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Formulation of a Suitable Medium and its Optimization for Maximizing L-asparaginase Production from Endophytic Fungi *Fusarium* sp. LCJ273

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Endophytic fungi *Fusarium* sp. LCJ273 capable of producing L-asparaginase was isolated from the medicinal plant *Adhatoda vasica.* The aim of the present study was to maximize L-asparaginase production by submerged fermentation through statistical optimization. L-Asparaginase production by *Fusarium* sp. LCJ273 was studied in five different media. Various nutritional parameters specifically carbon, nitrogen and inducers were optimized for enhancing the production of L-asparaginase. In addition, different statistical based experimental designs were also applied to increase the production of L-asparaginase by *Fusarium* sp. LCJ273. Dextrose, ammonium sulphate and wheat bran were found to be effective for growth and higher yield of L-asparaginase in Modified Czapek's Dox Broth. Dextrose at a concentration of 3.0 g/L increased L-asparaginase production up to 9.18±0.9 U/mL, ammonium sulphate at the concentration of 20 g/L showed maximum L-asparaginase production up to 13.69±0.4 U/mL and wheat bran at 2.5 g/L yielded up to 14.24±0.5 U/mL. The maximum L-asparaginase production was observed by *Fusarium* sp. LCJ273 on 5th day. The study revealed that through optimization, a 2 fold increase in L-asparaginase could be achieved.

Keywords: L-Asparaginase, Endophytic fungi, Enzyme production, Submerged fermentation.

L-Asparagine is hydrolyzed by L-asparaginase (L-asparagine amido hydrolase E.C.3.5.1.1) to form aspartic acid and ammonia. L-Asparaginase is present in animals, plants and microbes but not in humans. Fungal L-asparaginases are enzymes of great therapeutic significance due to their use in anti-leukemic and antilymphoma treatment[1,2]. L-Asparaginase has a growing demand in medical application and food industries[3]. L-asparaginase production from microbes has more attention because the process is cost-effective and environmental friendly[4]. Bacteria such as *Escherichia coli* and *Erwinia carotova* are the best producers of

L-asparaginase and are used in pharmaceutical industries. There are only few researches carried out on L-asparaginase production by endophytic fungi[5,6,7]. Recently, L-asparaginase obtained from fungi, such as *Fusarium*, *Aspergillus* and *Penicillium*, show additional promise since their L-asparaginases have less adverse effects when compared to bacterial

L-asparaginases[8]. Various types of cancers including melanosarcoma, reticulosarbom, acute myelocytic leukemia, lymphosarcoma, Hodgkin disease, chronic lymphocytic leukemia, acute myelomonocytic leukemia and acute lymphoblastic leukemia specially in children can be

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treated using L-asparaginase[9,10]. L-Asparaginases are effective because neoplastic cells cannot synthesize L-asparagine and therefore rely on L-asparagine found within blood plasma. Research has demonstrated that plasma L-asparagine levels in the blood can be reduced through intravenous injections of L-asparaginases[11]. Submerged fermentation (SmF) is a very effective technique and require low energy and less risk of contamination[12]. Screening and estimation of the environmental factors and nutritional parameters are more essential stages of bioprocess[13]. Minimum information is available related to the optimization of medium conditions using statistical methods for enzyme production. Response surface methodology (RSM) uses mathematical equations to predict the relationship between response and variables[14,15]. The present study was carried out to formulate a suitable medium and to optimize various nutritional factors specifically carbon, nitrogen, natural inducers and culture conditions for maximizing the L-asparaginase production by *Fusarium* sp. LCJ273 under submerged fermentation. Optimization by RSM was used to observe the correlation between the L-asparaginase production and significant parameters.

MATERIAL AND METHODS

Microorganism and Growth medium

The fungal strain *Fusarium* sp. LCJ273 was isolated from *Adhatoda vasica* collected from Chennai. It was grown in Potato Dextrose Agar (PDA) medium containing Potato (200 g/L), Dextrose (20 g/L) and agar (15g/L). The fungal strain were periodically sub-cultured once in two weeks and stored at 4ºC[16].

