OPTIMIZATION OF SUBMERGED STATE FERMENTATION PROCESS FOR TERREIC ACID PRODUCTION APPLYING BIOSTATISTICAL TOOLS

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ABSTRACT

A quinine epoxide called terreic acid a secondary metabolite produced by the fungus *Aspergillus terreus*, is considered to be the "next generation antibiotic" due to its broad range antibiotic specificity. The antibiotic property of terreic acid was recognized more than 60 years ago. Previously many researchers were attempted different techniques and conditions for obtaining terreic acid. Very scanty research has been carried out on the optimization of SmF process for the production of terreic acid. Mathematical designs and biostatistical tools were never been used in the process optimization studies. In the present investigation, the focus is on optimization of various nutrients factors of *Aspergillus terreus* MTCC-11395; SmF cultures, namely Dextrose, Sucrose, Starch, Mannitol, Yeast extract, Dried yeast, L-tyrosine, Acetic acid, Malt extract, Sodium nitrate and process parameters such as pH, Agitation (Rpm), Temperature, Inoculum volume, Fermentation time considering, Agar (dummy1), Agarose (dummy2), Mineral oil (dummy3) and Water (dummy4) as a four dummy variables, for enhanced production of terreic acid applying Plackett-Burman design (PBD) and Response Surface Methodology (RSM). The terreic acid in the fermented broth was confirmed by bioassay and estimated through UV spectrophotometry (214nm). PBD identified Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume were the principal factor influencing the production of terreic acid (0.463 mg/ml). Further, PBD identified principle factors were optimized applying Central Composite Design (CCD) of Response Surface Methodology (RSM). An optimized medium containing 65 g/L of sucrose, 1 g/L of L-tyrosine, Agitation 180 RPM and 15% of inoculum volume was found to support high yield (0.620 mg/L) of terreic acid under SmF process.

Keywords: *Aspergillus terreus*, terreic acid, Submerged fermentation process, Plackett-Burmann design, Response Surface Methodology, Bioassay

INTRODUCTION

Antibiotics are a class of drugs used to prevent the growth and proliferation of microorganisms[1,2,3]. Antibiotics can be classified based on their source of origin, structure and mechanism of action[4]. Antibiotics form an important class of metabolites, can be obtained in several forms and are used for the treatment and diagnosis of several types of diseases[5]. Several types of acids, phenols and aromatic compounds can also be used as antibiotics[6]. Recently bacteriocines produced by lactic acid bacteria were identified with brand range of antimicrobial activity[7,8]. Antimicrobial resistance is an increasingly problematic issue that leads to millions of deaths every year[9]. A few infections become completely untreatable due to the antibiotic resistance[6]. A World Health Organization (WHO) report released April 2014 stated, "this serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country[10], stating the urge of finding new antibiotics. Fungi are accomplished chemists that produce a wide range of complex organic molecules with important applications in the pharmaceutical industry[11]. The biological properties of these compounds and the availability of 13C labeling make fungal metabolites good candidates for studying biosynthetic pathways exploitations using NMR[12].

The fungus *Aspergillus terreus* has a worldwide distribution in different soils and known to produces several metabolites such β-lactam antibiotics penicillin, cephalosporin, aniticholesterol drug lovastatin[13,14], antifungal antibiotic griseofulvin, antibacterial compounds terrinol and terreic acid[15]. Terreic acid is a metabolite with antibiotic properties, produced by the fungus *Aspergillus terreus*[16]. The antibiotic properties of terreic acid were recognized more than 60 years ago, but its cellular and molecular modes of action remained obscure. Chemically, terreic acid is a quinine epoxide, therefore sharing with fosfomycin a potential reactivity towards

nucleophiles[17]. Terreic acid found to inhibit the enzymatic activity of Bruton's tyrosine kinase (Btk) in mast cells, cell-free assays and functions as an immunomodulator and known to show the properties of antinflammation both invivo and invitro and in its combined form is being used as a component for several antinflammatory drugs[18]. Terreic acid inhibits the growth of *C. albicans* AS2 at low concentration and has fungicidal action at high concentration[19]. Terreic acid is shown to be a potent and irreversible inhibitor of acetylcholinesterase (AChE)[20]. The molecular formula for terreic acid is found to be $C_7H_6O_4$. It comprises of two oxygenated methyne protons and one methyl group attached to a quarternary carbon. The fifth and sixth carbon atoms are attached to an oxygen atom forming cis epoxide[21]. Terreic acid had higher potency of hydrogen peroxide radical scavenging activity than terremutin and similar with ascorbic acid. The antioxidant property of terreic acid is studied using a lipophillic free radical, derived from the DPPH compound[22].

