOL/interactive tree (https://www.ebi.ac.uk/, EMBL-EBI, Cambridgeshire, CB10 1SD, UK) to describe the phylogenetic relationships involving the new isolate and the similar sequences available in the NCBI.

3. RESULTS

Qualitative method for L-methioninase producing bacteria by rapid plate method:

We isolated about 12 bacterial strains on a modified m9 medium from **wastewater (sewage)** samples.

Out of 12 isolates ten bacterial isolates, L-methioninase producing.

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

Supplemented the quantitative estimation of L-methioninase by agar well diffusion assay of cell-free filtrate culture (CFF) on culture with L-methionine as a sole organic source and other with L-methionine as the nitrogen source and glucose as carbon source.

Results in Fig. (1) show the diffusion of L-methioninase that produced by the producer isolates after 48 hours of incubation at 35±2 C° either incubated in shaking or static incubator. Among the ten isolates, results revealed that the isolates produced L- methioninase after 48 hours of incubation. The results clear that eight and nine isolates produced L-methioninase that were incubated in a constant and a shaking (150 rpm) incubators respectively. The biggest diameter of the yellow zone of CFF was 42.5 mm of WW7, while the biggest diameter of L-methioninase zone of CFF from culture incubated in a constant incubator 37.5 mm of isolate WW11 which also isolated from sewage.

The bacterial isolate has the highest ability of L-methioninase production was genetic identified and used for further studies**.**

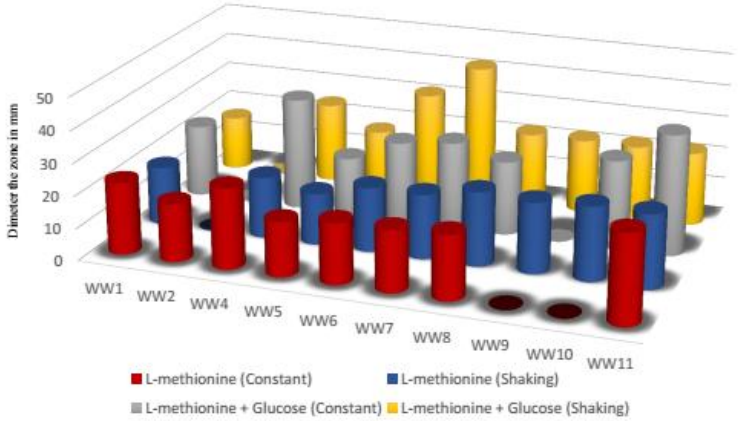

Figure (1): Quantitative estimation of L-methioninase in submerged culture of bacterial isolates by rapid plate method

Molecular identification of the highest L-methioninase producing bacterial isolate based on 16S rDNA

Analysis of BLAST alignment tools of GenBank for the partial-length 16S rRNA gene (700 bp) showed that identified the new isolate as *Escherichia coli*. This new strain of *Escherichia coli* was named WSM2 and given the accession no. MK072734 for the strain that isolated from wastewater. PCR analysis confirmed that the new *Escherichia coli* strain of L-*methioninase* gene (Figure 3). PCR yielded an amplicon of the gene with the expected full-length of 1197 b

Phylogenetic tree analysis of *E. coli* isolates based on 16S rDNA

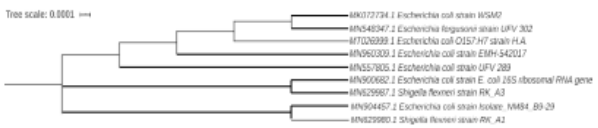

Figure (2): Neighbor-joining tree showing the phylogenetic position of *Escherichia coli* and their related species based on partial 16S rRNA gene sequences. GenBank accession numbers of nucleotide sequences MK072734.1 was shown along with the name of the bacterial strain.

Detection of L-methioninase gene in *Escherichia coli* WSM2 strain.

The gel electrophoresis image shows that L-methioninase gene presented in *Escherichia coli.* L methioninase gene presence in *Escherichia coli* with expected PCR yield of L-methioninase gene is 1197 bp which indicates a positive result (Figure 3).