Culture conditions for L-asparaginase production

Fusarium sp. LCJ273 was grown in 5 different basal media specifically Modified Czapek's Dox Broth (MDCB) (Medium 1), Agar based modified broth (Medium 2), Glucose Asparagine Broth (GAB) (Medium 3), Asparagine Dextrose Salts Broth (ADSB) (Medium 4) and Inorganic Salts Starch Asparagine liquid medium (ISA) (Medium 5)[17,18,19] for maximizing the L-asparaginase production. Endophytic fungi *Fusarium* sp. LCJ273 showed higher activity when Modified Czapek's Dox medium was used. Composition of Modified Czapek's Dox medium (g/L): Glucose 2.0, L-asparagine 10.0, KH_2PO_4 1.52, $MgSO_4.7H_2O$ 0.52, KCL 0.52, traces of $Cu(NO_3).3H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$.

Submerged fermentation was done using 100 mL liquid medium in 250 mL conical flasks. The medium was sterilized and Streptomycin (Hi-media) was added to liquid medium to avoid bacterial contamination. After sterilization, the mycelium of *Fusarium* sp. LCJ273 was inoculated into the medium and incubated in rotatory shaker at 120 rpm for 5 - 8 days. After every 24h of fermentation, 0.5 mL of crude culture filtrate was taken and centrifuged at 10,000 rpm for 10 mins and the clear supernatant was collected. The activity of

L-asparaginase was measured at every 24 h interval using the supernatant as a enzyme source.

Assay for L-asparaginase

The activity of L-asparaginase was evaluated quantitatively by estimating the amount of ammonia liberated from L-asparagine using Nessler's reagent by Imada *et al.,*[20] in short:

0.5 mL of crude culture filtrate, 0.5 mL of 0.5 M tris HCL buffer (7.2), 0.5 mL of 0.04 M asparagine and 0.5 mL of distilled water were combined and incubated at 37ºC for 30 min. The reaction was terminated by adding 0.5 mL of 1.5 M tricholoro acetic acid (TCA). After termination of the reaction, the mixture was centrifuged at 10,000 rpm for 10 min and 0.1 mL of clear supernatant was added to 3.7 mL of distilled water. 0.2 mL of Nessler's reagent was then added and incubated for 20 min. Absorbance was measured at 450 nm using spectrophotometer. One international unit (IU) of L-asparaginase needed to release 1 ìM of ammonia per minute under specified conditions.

$$\text{Units/mL enzyme} = (\mu M \text{ of } NH_3 \text{ liberated})\ (2.5)/(0.1)\ (30)\ (0.5)$$

2.5 = Initial volume of reaction mixture (mL)
0.1 = Volume of supernatant used (mL)
30 = Time of incubation (min)
0.5 = Volume of crude enzyme sample used (mL)

Estimation of protein

Protein estimation was done by the method of Lowry *et al*.,[21] using Bovine serum albumin as the standard.

Optimization studies for L-asparaginase production

Optimization of medium was done by changing any one carbon, nitrogen and natural inducers variable while keeping all the other variables at a fixed level. Optimization of the fermentation medium is to study the process conditions to maximize the enzyme production. Carbon, nitrogen and inducers are considered as the key parameters for medium optimization to increase the L-asparaginase production. They also play a major role in the synthesis of fundamental nutrients needed for the organism growth in the liquid medium. Optimization of nutritional factors by "one-factor-at-a-time" technique was done. Carbon sources such as dextrose, maltose, lactose, glycerol, sucrose and mannitol were studied at different concentrations from 0.5 to 6.0 g/L to determine the effect of carbon source in L-asparaginase production. Nitrogen sources such as ammonium sulphate, yeast extract, sodium nitrate and peptone were also studied at different concentrations from 5 to 25 g/L for the production of L-asparaginase by *Fusarium* sp. LCJ273.

Effect of carbon sources and their concentration on L-asparaginase in modified Czapek Dox medium

The effect of carbon source on the production of L-asparaginase by *Fusarium* sp. LCJ273 was studied using dextrose, lactose, maltose, sucrose, glycerol and mannitol at a concentration of 10 g/L. Other components in the medium were kept unchanged while original medium was used as a control.

The best carbon source was further optimized by using different concentrations from 0.5 to 6.0 g/L. L-Asparaginase and protein activity were determined and best carbon source was chosen for further studies.