Very scanty research has been carried out on the isolation and extraction of terreic acid. Previously many techniques and conditions were used for obtaining terreic acid. Mathematical designs and statistical tools were never used in the process optimization of terreic acid production. Optimization techniques like Placket Burman Design and Response Surface methodology gives a detailed study of several factors and their dependence on the yield of terreic acid. This study further helps in the design of the media with equal distribution of several physical and process parameters, which aids in achieving the enhanced yield. Media optimization experiments with the *Aspergillus* species showed that glucose and sucrose are the best carbon source for terreic acid production.

In the present investigation, the focus is on optimization of various nutrients factors of *Aspergillus terreus* MTCC-11395; SmF cultures, namely Dextrose, Sucrose, Starch, Mannitol, Yeast extract, Dried yeast, L-tyrosine, Acetic acid, Malt extract, Sodium nitrate and process parameters such as pH, Agitation (Rpm), Temperature, Inoculum volume, Fermentation time considering, Agar (dummy1), Agarose (dummy2), Mineral oil (dummy3) and Water (dummy4) as a four dummy variables, for enhanced production of terreic acid applying Plackett-Burman design (PBD) and Response Surface Methodology (RSM). The terreic acid in the fermented broth was confirmed by bioassay and estimated through UV spectrophotometry (214nm). PBD identified Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume were the principal factor influencing the production of terreic acid (0.463 mg/ml). Further, PBD identified principle factors were optimized applying Central Composite Design (CCD) of Response Surface Methodology (RSM). An optimized medium containing 65 g/L of sucrose, 1 g/L of L-tyrosine, Agitation 180 RPM and 15% of inoculum volume was found to support high yield (0.62 mg/L) of terreic acid under SmF process.

MATERIALS AND METHODS

All the chemicals and reagents used in this study were of analytical grade (Merck, India).

Screening of selected microorganism: The culture of newly identified strain[23]; *Aspergillus terreus*-MTCC 11395 was obtained from Microbial Type Culture Collection (IMTech-Chandigarh) and host culture was revived on the potato dextrose agar (PDA) slants and stored at 4°C. Further revived fungal isolate was cultured on PDA plates. The fully grown culture of the selected fungal isolate was studied morphologically (Form, Margin, Elevation, Surface Texture and Color) and microscopically (hyphae type, conidia size, shape and color) using light microscopy at 100 X magnification applying fungal specific lacto phenol cotton blue staining[24].

Plackett-Burman experimental design: Dextrose, Sucrose, Starch, Mannitol, Yeast extract, Dried yeast, L-tyrosine, Acetic acid, Malt extract, Sodium nitrate were the ten medium constituents, pH, Agitation (Rpm), Temperature, Inoculum volume, Fermentation time were the five process parameters, considering, Agar (dummy1), Agarose (dummy2), Mineral oil (dummy3) and Water (dummy4) as a four dummy variables a total of 19 parameters were selected for the study. The Plackett-Burman experimental design for 19 variables (Fig 1), i.e. ten nutritional components, five process parameters and four dummy variables, were used to evaluate the impact on the enhanced production of terreic acid. Data analysis was carried out by the standard procedure of Plackett-Burman experimental design along with the design expert software (8.0.7.1)[25, 26].

