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Screening and statistical optimization of anti-cancer L-methionase enzyme produced by *Aspergillus fumigatus* MF13 using response surface methodology

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ABSTRACT

Cancer research focuses on new pharmacological agents. Enzymatic approaches have shown promise in reducing cancer burden in experimental models. In this study, *Aspergillus fumigatus,* a new high-productivity L-methionine-producing fungus, was isolated from soil samples. The enhancement of enzyme production by *A. fumigatus* was carried out using the method. One-factor-at-a-time for seven different parameters: temperature (25°C, 30°C, 35°C, and 40°C), pH (6 to 10), incubation time (4 to 8 days), inoculum (1% to 3%), substrate concentration (0.2% to 0.5%), and various carbon sources (glucose, fructose, maltose, and lactose), and organic and inorganic nitrogen sources (Yeast extract, peptone, potassium nitrate, and ammonium sulfate). The statistical method, Plackett-Burman (PB), and response surface method design were used for optimization using Design Expert 13 software. The PB formula consisted of 12 run trials and 9 factors: temperature, pH, glucose, and yeast extract. Incubation time: dipotassium phosphate, potassium nitrate, magnesium sulfate, and potassium chloride Three important parameters were identified in this analysis: temperature, yeast extract, and dipotassium phosphate with p-values <0.05. The enzyme yield was then optimized using these important parameters through central composite design with 20 run trials; the highest L-methionase enzyme production was achieved at 30°C, with a yeast extract concentration of 2.4 g/l and a dipotassium phosphate concentration of 1.2 g/l. Regression model analysis resulted in the R^2 value of 96.5%, indicating that it was well suited to describe the relationship between the response and the variables.

1. INTRODUCTION

Cancer is the second-leading cause of death in developing countries. This is mostly due to cancer-causing gene mutations. Tumor suppressor genes, DNA repair genes, and genes involved in cell formation increase, and differentiating enzymes such as L-asparaginase, L-glutaminase, and L-methionase remove essential amino acids from metabolic sources. By inhibiting the growth of cancer cells. Although it has high specificity, it can cause fever, immune, and allergic reactions. [1,2]. L-methionase is a carbon-sulfur lyase that converts L-methionine to α-ketobutyrate, methanethiol, and ammonia through an elimination reaction [3]. The study thoroughly examined the enhancements in cancer cell dependence on methionine and the biochemical findings of L-methionase as a therapeutic drug. Therefore, using L-methionase to remove L-methionine-dependent cancer cells is a potent, acceptable therapeutic technique [4,5].

L-methionase, a pyridoxal-5-phosphate-dependent enzyme [6], has shown efficacy as an anticancer drug against various tumor cells [7]. These comprise neuroblastoma, lung, kidney, and breast cancer, along with glioblastoma, and could have applications in the food and pharmaceutical industries [8]. Methionine is required for the production of proteins and the growth of cancer cells. However, it differs from normal cells, which stay unaffected as they can synthesize amino acids from external sources. Targeting methionine can inhibit cancer cell growth and cause apoptosis [9]. The therapeutic preparation of L-Methionase has been conjugated with polyethylene glycol to decrease its immunogenicity [10].

However, some studies have shown that tumor cells lack the enzyme methionine synthase, which normal cells require to synthesize methionine from the precursor homocysteine [4]. Normal cells are independent of methionine. While tumor cells require methionine for protein synthesis and DNA regulation. Homocysteine, which is formed from the demethylation of L-methionine, is associated with cardiovascular disease and Alzheimer's disease [11]. L-methionase is used pharmacologically to reduce homocysteine levels, reduce histone methylation, and control weight in breast and prostate cancer cells

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[12]. L-methionase has potential therapeutic uses in the treatment of cancer, drug resistance, infections, aging, and heart disease [13]. It can also be employed as a biotechnology-relevant treatment for dangerous microorganisms [14].

Enzyme production and activity by isolated microorganisms is affected by culture conditions and the composition of the culture medium. This is different from traditional optimization approaches that adjust variables one at a time. This is also called one factor at a time (OFAT), by keeping other parameters constant. Statistical methods use tools such as response surface methods (RSM) to analyze relationships between variables and optimize culture parameters. The statistical RSM optimizes culture parameters by establishing relationships between responses and independent variables. This technique analyzes individual or combined variables, enhancing the optimal culture parameter through efficient statistical experimental design [10].

The main objective of this study was to screen L-methionase-producing fungi and increase L-methionase production from *Aspergillus fumigatus* MF 13 media components. The nutrient source and the culture conditions of this fungus were optimized using statistical methods**.**

2. MATERIALS AND METHODS

2.1. Materials

The chemicals used in this study were L-methionine (SRL, Maharashtra, India), phenol red, dextrose, sodium hydroxide, pyridoxal-5-phosphate, Nessler's reagent, Folin reagent, yeast extract, sucrose, magnesium sulfate, potassium chloride, iron, potassium nitrate, ammonium sulfate, maltose, fructose, lactose, potato dextrose agar (PDA) (potato infusion: 4 g/l, dextrose: 20 g/l, and agar-agar: 20 g/l) and C'zapek Dox broth (sucrose: 30 g/l, sodium nitrate: 2 g/l, dipotassium phosphate: 1 g/l, magnesium sulfate: 0.50 g/l, potassium chloride: 0.50 g/l, ferrous sulfate: 0.010 g/l, and agar-agar: 20 g/l), all of which were purchased from Himedia, Mumbai, India.