Effect of nitrogen sources and their concentration on L-asparaginase in modified Czapek Dox medium

L-Asparaginase production by *Fusarium* sp. LCJ273, was studied using four different nitrogen sources. Modified Czapex Dox broth was amended with different nitrogen sources using ammonium sulphate and ammonium nitrate (inorganic sources), peptone, yeast extract (organic sources) at a concentration of 10 g/L and other variables in the medium were kept constant. The original medium was used as a control.

L-Asparaginase production by *Fusarium* sp. LCJ273 was studied at different concentrations of selected nitrogen source ranging from 5 to 25 g/L. L-Asparaginase and protein activity were determined and best nitrogen source which showed maximum L-asparaginase production was selected for further study.

Effect of natural inducers and their concentration on L-asparaginase in modified Czapek Dox medium

The effects of cheap natural inducers on the L-asparaginase production by *Fusarium* sp. LCJ273 was studied using groundnut oil cake, coconut oil cake, wheat bran, paddy straw and bagasse at the concentration of 5 g/L and other variables were kept constant, while original medium was used as a control.

The best inducer was further optimized by using different concentrations from 0.5 to 3.0 g/L. L-Asparaginase and protein activity were determined and best natural inducer which showed

Table 1. Effect of different carbon sources, nitrogen sources and natural inducers in the culture medium for L-asparaginase production by *Fusarium* sp. LCJ273

Sources	L-Asparaginase activity U/mL
Carbon sources (10 g/L)	
Fructose	6.81±1.1
Glycerol	6.38±0.5
Maltose	6.84±0.4
Starch	6.51±1.2
Lactose	6.38±0.4
Sucrose	7.03±1.0
Mannitol	6.70±1.1
Dextrose	7.06±0.9
Nitrogen sources (10 g/L)	
Peptone	2.46±0.9
Yeast extract	2.97±1.0
Ammonium sulphate	10.21±1.1
Ammonium nitrate	8.45±0.8
Natural inducers (5 g/L)	
Groundnut cake	12.45±0.7
Coconut cake	12.63±0.6
Wheat bran	13.29±0.4
Paddy straw	12.43±0.4
Bagasse	11.78±0.5

maximum L-asparaginase production was selected for further study.

Optimization of L-asparaginase production using response surface methodology

The optimized medium components for enzyme production was studied using Central Composite Design (CCD), a method commonly used in Response Surface Methodology (RSM). Central composite design methodology which is applied to explain the suitable conditions for the production of L-asparaginase. The level of major parameters namely dextrose, ammonium sulphate and wheat bran were selected for studies using Face Centre Central Composite Design (FCCCD).

Table 2. Experimental range and levels of the three independent variables used for production of L-asparaginase by *Fusarium* sp. LCJ273 using RSM in terms of actual and coded factors

Varibles	Actual	Coded	Actual	Coded	Actual	Coded
Dextrose (X_1)	2	-1	3	0	4	1
Ammonium sulphate (X_2)	15	-1	20	0	25	1
Wheat bran (X_3)	2	-1	2.5	0	3	1

Table 3. Face Centered Central Composite Design for L-asparaginase production with observed and predicted values

Run	Dextrose	Ammonium sulphate	Wheat bran	L-Asparaginase activity (U/mL) Observed	Predicted
1	0	0	0	10.95	10.37
2	0	0	0	6.73	7.51
3	1	1	-1	12.05	12.20
4	0	-2	0	11.69	11.00
5	0	0	2	11.82	12.28
6	-1	-1	1	10.77	10.89
7	-1	1	1	11.53	11.36
8	-1	-1	-1	10.25	11.79
9	1	1	-1	11.46	12.01
10	0	0	0	10.88	11.24
11	1	-1	1	10.02	11.06
12	-1	1	-1	9.68	10.69
13	2	0	0	11.73	10.88
14	0	0	0	6.82	7.51
15	0	0	0	7.01	7.51
16	1	-1	1	9.9	9.56
17	0	0	0	10.55	7.51
18	1	-1	-1	10.77	10.50
19	1	-1	-1	11.21	12.31
20	0	0	-2	13.24	12.55
21	-1	-1	1	12.54	11.95
22	-1	-1	-1	12.04	11.05
23	0	2	0	12.53	12.99
24	0	0	0	6.96	7.51
25	-2	0	0	11.14	11.76
26	1	1	1	11.36	11.91
27	1	1	1	12.64	11.77
28	-1	1	1	14.48	14.05
29	-1	1	-1	14.54	13.06
30	0	0	0	7	7.51