Design Summary

File Version	8.0.7.1		
Study Type	Factorial	Runs	20
Design Type	Plackett Burman	Blocks	No Blocks
Center Points	0		
Design Model	Main effects	Build Time (ms)	1.49

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Coded		Value	Mean	Std. Dev.
A	Dextrose	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
B	Sucrose	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
C	Starch	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
D	Mannitol	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
E	Dummy 1	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
F	Dried Yeast	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
G	Malt Extract	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
H	L-Tyricine	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
J	Acetic Acid	ml/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
K	Dummy 2	ml/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
L	Yeast Extract	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
M	pH		Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
N	RPM		Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
O	Dummy 3	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
P	Temperature	0 C	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
Q	Inoculum volume	%	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
R	Sodium nitrate	g/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
S	Fermentation time	Days	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	
T	Dummy 4	ml/l	Numeric	Continuous	-1.00	1.00	-1.000=-1.00	1.000=1.00	0.00	1.00	

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y1	yield	mg/ml	20	Factorial	0.099	0.463	0.264145	0.131559	4.67677	None	Mean

Fig.1. Plackett-Burmann experimental design of design expert (8.0.7.1) showing the 19 selected variables.

Optimization by Response Surface Methodology design: The screened and identified factors of PBD; Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume, were optimized using Response Surface Methodology for the enhanced yield of terreic acid in the SmF culture of *Aspergillus terreus* MTCC-11395. A four factor RSM design was generated with the Design- Expert 8.0.1.7 software. The model applied was central composite design (CCD) and a second order polynomial response equation gives the yield of terreic acid. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as-1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Optimization studies were carried out as a batch experiments based on CCD of RSM as shown in Table 2 within the 250 ml conical flask with the volume of 100 ml as a production media[27,28].

Downstream processing of terreic acid: At the end of fermentation process, pH of the final fermentation broth was measured and adjusted to 2.0 using dilute HCl (1N). Acidified broth was filtered using Whatman no 1filter paper. Filtered broth was extracted separately with the volumes of 1:2 ratios ethyl acetate in a shaker incubator (220 rpm) at 36^{o}C for 2 hours. Solvent layer (organic phase) was separated by centrifugation at 45000 rpm for 8 min. The separated organic layer was subjected to evaporation at 45˚C for 2hrs. The crystals (pale yellow) obtained were dissolved in acetonitrile (5ml) and storage at -20˚C [29].

Qualitative analysis of Terreic acid by Bioassay: *Bacillus subtilis* and *E.coli* cultures were uniformly (carpet culture) grown on LB agar plates. Discs dipped in extracted samples were placed on the LB agar plates; ethyl acetate was used as a negative control. These plates were incubated at 37°C, for 18-24 hours. The zone of inhibition was observed and diameter measured[15,30].

UV Spectrophotometric analysis of lovastatin: The presence of terreic acid in prepared sample were qualitatively analyzed using UV Spectrometric scan (Shimadzu, Model no UV-2450 and Software UV-probe 2.21) between 200nm–320nm and subsequently estimated at 214 nm, using pure terreic acid (sigma-Aldrich) as a standard [31] .

Results

Screening of selected microorganism: Morphological and microscopic screening of the selected fungal cultures is an important parameter to assess the growth stability and purity of the revived fungal cultures. The morphological properties of newly identified strain; *Aspergillus terreus*-MTCC 11395 on PDA medium was shown in Fig 2. The microscopic studies of the selected fungi explained that the *Aspergillus terreus* 11395 exhibits hyaline, septate hyphae and smooth, elliptical conidia form long chains with biseriate phialides. The

results explained that the revived culture selected for the strain improvement methodologies was of pure in culture and study in growth

Fig.2. Morphological and microscopic observation of *Aspergillus terreus* MTCC-11395 culture. *2A) Fungal culture on PDA plates showing cinnamon brown colour colonies. 2B) septate hyphae with biseriate phialides (100X)*

Plackett-Burman experimental design: The Plackett Burman design identified Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume, were the principal factor influencing the production of terreic acid. The effects of various nutritional factors and process parameters on terreic acid production based on the observations of Plackett- Burman design experiments were shown in (Table 1). Results showed that the main parameters influencing the production of the terreic were found to be Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume with high *F* values (0.180, 0.577, 1.137 and 5.1648 resp.).

Table 1. Plackett-Burman experimental design observations of terreic production.