2.2. Isolation of Fungi

Soil samples were collected at a depth of 5 cm from different locations. Including garbage dump sites (Rajkot, India), agricultural land (Rajkot, India), marine soil (Porbandar, India), pond soil (Morbi, India), and river soil (Aji River, Rajkot, India) after the sample was placed in a sterile zip-lock plastic bag and transported to the laboratory. Soil samples were stored at room temperature until further inoculated on PDA plates and incubated at 28°C for 5 to 6 days, and then the pure cultures were stored at 4°C for use further [15].

2.3. Screening of Fungal Isolates for L-methionase Production

This study used an agar-well diffusion technique to qualitatively investigate L-methionase production. Fungal isolates were inoculated into a modified C'Zapec-Dox medium and incubated for 5 to 7 days after the incubation period. Centrifugation was used to extract fungal cells free of filters. To determine L-methionase activity, 100 µl of fungal cell-free culture was introduced to the wells [16].

2.4. L-methionase Enzyme Assay

L-methionase production was examined by measuring the ammonia released from L-methionine. 1 ml of 1% methionine in 0.5 M potassium phosphate buffer along with 1 ml of crude enzyme made up the optimal reaction system. After adding 0.1 ml of 1.5 M trichloroacetic acid and centrifuging it, enzyme activity was inhibited. 3.7 ml of distilled water was mixed with 0.1 ml of the supernatant. Nessler's reagent was used to identify the released ammonia, and 480 nm was used to analyze the color compounds that were produced [17].

2.5. Protein Determination

Using bovine serum albumin as the standard, the Folin–Lowry method was used to determine the protein concentration [14].

2.6. Morphological and Molecular Characterization

Macroscopic and microscopic examination of fungal morphology was carried out, with particular focus given to characteristics such as color, size, shape, and hyphae staining with lactophenol cotton blue [18].

Molecular identification of fungal isolates was performed by SLS Research Pvt. Co., Ltd. Genomic DNA was extracted from fungal isolate on PDA plates using a DNeasy® Fungal Mini Kit (QIAGEN, Hilden, Germany.) According to the manufacturer's instructions, an assessment of the concentration and quality of the extracted DNA using a spectrophotometer (LABMAN, Chennai, Tamil Nadu) was performed using a total of 20 ng of DNA. The ITS region of the ribosome was amplified using the forward primer ITS1-F (5′-CTACCTGATCCGAGGTCAAC-3′) and the reverse primer ITS4-R (5′-AGGTGGACCCAGGGCCCTCA-3′) in a Bio-Rad thermocycler (Hercules, CA, USA). 25 µl of polymerase chain reaction (PCR) reaction mix contained 2.5 µl of 10× PCR buffer, 1.5 µl of $MgCl_2$ (50 mM), 5.0 µl of dNTPs (10 mM), 1.0 µl of each primer, and 0.9 µl of Taq DNA polymerase (10 U/µl) (Amid Bioscience, USA), and 19.6 µl of molecular biology water. Amplification was achieved with the following PCR conditions: initial denaturation at 94°C for 5 minutes, followed by 33 cycles of denaturation at 94°C for 30 seconds, and annealing at 55°C for 30 seconds, extension at 72°C for 2 minutes, each time at 72°C, ending with a final extension for 5 minutes, PCR amplicons were purified using the PCR Clean-up Kit (Sigma-Aldrich, St. Louis, MO). They carried out the sequencing reaction with sequencing 27F (TACGTCCCTGCCCTTTGTAC) using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific, USA) on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA). They performed similarity searches for the nucleotide sequences by BLAST (http://www.ncbi.nlm.nih.gov/blast) against the GenBank database to identify the isolate.

The 18S rRNA gene sequence of strain MF13 was used to search for similar sequences in the nucleotide sequence database by running the BLAST program. The highest-scoring sequences similar to the 18S rRNA gene sequence were identified from the results and retrieved. From the GenBank database, the identified sequences were aligned using MEGA 6.0 software, and the phylogenetic tree was estimated using neighbor-joining bootstrap analysis with the help of MEGA 6.0 software.

2.7. Media Optimization Using OFAT

The effects of culture conditions and media composition on L-methionase production were studied using the OFAT method with different parameters such as pH (from 6 to 10), temperature (25°C, 30°C, 35°C, and 40°C), substrate concentration (0.2% to 0.5%), inoculum size (1% to 3%), and incubation period (4 to 8 days) Nitrogen source (peptone, yeast extract, ammonium sulfate, and potassium nitrate), and carbon sources (glucose, maltose, fructose, and lactose).

2.8. Statistical Modeling and Optimization of L-methionase Production

Statistical modeling and optimization techniques were used to increase L-methionase production in *A. fumigatus* MF 13 fungal strains using the RSM and Plackett-Burman design (PBD). All experiments, data analysis, and verification of the results were carried out using Design Expert 13 software (Stat-Ease).