Table 4. Analysis of variance (ANOVA) for the experiment

ANOVA	
Std. Dev	1.25
Mean	10.74
Coefficient of variance	11.60
R^2	0.8149
Adjusted R^2	0.6421
Predicted R^2	0.3161
Adeq Precision	7.6052
PRESS	86.04
F-Value	4.72

Fig. 1. Pink zone formation by L-asparaginase producing *Fusarium* sp. LCJ273 on MCD agar media

The results of the experiments were estimated using statistical software Design Expert (version 10, Stat-Ease, Inc., Minneapolis, MN). The three independent parameters were studied at three different levels (-1, 0, +1) shown in Table. 2.

Thirty experiments were generated and all the parameters were taken at the central coded points to estimate the pure error. The minimum and maximum ranges of parameters were studied and the proper response to the independent parameters. The response of L-asparaginase production is presented in the following polynomial equation employed to fit the experimental data.

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b11X_1^2 + b22X_2^2 + b33X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

In this equation, Y is the predicted response (L-asparaginase production); b_1, b_2, b_3 linear coefficients; b_{11}, b_{22}, b_{33} squared coefficients; b_{12}, b_{13}, b_{23} interaction coefficients.

To validate the statistical model, the coefficients were designed by regression analysis and their significance level. L-Asparaginase production was subjected to Analysis of Variance (ANOVA) with statistic software program (version 10, Stat-Ease, Inc., Minneapolis, MN) suitable to the design of the experiments. The response surface curves were acquired for determining the finest

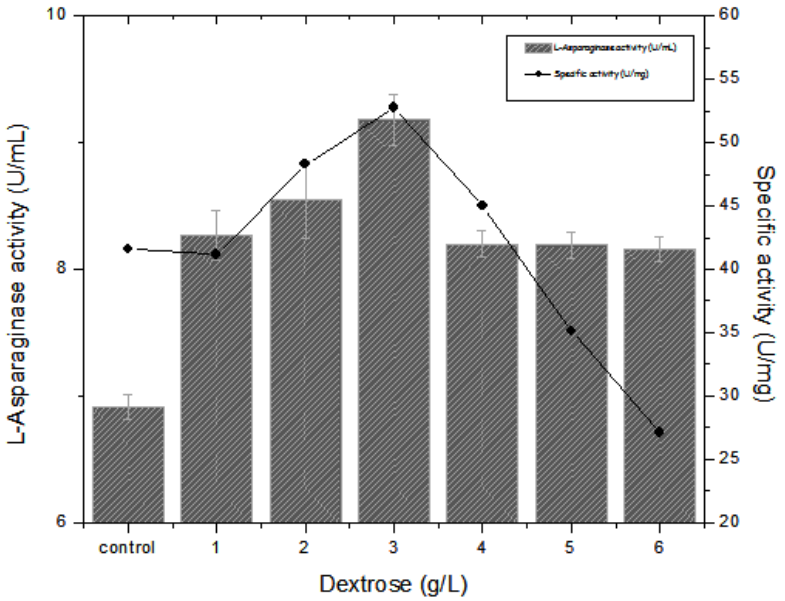

Fig. 2. Effect of different concentration of dextrose on the production of L-asparaginase by *Fusarium* sp. LCJ23 on the 5th day

levels of the parameters for high L-asparaginase production.

Original and optimized medium for L-asparaginase production in modified Czapek Dox medium

Comparison was done between original and optimized medium to prove the efficiency of the optimized medium in the L-asparaginase production by *Fusarium* sp. LCJ273. The mycelium of *Fusarium* sp. LCJ273 was inoculated in 1000 mL of original and optimized medium in separate set of experiments and incubated for 3-8 days. L-Asparaginase activity was determined by measuring the absorbance at 450 nm.