Slno	Factor	E (H)	E (L)	Mean square	E value	F test	% of contribution
1.	Sucrose	2.461	2.821	0.00065	-0.036	0.180	2.00
2.	Dextrose	2.701	2.4919	0.00021	0.02	0.06	0.70
3.	Starch	2.6439	2.639	0.00003	0.0004	0.006	0.07
4.	Mannitol	2.6859	2.598	0.00004	0.0003	0.010	0.11
5.	Dummy 1	2.6832	2.426	0.0005	0.025	0.013	0.15
6.	Dried Yeast	2.4339	2.386	0.000014	0.0004	0.0003	0.003
7.	Malt Extract	2.569	2.7139	0.00014	-0.014	0.029	0.33
8.	Acetic acid	2.7559	2.527	0.000002	0.02	0.0004	0.004
9.	L-tyrosine	3.021	2.3762	0.0200	0.06	0.577	6.7
10.	Dummy 2	3.418	2.858	0.0156	0.056	0.43	5.03
11.	Yeast Extract	2.328	2.9549	0.000019	-0.06	0.000045	0.0005
12.	pH	2.315	2.9679	0.021	-0.065	0.592	6.9
13.	RPM	2.189	3.0939	0.04	-0.09	1.137	13.30
14.	Dummy 3	2.984	2.2929	0.023	0.069	0.011	0.128
15.	Temperature	2.8919	2.411	0.011	0.04	0.321	3.757
16.	Inoculum volume	2.3498	2.781	-0.043	-0.043	5.1648	60.45
17.	Sodium nitrate	2.702	2.8449	0.0009	-0.013	0.026	0.30
18.	Fermentation time	2.5889	2.694	0.0005	-0.10	0.015	0.1755
19.	Dummy 4	2.4789	2.789	0.0004	-0.031	0.011	0.128

Optimization by Response Surface Methodology design: Based on the experimental results of CCD in Table 2, a quadratic polynomial was established to identify the relationship between adsorption capacity and process variables. Final Equation in terms of coded factors represents the yield of terreic acid Fig.3.

Final Equation in Terms of Actual Factors:

Yields =
+17.81994
-0.25347 * Sucrose
-7.92394 * L-Tyrosine
-0.097181 * RPM
+0.10680 * Inoculum volume
+0.054139 * Sucrose * L-Tyrosine
+1.37375E-003 * Sucrose * RPM
-8.17500E-004 * Sucrose * Inoculum volume
+0.030000 * L-Tyrosine * RPM
-6.61111E-003 * L-Tyrosine * Inoculum volume
-2.60000E-004 * RPM * Inoculum volume

Fig.3. Final Equation in terms of coded factors representing the yield of terreic acid

Table 2. CCD RSM desing table representing the observation of the procces terreic acid yield

Std	Run	Factor 1(SU)	Factor 2 (LT)	Factor 3 (RPM)	Factor 4(IV)	Yield g/l
4	1	65.00	1.00	140.00	5.00	0.41
3	2	45.00	1.00	140.00	5.00	0.325
15	3	45.00	1.00	180.00	15.00	0.5
20	4	55.00	1.45	160.00	10.00	0.511
17	5	35.00	0.55	160.00	10.00	0.315
10	6	65.00	0.10	140.00	15.00	0.421
22	7	55.00	0.55	200.00	10.00	0.456
18	8	75.00	0.55	160.00	10.00	0.3
11	9	45.00	1.00	140.00	15.00	0.435
24	10	55.00	0.55	160.00	20.00	0.335
6	11	65.00	0.10	180.00	5.00	0.398
23	12	55.00	0.55	160.00	0.00	0
29	13	55.00	0.55	160.00	10.00	0.413
21	14	55.00	0.55	120.00	10.00	0.378
27	15	55.00	0.55	160.00	10.00	0.413
26	16	55.00	0.55	160.00	10.00	0.413
2	17	65.00	0.10	140.00	5.00	0.345
19	18	55.00	-0.35	160.00	10.00	0.175
13	19	45.00	0.10	180.00	15.00	0.254
30	20	55.00	0.55	160.00	10.00	0.413
7	21	45.00	1.00	180.00	5.00	0.289
1	22	45.00	0.10	140.00	5.00	0.002
16	**23**	**65.00**	**1.00**	**180.00**	**15.00**	**0.62**
9	24	45.00	0.10	140.00	15.00	2.57
28	25	55.00	0.55	160.00	10.00	0.413
14	26	65.00	0.10	180.00	15.00	0.435
5	27	45.00	0.10	180.00	5.00	0.157
12	28	65.00	1.00	140.00	15.00	0.523
8	29	65.00	1.00	180.00	5.00	0.512
25	30	55.00	0.55	160.00	10.00	0.413