2.9. Plackett Berman Design

The experiment aimed to optimize the design of Plackett–Berman (PB) and identify the effect of fermentation factors on the production of the L-methionase enzyme. Eight parameters were examined at the highest (+) and lowest (-) levels. These parameters include temperature, pH, incubation time, potassium phosphate, magnesium sulfate, glucose, yeast extract, potassium chloride, and potassium nitrate Flask culture tests were performed, and response was measured in U/ml/minute and calculated as enzyme yield.

2.10. Optimization of Selected Parameters Using RSM

Pareto chart from the experimental design of PB It was used to analyze medium-sized components that affect enzyme production. This was then optimized using a central composite design (CCD). The experiment involved 20 trials and 6 central points, with the L-methionase activity averaged and denoted as response R1.

2.11. Validation of the Model

Conduct experiments to validate the statistical model at its optimal level of the most significant variables under a predicted set of conditions.

3. RESULTS

3.1. Screening of Fungal Isolates for L-methionase Production

For the production of L-methionase, we screened 50 fungal isolates obtained from soil samples. When 10 strains were grown on C'zapex dox agar, a yellow zone formed around their colonies. Seven isolates with larger zone dimensions, yellow appearances, and stronger intensity were found during the qualitative screening. Fungal isolate MF 13 showed the highest zone diameter, measuring 35 mm (Fig. 1), and L-methionase activity, releasing 4.31 U/ml/minute of ammonia under the assay condition (Fig. 2).

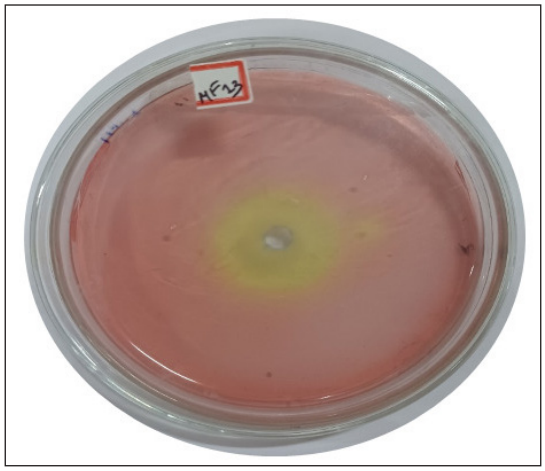

Figure 1. Qualitative assay of L-methionase by rapid plate method.

3.2. Morphological and Molecular Characterization

The morphological features of the fungal isolate MF 13 are typical. On PDA agar, colonies develop into gray-colored colonies in 7 days, with a maximum diameter of 70 mm. Under a microscope, the MF 13 conidia head on PDA agar revealed a columnar structure, ovate to flask-shaped

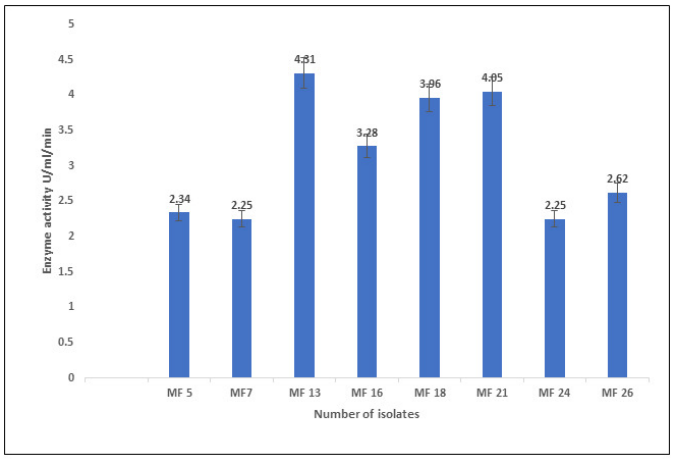

Figure 2. Quantitative assay of L-methionase activity produce by the selected fungal isolates by Nessler's method.

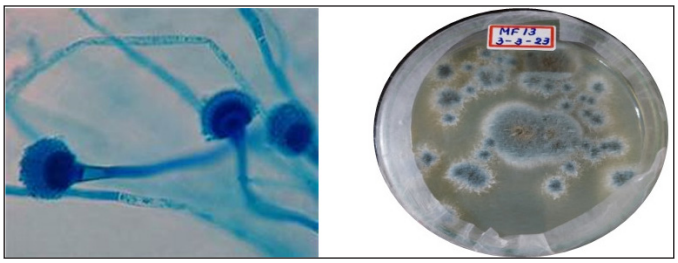

Figure 3. Micro morphological and macro morphological identification of MF13 by lactophenol cotton blue method.

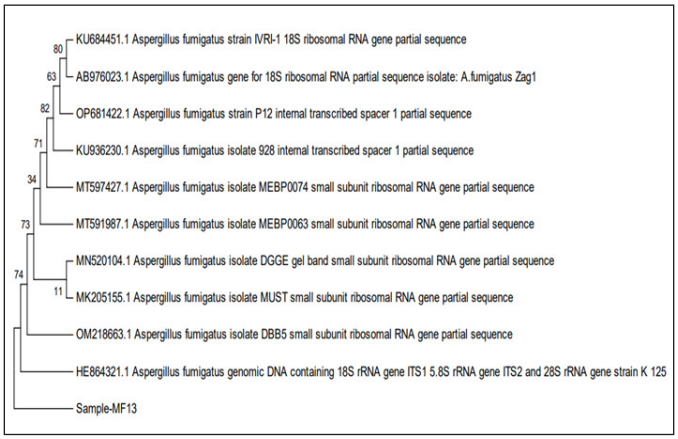

Figure 4. Phylogenetic tree analysis of *A. fumigatus* MF 13 by the neighbor-joining method.