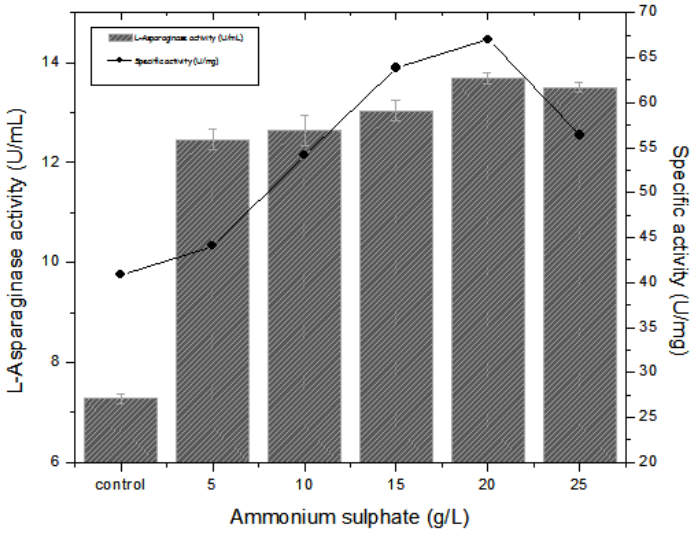

Fig. 3. Effect of different concentration of ammonium sulphate on the production of L-asparaginase by *Fusarium* sp. LCJ23 on the 5th day

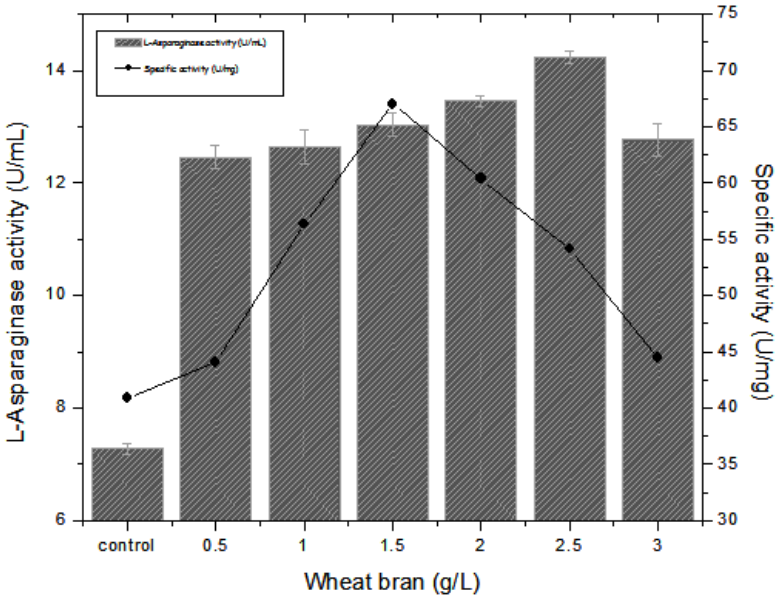

Fig. 4. Effect of different concentration of Wheat bran on the production of L-asparaginase by *Fusarium* sp. LCJ23 on the 5th day

RESULTS

Screening and identification of L-asparaginase producing endophytic fungi

L-Asparaginase activity was done using plate assay on Czapek's Dox media. Among the isolates while were positive, *Fusarium* sp. LCJ273 was considered as a highly potential strain (Fig 1). 18S rRNA gene sequence of the fungi has been deposited in GenBank under accession number KY238311.

Effect of carbon sources and their concentration on L-asparaginase in modified Czapek Dox medium

The results showed that, an addition of dextrose increased the L-asparaginase production when compared to the other carbon sources. L-Asparaginase activity of 7.06 U/mL was observed in dextrose (Table. 1). The effect of different concentration of dextrose from 0.5 to 6.0 g/L in MCDB was studied further.