The experimental results and the predicted values obtained from the model equation were compared. The Model F-value of 3.28 implies the model is significant. There is only a 1.25% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" (less than 0.0500) indicate; A, B ,C, D, AB, AC, AD, BC, BD, and CD were significant model terms. The "Lack of Fit F-value" of 0.19 implies the Lack of Fit is not significant relative to the pure error (0.000) Table 3.

Table 3. ANOVA for Response Surface 2FI Model of CCD RSM of terreic acid production

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4.67	10	0.47	3.28	0.0125	Significant
A-Sucrose	*0.35*	*1*	*0.35*	*2.45*	*0.1338*	
B-L-Tyrosine	*0.22*	*1*	*0.22*	*1.54*	*0.2299*	
C-RPM	*0.57*	*1*	*0.57*	*4.02*	*0.0594*	
D-Inoculum volume	*0.17*	*1*	*0.17*	*1.16*	*0.2949*	
AB	*0.95*	*1*	*0.95*	*6.66*	*0.0183*	
AC	*1.21*	*1*	*1.21*	*8.48*	*0.0090*	
AD	*0.027*	*1*	*0.027*	*0.19*	*0.6698*	
BC	*1.17*	*1*	*1.17*	*8.19*	*0.0100*	
BD	*3.540E-003*	*1*	*3.540E-003*	*0.025*	*0.8764*	
CD	*0.011*	*1*	*0.011*	*0.076*	*0.7859*	
Residual	2.71	19	0.14			
Lack of Fit	*2.71*	*14*	*0.19*			
Pure Error	*0.000*	*5*	*0.000*			Insignificant
Cor Total	7.38	29				

Qualitative analysis of Terreic acid by Bioassay: Bioassay of SmF extracted samples (terreic acid) on *Bacillus subtilis* and *E.coli* (LB agar) plate cultures were shown on Fig 4.

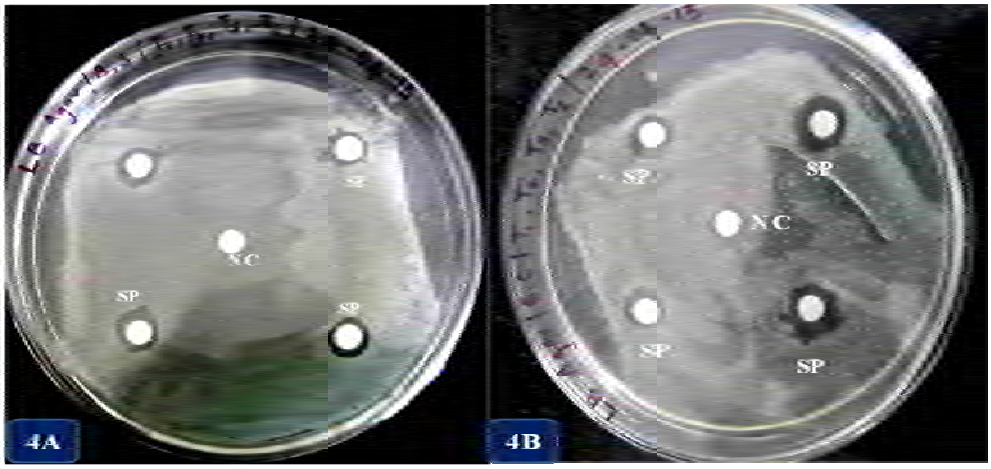

Fig.4. Bioassay of SmF extracted samples (SP) exhibiting zone on inhibition, NC represents negative control
4A). Bacillus subtilis culture plate showing zone on inhibition. 4B). E.coli culture plate showing zone on inhibition