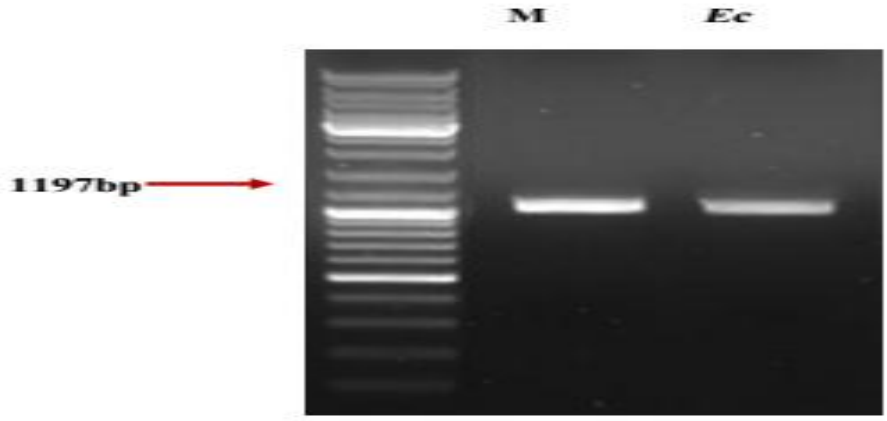

Figure (3): PCR amplification of L-methioninasegene from *Escherichia coli* strain. Lane 1: 100pb ladder; Lane 2; 1197 bp partial L-methioninase gene.

Production of L-methioninase using byproduct local organic wastes Production of L-methioninase using pomegranate juice wastes:
results in Fig. (4) show *E. coli* produced the highest amounts of L-methioninase after 48h of incubation in all cultures, grew L-methioninase amounts in pomegranate juice wastes culture supplemented with methionine higher amounts than in pomegranate juice waste culture only, but the maximum amount of the enzyme produced 0.128 U/min/ml in cultures supplemented with pomegranate juice wastes with L-methionine and yeast extract after 48h of incubation. The lowest amount of the enzyme was produced by *E. coli* WW11 in the cultures of pomegranate juice wastes with yeast extract without L-methionine.

E. coli WWSM2 produced Lmethioninase 0.126U/min/ml when fermentation medium contained 100% of dates. This amount increased to 0.228 U/min/ml by using 50 % Whey and 50% Dates, the maximum amount of L-methioninase 0.237U/min/ml was produced by *E. coli* when used 75 % Whey and 25% as fermentation media.

4. Discussion:

Phenol red shows the fundamental pH change, which is red in alkaline condition turn to yellow under acidic condition [16]. Similarly, this technique was used to study the dissimilation of L- methionine by L-methioninase. Recorded the observations were after 72 hours for the presence of yellow zone formed due to the formation of α-keto butyric acid, due to the presence of red phenol indicator. In this study fall in pH may be due to the formation of α-keto butyric acid; hence, pH change will lead to change in colour to yellow; this result agrees with that found by Bahl *et al*. [10].

Culture broth on L-methionine glucose media has shown good L-methioninase production under submerged conditions [13]. This media gives the necessary minimal nutrition required and provides L-methionine as a carbon source, which is used by bacteria as a growth substrate. Shaking velocity is kno wn as a potential parameter influencing enzyme productivity. May be ascribed this to the mechanical powers that can lead to vacuolation of older hyphal compartments, which may perform to weakened hyphae and /or accelerating hyphal fragmentation [17] produced the highest amount of L-methioninase in shaking incubator.

In cultures contain L-methionine as nitrogen source and glucose as carbon source, incubated in shaking and constant incubators, nine and eight bacterial isolates respectively found to be positive. The acid production takes place in both cases; L-methionine utilization produces α-keto butyric acid, whereas glucose utilization produces organic acids. Therefore in this study, the result confirmed the presence of the enzyme when L-methionine was used as the sole organic source, the result also showed that the enzyme production was enhanced in the presence of carbon source maybe because the carbon source act as growth-supporting its ability for L-methioninase producing, this result agree with that found by Abu-Tahon and Isaac [9[, El- Sayed[13],

Lockwood and Coombs[18].

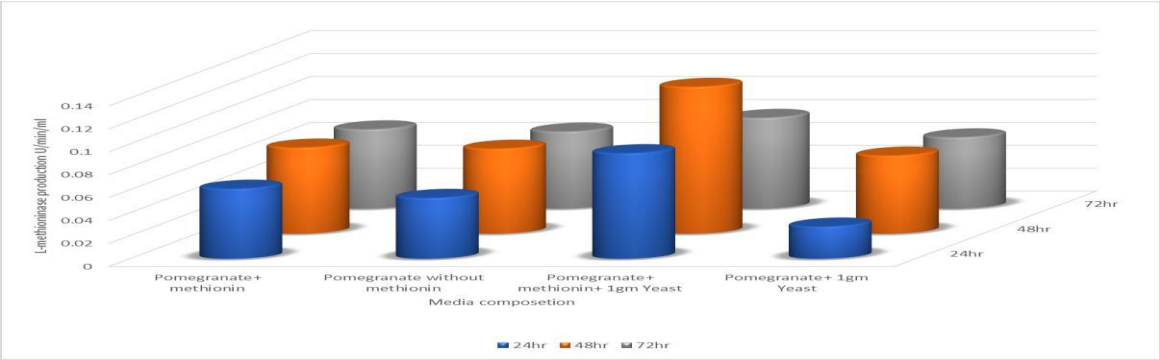

Figure (4): Production of L-methioninase using pomegranate juice wastesProduction of L-methioninase by the selected isolate using Dates and whey