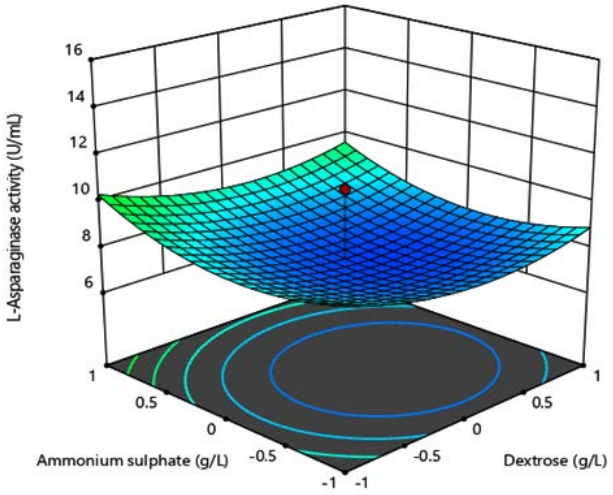

Fig. 5. Three-dimensional response surface plots showing the interaction between dextrose and ammonium sulphate on the production of L-asparaginase by *Fusarium* sp. LCJ273

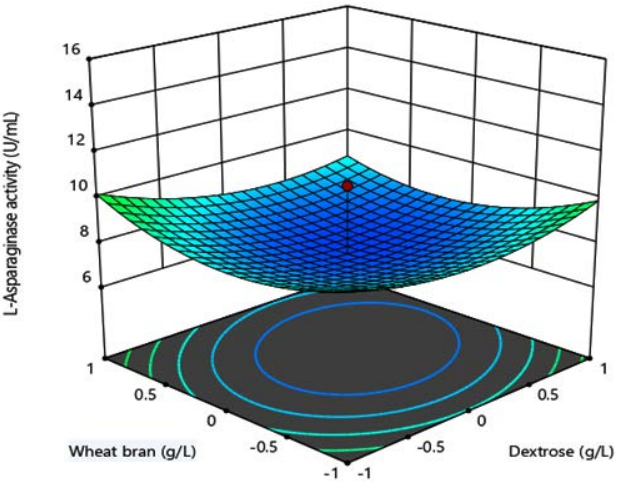

Fig. 6. Three-dimensional response surface plots showing the interaction between dextrose and wheat bran on the production of L-asparaginase by *Fusarium* sp. LCJ273

Highest L-asparaginase activity of 9.18 U/mL was observed at 3.0 g/L of dextrose on the 5th day of incubation Fig 2. Dextrose concentration above 3 g/L decreased L-asparaginase activity. Minimum L-asparaginase production was observed in glycerol and lactose. The maximum specific activity of L-asparaginase in *Fusarium* sp. LCJ273 was found to be 52.77 U/mg in dextrose.

Effect of nitrogen sources and their concentration on L-asparaginase in modified Czapek Dox medium

Ammonium sulphate was found to be best nitrogen source when compared to other nitrogen sources. Maximum L-asparaginase yield of 10.21 U/mL was observed in *Fusarium* sp. LCJ273 (Table 1). The effect of different concentration of ammonium sulphate (5 to 25 g/L) was further studied for maximizing the production of enzyme.

Maximum L-asparaginase yield of 13.69 U/mL was observed at 20 g/L of ammonium sulphate on 5th day of incubation (Fig 3). L-Asparaginase production decreased by the addition of yeast extract and peptone (organic nitrogen sources). The maximum specific

L-asparaginase activity by *Fusarium* sp. LCJ273 was 67.02 U/mg when in ammonium sulphate was used. L-Asparaginase activity decreased above 20 g/L ammonium sulphate.

Effect of natural inducers and their concentration on L-asparaginase in modified Czapek Dox medium

The results showed that addition of wheat bran favoured maximum

L-asparaginase production compared to other tested inducers. Higher L-asparaginase yield of 12.45 U/mL was observed in wheat bran (Table 1). The influence of different concentration of wheat bran from 0.5 to 3.0 g/L by *Fusarium* sp. LCJ273 in MCDB was further evaluated.

Maximum activity of 14.24 U/mL was observed at 2.5 g/L of wheat bran on 5th day of incubation (Fig. 4). L-Asparaginase activity decreased when the concentration of wheat bran was increased above 2.5 g/L. Minimum activity was observed in bagasse and paddy straw. The maximum specific activity of L-asparaginase in *Fusarium* sp. LCJ273 was found to be 67.02 U/mg in wheat bran. However, the medium contained 5 g/L of L-asparagine as a substrate.