UV Spectrophotometric analysis of lovastatin: The concentration of terreic acid was calculated spectrophotometrically (three replicates). The samples and the standard exhibited a peak at 214 nm in the spectrophotometer scanning (200nm-320nm). Terreic acid in the sample was subsequently estimated at 214nm using terreic acid standard obtained from (Sigma Aldrich-India). The results explained that the yield of terreic acid after PBD (Fig.5) was found to be (0.463 mg/l) and after optimization of PBD screened principle factors through CCD RSM the terreic acid yield was enhanced (0.62 mg/l), reporting the 1.3 fold raise compare to the yield of PBD.

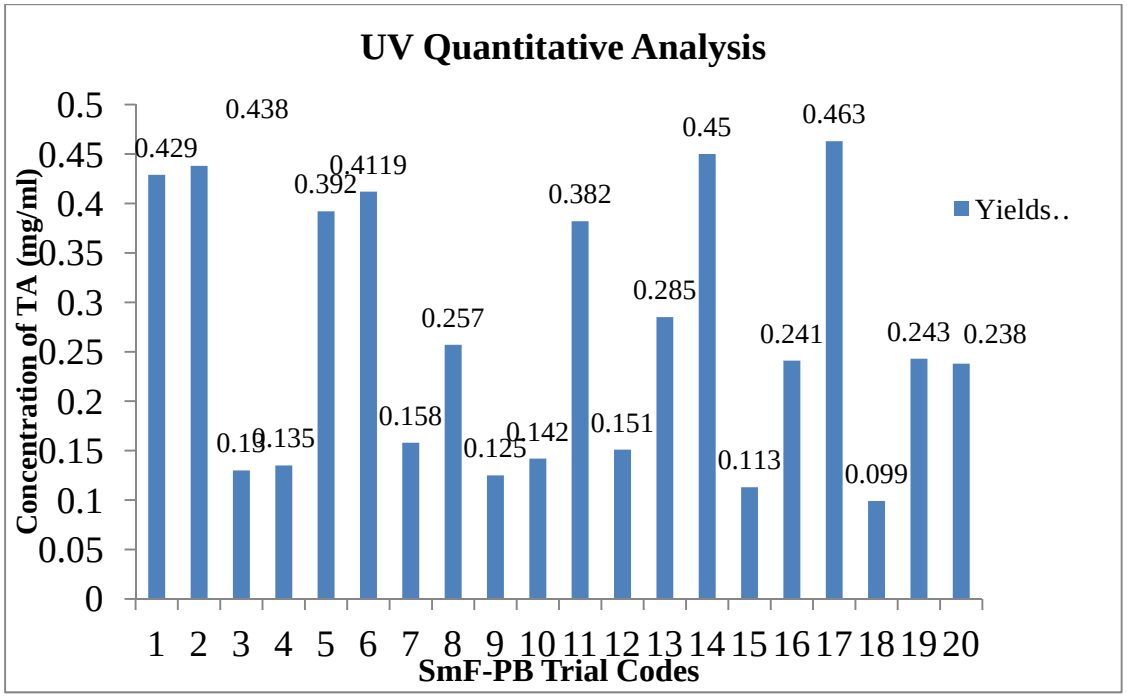

Fig.5. Bar charts representing the UV quantitative analysis of PBD samples

DISCUSSION

Terreic acid a secondary metabolite produced by the fungus *Aspergillus terreus*, is considered to be the "next generation antibiotic" due to its broad range antibiotic specificity. In the present investigation, the focus is on optimization of various nutrients factors of *Aspergillus terreus* MTCC-11395; SmF cultures, namely Dextrose, Sucrose, Starch, Mannitol, Yeast extract, Dried yeast, L-tyrosine, Acetic acid, Malt extract, Sodium nitrate and process parameters such as pH, Agitation (Rpm), Temperature, Inoculum volume, Fermentation time considering, Agar (dummy1), Agarose (dummy2), Mineral oil (dummy3) and Water (dummy4) as a four dummy variables, for enhanced production of terreic acid applying Plackett-Burman design (PBD) and Response Surface Methodology (RSM). The terreic acid in the fermented broth was confirmed by bioassay and estimated through UV spectrophotometry (214nm). PBD identified Sucrose, L-tyrosine, Agitation (rpm) and Inoculum volume were the principal factor influencing the production of terreic acid (463 mg/l). Further, PBD identified principle factors were optimized applying Central Composite Design (CCD) of Response Surface Methodology (RSM). An optimized medium containing 65 g/L of sucrose, 1 g/L of L-tyrosine, Agitation 180 RPM and 15% of inoculum volume was found to support high yield (620 mg/L) of terreic acid under SmF process. Temperature as a factor in the elaboration of mycotoxins by two fungi in groundnut fodder was investigated and the study reported 77 mg/l of terreic acid yield, which was 8, fold lesser than the yield of present optimization study. Isolation of Antioxidant compounds from *Aspergillus terreus* LS01 cultures was investigated and the study reported 150 mg/l of terreic ac id yield[22], compared present study reported approximately 4 fold higher terreic acid (620 mg/L).

CONCLUSION

Considering these references as discussed above, it could be concluded that *Aspergillus terreus* MTCC 11395 SmF culture was optimized successfully and reported enhanced terreic acid yield. It can be also seen that the present investigation led to explore the application of mathematical design (PBD) and biostatistical tools (RSM) on bioprocess optimization for biomolecule production.