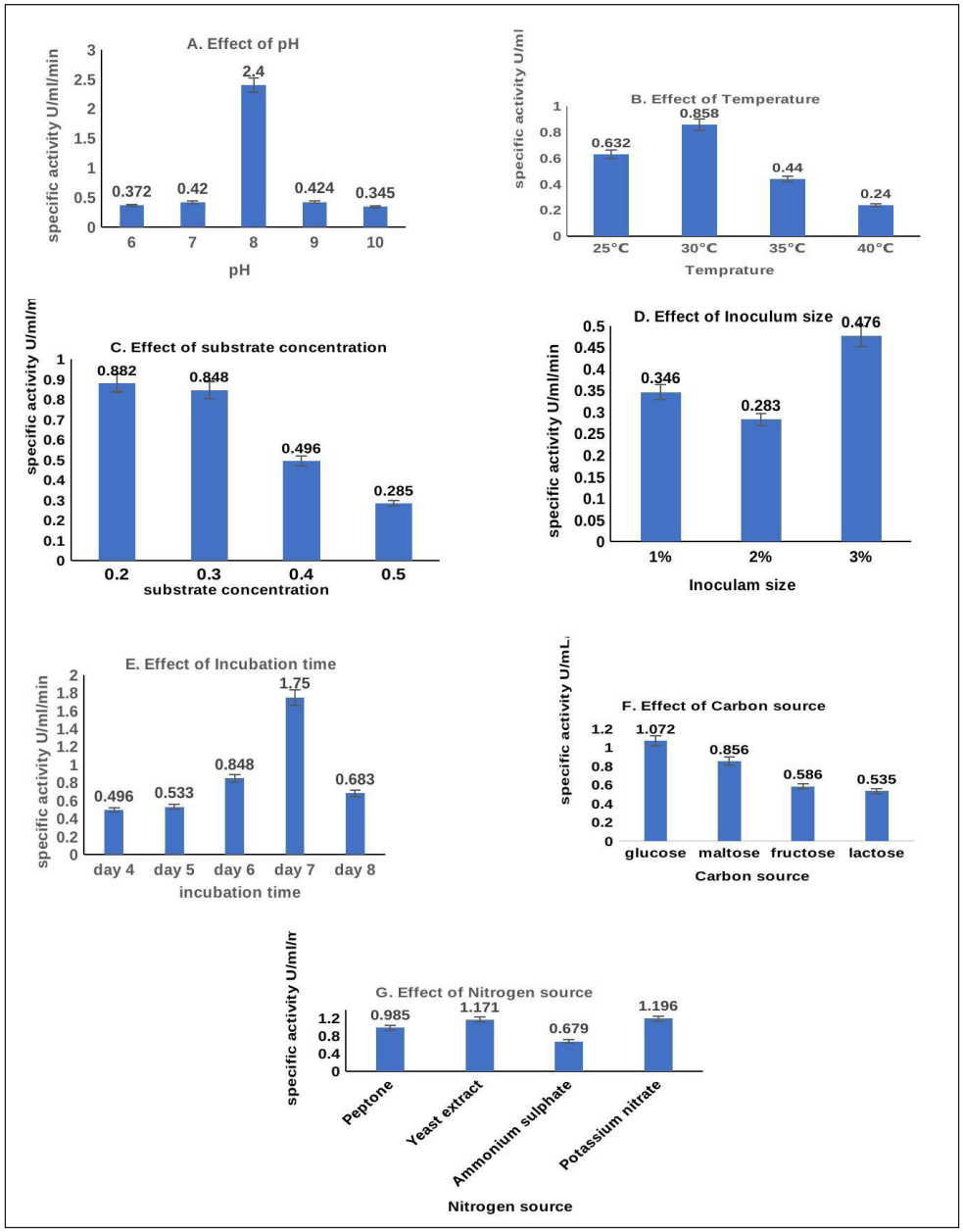

Figure 5. Optimization of A. pH, B. temperature C. substrate concentration D. inoculum size E. incubation time F. carbon sources G. nitrogen sources on L-Methionase production by *A. fumigatus* MF 13 through OFAT method.

vesicles, hyaline conidiophores, and uniseriate sterigmata. The shape of the conidia ranged from globose to prolate, with sizes between 1.5 and 2.5 µm (Fig. 3).

Using the neighbor-joining method in MEGA 6.0 software and the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the analysis of the 18S rRNA sequence showed 99.82% similarity to *A. fumigatus*. With *A. fumigatus*, a phylogenetic tree was constructed with a bootstrap value of 98%. The NCBI database has *A. fumigatus* strain MF13 with accession number **OQ690549** associated with the 18S rRNA gene sequence (Fig. 4).

3.3. Media Optimization Using OFAT

The study found that there was a significant relationship between pH and the amount of enzyme produced, with pH 8 (2.8 U/ml/minute)

Table 1. Experimental variable at two levels used for the production of L-methionase by *A. fumigatus* MF 13 using PBD.