Optimization of L-asparaginase production using response surface methodology

The influence of three parameters namely dextrose, ammonium sulphate and wheat bran was evaluated using response surface methodology. Both dextrose and ammonium sulphate were found to be highly significant (P-value=0.001) and also

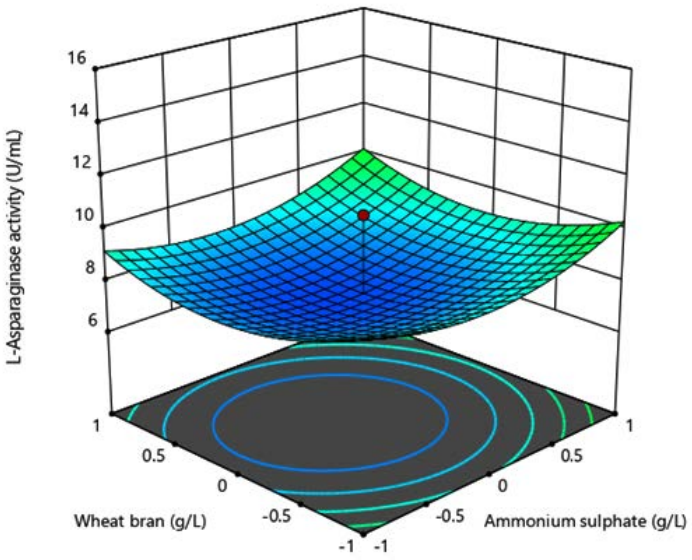

Fig. 7. Three-dimensional response surface plots showing the interaction between ammonium sulphate and wheat bran on the production of L-asparaginase by *Fusarium* sp. LCJ273

increased the L-asparaginase production. The interaction between wheat bran and dextrose, wheat bran and ammonium sulphate were significant as shown by the P-value ($P<0.05$). The results indicated that optimal concentration of A, B and C (dextrose, ammonium sulphate and wheat bran) were between 2 to 2.5 g/L.

The face centered central composite design used for studying the effects of three independent parameters namely dextrose, ammonium sulphate and wheat bran for L-asparaginase production are shown in Table 3 along with observed and predicted value. The regression equation coefficients were analysed by following equation:

$$Y= 102.53 - 2.18 X_1 - 4.10 X_2 - 0.5400 X_3 – 23.81 X_1X_2 + 33.21 X_2X_3 + 39.85 X_1X_3 - 0.0650 X_1^2 - 2.54 X_2^2 - 0.1089 X_3^2$$

Where Y, L-asparaginase production; X_1, X_2 and X_3 the coded levels of dextrose, ammonium sulphate and wheat bran. The results analysed using Analysis of Variance (ANOVA) are shown in Table 4. In this analysis the R^2 value was 0.81, which indicated 95% of the variability of the response for L-asparaginase production explained by the model. The coefficient of variance was 11.60. The adequate precision value was 7.6052. The Lack of Fit F-value of 4.72 suggests that the Lack of Fit is not significant. It identifies the model can be used to direct the design space for L-asparaginase production, ratio greater than 4.0 is desirable. The predicted sum of squares was 86.04 and this is used to measure the particular model.

Three-dimentional response surface graphs shown in Figures 5, 6, and 7 represents the response of L-asparaginase production, main effect, interaction effect and squared effect of three parameters such as dextrose, ammonium sulphate and wheat bran, which shows the best combination for production of L-asparaginase. Finally the highest yield of L-asparaginase was determined using dextrose (2 g/L), ammonium sulphate (25 g/L) and wheat bran (2 g/L). Maximum L-asparaginase activity 14.05 U/mL was predicted using this combination. The maximum enzyme activity reached in this experiment was 14.54 U/mL and also revealed that L-asparaginase activity decreased at the highest levels of these three factors. After statistical optimization, L-asparaginase yield increased to two times higher compare to original medium. Within the designed space, the model was validated for three parameters, predicted values were very close to observed value and the model was successfully verified.

Original and optimized medium for L-asparaginase production in modified Czapek Dox medium

The L-asparaginase production by *Fusarium* sp. LCJ273 in original medium was 6.91 U/mL. After optimization of medium components, the L-asparaginase yield was 14.24 U/mL. Thus a 2 fold increase was possible through optimization.