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REFERENCE

[1] Utilizing antibiotics agents effectively will preserve present day medication". News Ghana. 21 November 2015. Retrieved 21 November 2015.
[2] Antibiotics. NHS. 5 June 2014. Retrieved 17 January 2015.
[3] Factsheet for experts. European Centre for Disease Prevention and Control. Retrieved 21 December 2014.
[4] http://www.orthobullets.com/basic-science/9059/antibiotic-classification-and mechanism.
[5] Antibiotics Simplified. Jones & Bartlett Publishers. 2011. pp. 15 17.ISBN 9781449614591.
[6] https://en.wikipedia.org/wiki/Phenols.
[7] Khandelwal, P., Gaspar, F. B., Crespo, M. T. B., and Upendra, R. S. (2015). 'Lactic acid bacteria General Characteristics, Food preservation and Health Benefits'. In Lactic Acid Bacteria. Fermented Foods, Part I: Biochemistry and Biotechnology, CRC-press, pp. 127-147.
[8] Upendra R.S., Pratima Khandelwal, Khushali Jana, N. Ajay Kumar, Gayathri Devi M, Mary Linda Stephaney (2016): Bacteriocin production from indigenous strains of lactic acid bacteria isolated from selected fermented food sources, International Journal of Pharma Research and Health Science, Accepted.
[9] Antibiotic Resistance Questions & Answers". Get Smart: Know When Antibiotics Work. Centers for Disease Control and Prevention. 30 June 2009. Retrieved 20 March 2013.
[10] WHO (April 2014). "Antimicrobial resistance: global report on surveillance 2014". WHO. Retrieved 9 May 2015.
[11] Abid Ali Khan, Nafees Bacha, Bashir Ahmad, Ghosia Lutfullah, Umar Farooq, Russell John Cox (2014): Fungi as chemical industries and genetic engineering for the production of biologically active secondary metabolites. Asian Pacific Journal of Tropical Biomedicine, 4(11): 859–870.
[12] http://pub.epsilon.slu.se/10819/1/hansson_d_130925.pdf.
[13] R.S.Upendra, Pratima Khandelwal, Amiri Z R, Swetha L and Mohammed Ausim S (2013): Screening and Molecular Characterization of Natural Fungal isolates Producing Lovastatin. Journal of Microbial and Biochemical Technology, 5(2):025-030.
[14] Upendra R. S, Pratima Khandelwal, Z. R. Amiri, Aparna. S, Archana. C and Ashwathi. M (2014): Isolation and Characterization of lovastatin producing food grade fungi from oriental foods. World Journal of Pharmaceutical Research, 3(10): 1404-1414.
[15] Yi Wang , Jinkai Zheng, Peipei Liu, Wei Wang and Weiming Zhu(2011): Three New Compounds from Aspergillus terreus PT06-2 Grown in a High Salt Medium. Marine Drugs, 9: 1368-1378.
[16] Olesen SH[1], Ingles DJ, Yang Y, Schönbrunn E (2014): Differential antibacterial properties of the MurA inhibitors terreic acid and fosfomycin. Journal of Basic Microbiology. 54(4):322-6.
[17] Han, H., Yang, Y., Olesen, S. H., Becker, A., Betzi, S., & Schönbrunn, E. (2010). The Fungal Product Terreic Acid is a Covalent Inhibitor of the Bacterial Cell Wall Biosynthetic Enzyme UDP-N-acetylglucosamine 1-carboxyvinyltransferase (MurA). Biochemistry, 49(19), 4276–4282.