Factors	Low level	High level
pH	7.5	8.5
Temperature	28°C	32°C
Incubation time	7 days	9 days
Glucose	20 g/l	40 g/l
Yeast extract	2.4 g/l	5 g/l
Potassium nitrate	2.4 g/l	5 g/l
Potassium chloride	0.4 g/l	0.7 g/l
Dipotassium phosphate	0.4 g/l	2 g/l
Magnesium sulphate	0.4 g/l	2 g/l

Table 2. PBD for optimization of parameters influencing L-methionase production by *A. fumigatus* MF 13.

Run	Glucose g/l	Yeast extract g/l	Dipotassium phosphate g/l	Magnesium sulphate g/l	Potassium chloride g/l	Potassium nitrate g/l	pH	Temperature	Incubation time	L-methionase U/ml
1	20	5	2	0.4	0.7	5	8.5	28	7	0.613
2	40	2.4	2	0.7	0.7	2.4	7.5	28	9	0.84
3	20	2.4	2	0.4	0.7	5	7.5	32	9	0.852
4	20	2.4	0.4	0.7	0.4	5	8.5	28	9	1.415
5	40	5	0.4	0.7	0.7	5	7.5	28	7	0.89
6	20	5	2	0.7	0.4	2.4	7.5	32	7	0.483
7	40	5	2	0.4	0.4	2.4	8.5	28	9	0.981
8	40	5	0.4	0.4	0.4	5	7.5	32	9	0.802
9	40	2.4	0.4	0.4	0.7	2.4	8.5	32	7	0.988
10	20	2.4	0.4	0.4	0.4	2.4	7.5	28	7	1.655
11	20	5	0.4	0.7	0.7	2.4	8.5	32	9	0.768
12	40	2.4	2	0.7	0.4	5	8.5	32	7	0.63

Table 3. ANOVA for the experimental result of PBD.

Source	Sum of squares	DF	Mean square	*F*-value	*p*-value	
Model	0.9490	3	0.3163	9.67	0.0049	Significant
B-Yeast extract	0.2831	1	0.2831	8.65	0.0187	Significant
C-Dipotassium phosphate	0.3742	1	0.3742	11.44	0.0096	Significant
H-Temperature	0.2917	1	0.2917	8.92	0.0174	
Residual	0.2616	8	0.0327			
Cor total	1.21	11				

Note: DF is the degree of freedom. *One-way ANOVA. **p*-value (< 0.05).

showing the highest level of L-methionase production after being examined at several temperatures. It was found that at 30°C the highest result for enzyme production was U/ml/minute. Substrate concentration ranged from 0.2% to 0.5%, with a maximum enzyme yield of 0.882 U/ml/minute, observed at a concentration of 0.2%. This study examined enzyme production at different inoculum sizes, with a maximum production of 0.476 U/ml/minute at an inoculum dose of 3%. Measurement of the production of enzymes took place from day 4 to 8 of incubation, with a peak of 1.75 ml/minute on day 7 and a subsequent decline to 0.683 ml/minute. When the impact of carbon sources on the production of L-methionase was evaluated, glucose produced the highest yield of the enzyme. The production of enzymes is also influenced by nitrogen sources like potassium nitrate and yeast extract (Fig. 5).

3.4. Plackett Burman design

The determination of important physicochemical parameters of PB (pH, temperature, incubation time, glucose, yeast extract, magnesium sulfate, potassium chloride, potassium nitrate, and potassium phosphate) to increase L-methionase production is an effective selection method. The study involved nine variables and 12 run trials, which were analyzed at two levels and coded as (−1) for low level and

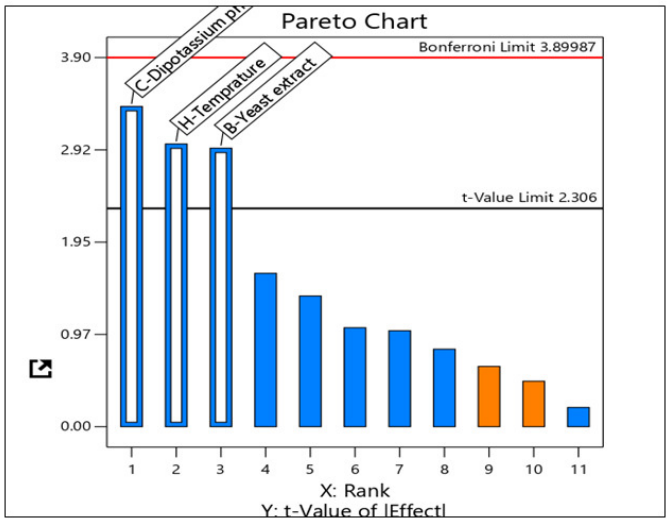

Figure 6. Pareto chart of the standardized effects of nine medium factors on L-methionase production by *A. fumigatus* MF13, temperature, yeast extract, and dipotassium phosphate the significant factors positively affecting enzyme production.