DISCUSSION

The anti-leukaemic activities of L-asparaginase have high medicinal value and also used for treating many diseases[22,23]. In the present study, *Fusarium* sp. LCJ273 was isolated from *Adhatoda vasica.* It was screened for L-asparaginase activity and optimization studies were done for maximum L-asparaginase production. Medium optimization was done under submerged fermentation using nutritional parameters specifically dextrose, ammonium sulphate and wheat bran. Dextrose and ammonium sulphate play a major role in synthesis of essential nutrients and promote growth of the organism. For maximizing L-asparaginase production nitrogen source play a vital role. In both submerged and solid state fermentation L-asparaginase production was done[24,25,26]. The formulation and optimization of medium is done for enhancement of microbial growth and L-asparaginase production in a large scale microbial fermentation processes[27]. Fungi are the most potent producers for L-asparaginase, which has the significant value [28].

In the present study, the dextrose and ammonium sulphate in the Czapex Dox medium were optimized for the L-asparaginase production by *Fusarium* sp. LCJ273. When dextrose is used as a carbon source, the production of L-asparaginase was 7.06 U/mL and the concentration of dextrose was further optimized in the range of 1 to 6 g/L. Dextrose at 3.0 g/L showed 9.18 U/mL of L-asparaginase activity using Czapek's Dox broth. Similarly in earlier studies glucose has resulted in high production of L-asparaginase at the concentration of 3.0 g/L[29,30,31]. Glycerol and lactose showed minimum production of L-asparaginase. Dextrose has been indicated as the best carbon

source when compared to other sources and also it is economical[32].

When ammonium sulphate is used as a nitrogen source, the production of L-asparaginase was maximum of 10.21 U/mL at the concentration of 20 g/L. The minimum activity of L-asparaginase was 2.46 U/mL observed when peptone was used as a nitrogen source. The various natural inducers such as paddy straw, coconut oil cake, bagasse, groundnut oil cake and wheat bran were selected and screened for L-asparaginase from *Fusarium* sp. LCJ273. The maximum yield of 13.29 U/mL was observed in the presence of wheat bran. L-Asparaginase production by *Fusarium* sp. LCJ273 has been studied using different nitrogen sources such as ammonium sulphate, ammonium nitrate, yeast extract and peptone[33,34]. Previous work also strongly suggested that wheat bran acts as a best substrate for the production of L-asparaginase[35]. L-asparaginase production was further optimized by using different concentration of wheat bran 0.5 to 3 g/L, 14.24 U/mL was observed at 2.5 g/L of wheat bran. Paddy straw and bagasse showed minimum activity of L-asparaginase.

Highest yield of L-asparaginase was observed by optimum combination of dextrose (3.0 g/L), ammonium sulphate (20 g/L) and wheat bran (2.5 g/L). L-Asparaginase production increases 2 fold after optimization process compared to un-optimized condition. The three variables such as dextrose, ammonium sulphate and wheat bran were selected and optimal level were identified using Response Surface Methodology (RSM)[36,37]. RSM is used to investigate the importance of independent variables at different level. Surface plots used to observe the significant interaction between three variables such as dextrose, ammonium sulphate and natural inducers, among them dextrose and ammonium sulphate acts as a best substrate.

In statistical optimization using Response Surface Methodology the yield of L-asparaginase was 14.54 U/mL, the medium composition was as follows dextrose 2 g/L, ammonium sulphate 25 g/L and wheat bran 2 g/L. There have been a number of studies conducted on statistical optimization using different carbon and nitrogen sources for the production of L-asparaginase[38,39,40]. In the present study, *Fusarium* sp. LCJ273 produces maximum L-asparaginase production using dextrose, ammonium sulphate, wheat bran and L-asparagine as substrate.

In conclusion, *Fusarium* sp. LCJ273 was isolated from the medicinal plant *Adhatoda vasica* showed maximum production of L-asparaginase using an optimized medium containing dextrose, ammonium sulphate along with natural inducer wheat bran. In this study,

L-asparaginase production was two times higher compared to the original medium

L-Asparaginase production from microbes is much cheaper compared to others and also enzyme has more importance in pharmaceutical industries. The study concentrates on the selection of different parameters such as carbon, nitrogen and natural inducer, would influence cost-effective production of L-asparaginase. However, further studies on purification of *Fusarium* sp. LCJ273 will help to extent its application in various industries.

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