[18] Kawakami, Y., Hartman, S. E., Kinoshita, E., Suzuki, H., Kitaura, J., Yao, L., Kawakami, T. (1999). Terreic acid, a quinone epoxide inhibitor of Bruton's tyrosine kinase. Proceedings of the National Academy of Sciences of the United States of America, 96(5), 2227–2232.
[19] http://journals.im.ac.cn/actamicrocn/ch/reader/view_abstract.aspx?file_no=07030447.
[20] Čolović, M. B., Krstić, D. Z., Lazarević-Pašti, T. D., Bondžić, A. M., & Vasić, V. M. (2013). Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Current Neuropharmacology, 11(3), 315–335.
[21] https://pubchem.ncbi.nlm.nih.gov/compound/Terreic_acid.
[22] Dewi RT, Tachibana S, Itoh K, Ilyas M (2012) Isolation of Antioxidant Compounds from Aspergillus Terreus LS01. Journal of Microbial& Biochemical Technology, 4: 010-014.
[23] Upendra, R.S and Pratima, K (2013): Isolation and molecular characterization of natural fungal isolates producing lovastatin. Organism- Aspergillus terreus SSM3. GenBank, JX419386.
[24] Upendra R.S., Khandelwal P and Mohammed Ausim: A novel approach for enhancement of Lovastatin production using Aspergillus species. Proceedings of the III International conference on SUPBT-2012.pp.135-140.
[25] Plackett RL, Burman JP (1946): The design of optimum multi factorial experiments. Biometrika, 33: 305–325.
[26] Upendra RS, Khandelwal P and Mohammed Ausim (2013b): A novel approach for enhancement of Lovastatin production using Aspergillus species. International Journal of Agriculture Environment and Biotechnology, 6:779-786.
[27] Upendra R.S, Pratima Khandelwal, Amiri Z.R., Amulya Achar, Kumari B.G., Sowmaya M. and Tejaswini J (2015): Optimization of fluoride removal system using Ocimum sp. Leaves and ragi seed husk by applying bio-statistical tools. Journal of Environmental Research And Development, 9 (4): 1109-1116.
[28] Upendra R.S, Pratima Khandelwal, Z.R. Amiri and Baboo M. Nair (2014a): Invited session key lecture on "Optimization of process for producing lovastatin from novel fungal isolates". Proceedings of the IV International conference on SUTPBM-2014: 28-34.
[29] Kawakami, Y., Hartman, S. E., Kinoshita, E., Suzuki, H., Kitaura, J., Yao, L., Kawakami, T(1999): Terreic acid, a quinone epoxide inhibitor of Bruton's tyrosine kinase. Proceedings of the National Academy of Sciences of the United States of America, 96(5), 2227–2232.
[30] Upendra R.S, Pratima Khandelwal (2012): Assessment of nutritive values, phytochemical constituents and biotherapeutic potentials of Epiphyllum oxypetalum. International Journal of Pharmacy and Pharmaceutical Science, 4(5), 421-425.
[31] http://www.drugfuture.com/chemdata/terreic-acid.html.
[32] Saini; Kalyani; Surekha; Reddy (2011): Temperature as a factor in the elaboration of mycotoxins by two fungi in groundnut fodder. International Journal for Biotechnology and Molecular Biology Research