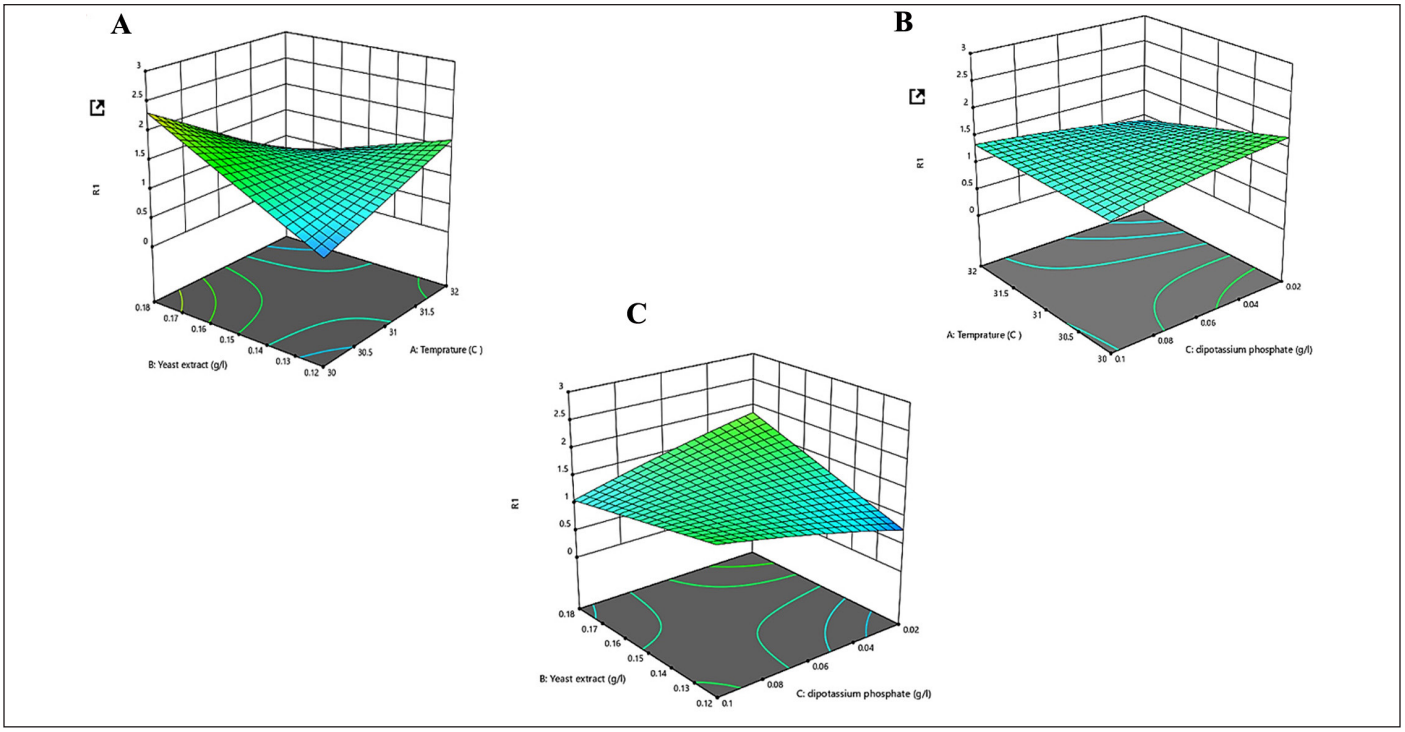

Figure 7. (A–C) RSM 3D surface plots obtain by design expert 13 representing the effect and relationship between different variables in L-methionase production A) Temperature versus yeast extract, B) Temperature versus Dipotassium phosphate C) Yeast extract versus Dipotassium phosphate.

(+1) for high level (Table 1). L-methionase activity in *A. fumigatus* MF13 showed a wide variation, from 0.613 to 1.655 U/ml/minute, as shown in Table 2.

The Pareto chart indicates the most important factors affecting L-methionase production, mainly temperature, yeast extract, and dipotassium phosphate, as shown in Figure 6. These factors were selected for the CCD with their p values < 0.05 (Table 3), indicating their significance in enzyme production.

3.5. Central Composite Design

Studies indicate that temperature, yeast extract, and dipotassium phosphate are key factors in increasing L-methionase production, which is further optimized using the CCD. The experimental design and response obtained for L-methionase production are shown in Table 4. The study included 20 experimental designs using CCD, and the highest L-methionase activity of 2.57 U/ml/minute was detected. In run number 15, the lowest L-methionase activity of 0.644 U/ml/minute was detected in run number 7. Regression analysis examined both the positive and negative effects of the significant variables on L-methionase production, where the results were interpreted in terms of probability values (p values) as shown in Table 5. The high F value of the model is 146.6 and the p-value is less than 0.05, with a poor fit, indicating the accuracy of predicting L-methionase production. The study found no significant effect of other interacting conditions on L-methionase production. Through regression analysis, using Design Expert software yields the following equations:

$$Yi = \beta 0 + \Sigma\, \beta i\, xj + \Sigma\, \beta ii\, xj2 + \Sigma\, \beta ij\, xi$$

Y = 1.67 + 0.0795 temperature + 0.1204 yeast extract + 0.1629 dipotassium phosphate + 0.2940 temperature *Yeast extract: 0.2390 temperature * dipotassium phosphate = 0.00083 yeast extract* dipotassium phosphate = 0.1513 (temperature) ^{2} + 0.0096 (yeast extract)^2 – 0.3245 (dipotassium phosphate)^2.