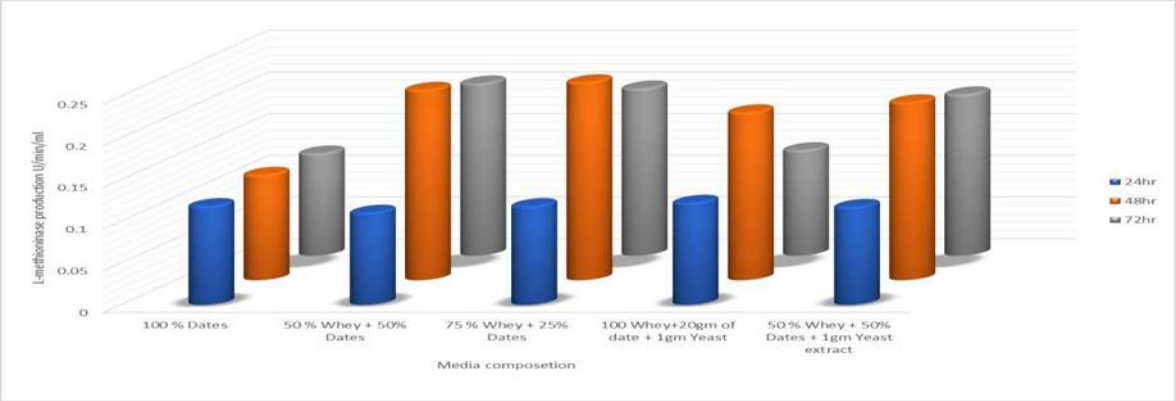

Figure (5): Production of L-methioninase by the selected isolate using Dates and whey

The partial 16S rDNA sequence (700 bp) analyzed using Blast alignment tools of GenBank showed that the new isolate belongs to ***Escherichia coli*** with 100% identity. The DNA sequence of the new strain WSM2 isolated from wastewater (sewage) was submitted to the Bacterial or Archaeal 16S ribosomal RNA sequences database and has given the accession number MK072734.1. The results also indicated successful detection of L-methioninase gene in the new ***Escherichia coli*** strain with the correct full-length size (1197 bp).

Physiologically L-methionine can be quickly oxidized through Millard reseponses formation amadori compounds that there for reduce their bioavailability as carbon and nitrogen for the organism [19]. Thus, utilizing L-methionine as a substrate for enzyme induction could be at least technically, not the superior substrate, in addition to the economic cost of these middle components [8]. Consequently, the look for novel producers and new forms of the growth medium for wide scale production for this enzyme will be able to a challenge. SSF on agro-byproduct remains promises a cost-effective bioprocess as it requires small vessels and provides a higher yield [11].

Virtuallyno comprehensive studies on the potential of bacteria for the L-methioninase production. There were no reports on the effected of different natural additives like cane molasses and beet, corn steep liquor and whey on alkaline L-methioninase production.

In the present study found, produced L-methioninase in media of local organic wastes, it noticed that dates and whey showed a considerable stimulatory effect on enzyme production more than wastes of pomegranate juice, this is due to the composition of these wastes. Date contains requirements nutritious medium. Date flesh was founded to be low in fat and protein but rich in sugars, mainly fructose and glucose. It is a high source of energy. Reported ten minerals, the major being selenium, copper, potassium, and magnesium. Vitamins B-complex and C are the major vitamins in dates [20]. The main chemical concoction segments of date fruit include carbohydrates, dietary fibre protein, enzymes, vitamins, fat, minerals, carotenoids and phenolic acids. The chemical composition of date fruit varies according to ripening stage, cultivar, growing environment, postharvest conditions [21]. Traditionally Whey has been a waste product of

cheese industry, but nowadays whey is evolving into a sought-after commodity on account of the lactose, minerals, and protein it contains in addition to the functional properties it imparts to food [22].

5. Conclusion

The microbial production of enzyme is cost effective and easily produced, which has drawn significant attention to exploring new strain for producing L-methioninase having therapeutic values. Therefore, in this study, To the best of our knowledge, this is the first report on the isolation and production of L-methioninase by *E. coli* using media of local organic wastes like dates and whey and pomegranate juice wastes in Saudi Arabia.