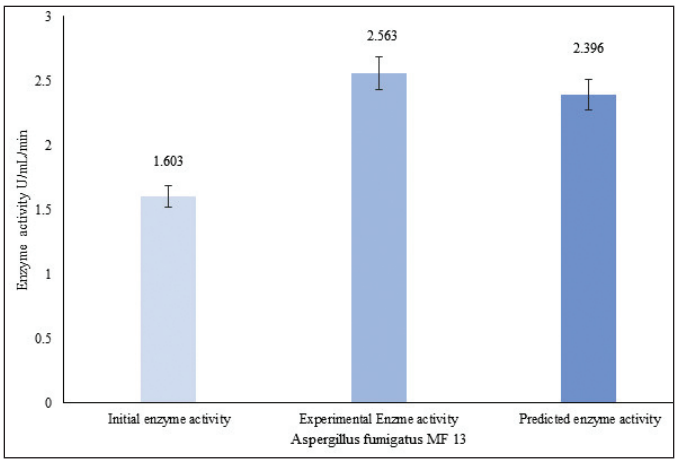

Figure 8. Validation of the ideal conditions for *A. fumigatus* MF 13 to produce L-methionase enzyme.

In the above equation, βi is the linear effect coefficient, βii is the quadratic effect coefficient, βij is the interaction effect coefficient, and Y shows the response of the model to L-methionase production, in good agreement with the indicated R^2 value of 95.4%, while the predicted R^2 value was 0.943 (94.8%).

Design experts have created RSM 3D surface plots to graphically display regression equations of key parameters in the activities of 13 enzymes. It shows the effect and relationship of the variables on L-methionase production (Fig. 7).

Table 4. CCD for optimization experiment for L-methionase by *A. fumigatus* MF 13.

Run	Temperature	Yeast extract	Dipotassium phosphate	Enzyme activity observed value (U/ml/minute) (R1)	Enzyme activity predicted value (U/ml/minute)
1	31	3.7	0.8	1.67	1.67
2	32	5	0.4	0.9692	0.9344
3	31	3.7	1.47272	0.7556	0.8048
4	32	2.4	0.4	1.25	1.22
5	31	3.7	0.8	1.67	1.67
6	29.3182	3.7	0.8	1.25	1.30
7	31	3.7	0.127283	0.6446	0.6938
8	30	2.4	0.4	1.05	1.02
9	32.6818	3.7	0.8	1.13	1.18
10	31	3.7	0.8	1.67	1.67
11	31	3.7	0.8	1.67	1.69
12	31	1.51367	0.8	1.73	1.78
13	32	5	1.2	1.53	1.50
14	31	3.7	0.8	1.67	1.67
15	30	2.4	1.2	2.57	2.54
16	31	3.7	0.8	1.67	1.67
17	31	5.88633	0.8	1.56	1.61
18	30	5	1.2	1.11	1.08
19	30	5	0.4	0.655	0.6202
20	32	2.4	1.2	0.752	0.7172

Table 5. Analysis of ANOVA and significance level of the response surface of the full quadratic model for the L-methionase production.

Sources	Sum of squares	df	Mean square	*F*-value	*p*-value	
Model	5.57	13	0.4284	1.466E+05	<0.0001	Significant
A-Temperature	0.1860	1	0.1860	63652.71	<0.0001	Significant
B-Yeast extract	0.1200	1	0.1200	41072.34	<0.0001	Significant
C-dipotassium phosphate	0.0000	1	0.0000	17.11	<0.0061	
AB	3.15	1	3.15	1.077E+06	<0.0001	
AC	0.3629	1	0.3629	1.242E+05	<0.0001	
BC	1.29	1	1.29	4.409E+05	<0.0001	
A^2	0.0005	1	0.0005	165.74	<0.0001	
B^2	0.0000	1	0.0000	7.99	<0.0301	
C^2	0.0244	1	0.0244	8350.18	<0.0001	
ABC	2.101E-06	1	2.101E-06	0.7189	0.4290	
A^2B	9.471E-07	1	9.471E-07	0.3240	0.5898	
A^2C	2.335E-06	1	2.335E-06	0.7990	0.4058	
AB^2	0.0000	1	0.0000	3.51	0.1103	
Residual	0.0000	6	2.923E-06			
Lack of Fit	0.8166	5	0.0058	0.63	0.7560	Not significant
Pure error	0.0160	5	0.0033			
Cor total	5.57	19				

Note: DF is the degree of freedom. *One-way ANOVA. **p*-value (< 0.05). *A-temperature. *B-yeast extract*C- dipotassium phosphate.

3.6. Data Validation

The obtained parameters of temperature (30°C), yeast extract (2.4 g/l), and dipotassium phosphate (1.2 g/l) were validated for their accuracy. These parameters indicated that the experimental enzyme activity was 2.565 U/ml/minute (Fig. 8). The model was found to be effective in increasing enzyme production considering all factors.

4. DISCUSSION

This study presents *A. fumigatus* ability to secrete the L-methionase enzyme, a bioactive compound widely observed in various microorganisms, including bacteria, yeast, and fungi. L-methionase is known for its potential therapeutic and industrial applications, making its production by different organisms of significant interest.

L-methionase production levels by *A. fumigatus* were found to be comparable to other well-studied organisms such as *A. flavipes*, *Trichoderma harzianum, Candida tropicalis*, *Serratia marcescens*, *Pseudomonas putida* and *Chaetomium globosum* [4,9,11,19,20]. This finding expands the possible sources of L-methionase and emphasizes the versatility of *A. fumigatus* in biotechnology applications. Research results indicate that *A. fumigatus* could be a valuable organism for further research and development of L-methionase production.

The agar-well diffusion technique was used for quantitative screening by isolating L-methionase on agar plates, which was considered a potential producer of Selim *et al.* [15]. A previous study by Hendy *et al.* [14] reported that *Aspergillus oryzae* exhibited a 20-mm zone of diameter and L-methionase activity, releasing 2.13 U/ml/minute of ammonia under the assay condition. In this study, the isolate *A. fumigatus* MF 13 demonstrated the highest zone diameter, measuring 35 mm, and L-methionase activity, releasing 4.31 U/ml/minute of ammonia under the assay condition.

The challenge lies in finding new manufacturers for the large-scale production of L-methionase and in developing new growth media. This study also highlights the importance of culture parameters and diet composition in influencing L-methionase production in a one-time, single-factor approach. The physical conditions and chemical composition of the culture medium significantly affect cell growth and metabolism production.

In the present study of L-methionase from *A. fumigatus* MF 13, we observed higher enzyme production at 30°C, while a sharp decrease in enzyme production was detected at 40°C. Another thing is pH. We found that pH 8.0 was effective in increasing L-methionase production. The carbon source in the culture medium is important for microbial growth and metabolism production. *Aspergillus flavipes* [21] was identified as the best carbon source of glucose for L-methionase production. In our study, glucose also results in higher L-methionase production by *A. fumigatus*. Yeast extract has also been shown to be highly effective in increasing L-methionase production as a nitrogen source. According to a previous report [22], peptone was identified as the most suitable organic nitrogen supplement for L-methionase production.

PBD experiments were used in the present work to test different media components for maximum L-methionase production by *A. fumigatus* MF 13. Previous studies by Selim *et al.* [23] showed that PBD was used to optimize the media components produced successfully by *Candida* specie. This experiment identified three factors, namely, temperature, yeast extract, and dipotassium phosphate, as statistically significant using the PBD. It revealed that yeast extracts are an important source of nitrogen for the production of PBD L-methionase. A report by Salim *et al.* [22] illustrates the significance of yeast extract in the production of L-methionase from *T. harzianum*. Ions are essential for building cell mass and activating biosynthetic enzymes. This study identified dipotassium phosphate as an important ion source for increasing L-methionase production.

Other statistical methods include artificial neural networks that consist of neurons. It learns complex relationships between independent and dependent variables using a multi-layer feedforward architecture with a backpropagation algorithm for nonlinear mapping. According to Salim *et al.* [22], neural networks in an implant with hidden neurons have the minimum mean square error and provide good predictions of the results for training and validation. The overall R^2 of the model is 0.993, which indicates a good fit. Activity of L-methionase. The predicted maximum was 30.17 U/ml/minute, which was close to the experimental value of 30.2 U/ml/minute [22]. A full qualitative fractional of the Box–Behnken design, a fraction of the full factorial, to evaluate quadratic effects and two-way interactions between variables, determining the nonlinear nature of the response. According to Mohkam *et al.* [24] yeast extract 0.2%; lactose 0.75%; pH 6; temperature 35°C. Additionally, this model predicted 13.1 U/ml/minute of L-methionase activity [24].

The study optimized factors for maximizing L-methionase activity using the CCD of RSM. The model's strength was confirmed by an R^2 value of 0.96, and the lack of fit F value (0.63) confirmed its significance. The model was generally significant, contrasting with previously reported data by Kavya and Nadumane [25], which indicated a high F value (145.31) and a lower F value (1.59), suggesting non-significance in the optimization of L-methionase production.

The main objective of this research is to find naturally occurring microorganisms for large-scale production of the enzyme L-methionase, avoiding the use of genetically modified organisms (GMOs). If GMOs are used to produce L-methionase, there is a chance that will be released accidentally. To the environment: This can destroy local ecosystems and have unexpected environmental impacts. Our research therefore has the potential to support biodiversity conservation efforts.

5. CONCLUSION

The study's outcomes show how well fungal isolates were isolated, screened, and characterized to produce L-methionase. To find possible producers of L-methionase, several qualitative and quantitative testing techniques were performed. The fungal isolate MF13 was chosen to check its molecular identity out of the 50 isolates based on their highest enzymatic activity. Moreover, the fungus *A. fumigatus* was detected. L-methionase production from *A. fumigatus* was optimized by a OFAT and reached 7.69 µg/ml/minute. Statistical optimization was then completed using the PBD and RSM. This study included a total of seven parameters, and the most important parameter identified was the temperature (30°C), yeast extract (2.4 g/l), and dipotassium phosphate (1.2 g/l). RSM showed an optimal response to the production of L-methionase. RSM depicts the optimum response for L-methionase production. To the best of our knowledge, it is the first time that RSM and its representatives have been employed and demonstrated significant results for optimizing the medium components for L-Methionase production by *A. fumigatus* MF 13. Optimum culture conditions enhanced L-Methionase production by 4.4-fold compared to that under natural conditions. These optimized experimental designs can provide a quick and meaningful approach to improving the productivity of L-methionase.

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7. AUTHOR CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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