6. References:

1. Milad M., T. Yasaman, G. Nasim, B., Aydin, D. Ali, A. Mohammad and G. Younes; Isolation and identification of novel l-Methioninase producing bacteria and optimization of its production by experimental design method. Biocatal. Agric. Biotechnol. 2020;(26): 101566.
2. Khalaf, S. A. and A.S. El-Sayed; L-Methioninase production by filamentous fungi: I-screening and optimization under submerged conditions. Curr. Microbiol., 2009; 58(3):219-226. https://doi: 10.1007/s00284-008-9311-9.
3. Sharma, B., S. Singh and S.S. Kanwar; L-methionase: a therapeutic enzyme to treat malignancies. *BioMed Res Int*., 2014. https://doi.org/10.1155/2014/506287.
4. Mangum, J.H. and J.A. North; Vitamin B12 dependent methionine biosynthesis in HEp-2 cells. Biochem. Biophys. Res. Commun., 1968; 32(1): 105-110. https://doi.org/10.1016/0006-291X(68)90433-6.
5. Hoffman, R. M.; Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis: a review and synthesis. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1984; 738(1-2): 49-87. https://doi.org/10.1016/0304-419X(84)90019-2.
6. Bondar, D.C., J.M. Beckerich and P.Bonnarme; Involvement of a branched-chain aminotransferase in production of volatile sulfur compounds in Yarrowia lipolytica. Appl. Environ. Microbiol., 2005; 71(8): 4585-4591(). https://doi:10.1128/AEM.71.8.4585–4591.2005.
7. Bonnarme, P., C. Lapadatescu, M. Yvon and H.E. Spinnler; L-methionine degradation potentialities of cheese-ripening microorganisms. *J. Dairy Res.*, 2001; 68(4): 663-674(). https://doi.org/10.1017/S002202990100509X.
8. El-Sayed, A.S.; Microbial L-methioninase: production, molecular characterization, and therapeutic applications. Appl. Microbiol. Biotechnol., 2010; 86(2): 445-467(). https:// doi: 10.1007/s00253-009- 2303-2.
9. Abu-Tahon, M.A. and G.S. Isaac; "Comparative study of a new alkaline L-methioninase production by Aspergillus ustus AUMC 10151 in submerged and solid-state fermentation. Braz. Arch. Biol. Technol., 2015*;5*: e16150484. https://doi.org/10.1590/1678-4324-2016150484.
10. Bahl, C., S.G. Saxena and , S.G. Sharma; Screening Endophytic Fungal Broth for L-Methioninase Activity (Doctoral dissertation) 2012.
11. Jain, R., K.U. Zaidi, Y. Verma and P. Saxena; L-asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia. People's J. Sci. Res., 2012;5(1): 29-35.
12. Imada, A., S. Igarasi, K. Nakahama and M. Isono; Asparaginase and glutaminase activities of micro- organisms. Microbiology, 1973; 76(1): 85-99. https://doi.org/10.1099/00221287-76-1-85.
13. El- Sayed, A.S.; L- methioninase production by Aspergillus flavipes under solid- state fermentation. J. Basic Microbiol., 2009;49(4): 331-341. https://doi.org/10.1002/jobm.200800318.
14. Buonaurio, R., , V.M. Stravato and C. Cappelli; Brown spot caused by Sphingomonas sp. on yellow Spanish melon fruits in Spain. *Plant pathol.*, 2001; 50(3), 397-40. https://doi.org/10.1046/j.1365-3059.2001.00571.x.
15. Okonechnikov, K., O. Golosova, M. Fursov and Mugene team; Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics, 2012;28: 1166- 1167().
16. Theantana, T., , K.D. Hyde and S.Lumyong; Asparaginase production by endophytic fungi isolated from some Thai medicinal plants. Curr. Appl. Sci. Technol., 2007;7(1-1): 13- 18.
17. Paul, G.C., C.A. Kent and C.R. Thomas; Hyphal vocuolation and fragmentation inpenicillium chrysogenum. Biotechnol. Bioeng., 1994;44(5), 655-660.. https://doi.org/10.1002/bit.260440513.
18. Lockwood, B.C. and G.H. Coombs; Purification and characterization of methionine γ-lyase from Trichomonas vaginalis. Biochem. J., 1991; 279(3): 675-682. https://doi.org/10.1042/bj2790675.
19. Delgado-Andrade, C. and I.Seiquer; Maillard reaction products consumption: Magnesium bioavailability and bone mineralization in rats. *Food Chem.*, 2008; 107(2): 631-639. https://doi.org/10.1016/j.foodchem.2007.08.055.
20. Al-Farsi, M.A. and C.Y. Lee; Nutritional and

functional properties of dates: a review. Crit. Rev. Food Sci. Nutr., 2008; 48(10): 877-887. https://doi.org/10.1080/10408390701724264.
21. Tang, Z.X., L.E. Shi and S.M. Aleid; Date fruit: chemical composition, nutritional and medicinal values, products. J. Sci. Food Agric., 2013;93(10): 2351-2361. https://doi.org/10.1002/jsfa.6154.
22. Tunick, M.H.; Whey protein production and utilization: a brief history. Whey Processing, Functionality and Health Benefits, 2008;1-13.