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Improvement of antimicrobial metabolites by *Saccharothrix flava* VSM-3 using full factorial design and chemotype analysis

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ABSTRACT

Bioactive metabolite production by marine *Saccharothrix flava* VSM-3was modeled by response surface methodology (RSM) statistical optimization, and kinetic parameter estimation was executed using unstructured models to depict the importance of growth-associated metabolite production. RSM-based optimization of the variables and their interactions was analyzed where the modeled data and experimental data are in concurrence and better responses were yielded in terms of inhibition zones for active metabolite with good regression coefficients. The regression model developed the significance of five variables and their influence on the bioactive metabolite production and its effect against the responses. Logistic, Luedeking–Piret equations were used for batch fermentation to produce bioactive metabolites by *S. flava* VSM-3, where the anticipated parameter data followed experimental data. Chemotype (using ethyl acetate extract) analysis of actinobacterial isolate *S. flava* was elucidated for the first time by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) analysis. The main compounds identified in the positive ion mode were 7-Deazaadenosine, 5-Hydroxy-9-Methylstreptimidone, Amiclenomycin, Dihydroabikoviromycin, Epopromycin A, OAP Silane 55 and MKN-003B. In the present study, maritime silt specimen of Bay of Bengal comprising *S. flava* VSM-3 recorded prominent broad-spectrum activity against various plant pathogens and LC-QTOF-MS data also supported VSM-3 was the most active strain. This study also reveals that under-explored Bay of Bengal of north coastal Andhra Pradesh should be continuously explored for extracting bioactive compounds from diverse strains.

INTRODUCTION

Biotechnology mediation in the aquatic habitat has unbolted fresh horizons to isolate new organisms that are a powerful source of bioactive compounds. Actinobacterium is an effective ingredient of microbial populations and establishes balanced and continuous populations in multiple marine habitat ecosystems, and is a significant source of new secondary bioactive metabolites (Cumsille *et al*., 2017; Das *et al*., 2006; Girão *et al*., 2019; Mohammadipanah and Wink, 2016 ; Ramesh and William, 2012). The uncaged marine sediments contain a broad range of novel microorganisms, showing a varied and unexploited repository of microorganisms, is essential in obtaining a complex array of innovative metabolic substances and income producing a discovery platform for the pharmaceutical industry (Baltz, 2007; Coutinho *et al*., 2018; Genitsaris *et al*., 2016 ; Stach *et al*., 2003; Sponga *et al*., 1999). Actinobacterium is an important marine bacterial source for its ability to produce complex commercial lead molecules (Imada, 2005). Actinobacterium lives in distinct extreme settings, is better documented for its capacity to secrete natural compounds with architectural complication and varied biological actions, and is deemed priceless commercially significant prokaryote that is capable of producing antibiotics, anti-tumor agents, immunosuppressive agents, and enzymes (Abdelmohsen *et al*., 2014; Shuvankar *et al*., 2015).

Optimization of the physico-chemical parameters along with the media constituents is crucial for high yields

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of the antimicrobial metabolites (Deka and Jha, 2018; Linda and Rabab, 2017 ; Ripa *et al*., 2009). Conventional methods for optimization involve changing one independent variable, while other factors are kept constant using one factor at a time that is time-intensive, overpriced for a full total of variables, and cannot evaluate the connected impact of all variables and draw false findings (Marimuthu *et al*., 2009). Response surface methodology (RSM) investigates and approximates the unknown function using experimentation, modeling, and data analysis (Box and Wilson, 1951; Heinze *et al*., 2018). RSM is a mixture of statistical and mathematical approach to experiment design, model building, analysis of the interactive impacts of multiple nonpartisan variables against answers (Kishore and Kayastha, 2012).

A mathematical model is set for hypotheses concerning a natural phenomenon translated into mathematical expressions. The experimental data obtained generally confirm the predictions based on these models. Mathematical models not only provide a complete knowledge of dynamic behavior but also optimize and control most of the fermentation processes. Microbiologists recognize the importance of models when created unstructured models mimic bioactive metabolite manufacturing profiles and describe the kinetic interactions between substrate, product, and biomass. Batch mode operated responses are hard to model due to the time and features of cell processes, which often result in nonlinearities. In unstructured models that provide a precise description of whole fermentation, only complete cell levels are considered. Parameter estimation is a critical step for model verification, and consequently, these mathematical models are effectively implemented to study the manufacturing of a multitude of bioproducts (Rama Krishna *et al*., 2016; Ushakiranmayi *et al*., 2016).

Liquid chromatography coupled with mass spectrometry is a powerful device for the evaluation of active constituents of actinobacteria because of its high sensitivity, selectivity, and rapid screening ability. The improvement of LC-MS/MS analytical technique requires optimization of both chromatographic and mass spectrometric conditions (Ouakhssase *et al*., 2019). Once the approach is advanced, it must be proven for selectivity, sensitivity, linearity, accuracy, and precision. In the present study, the morphologically distinct potent actinobacterial isolate, VSM 3, was selected from 57 isolated actinobacterial strains and was classified as *Saccharothrix flava* (KU507600) by polyphasic taxonomy. The present manuscript has been reported for RSM-based optimization and interactive effects of the variables to produce enhanced bioactive metabolites by VSM-3, unstructured kinetic modeling and elucidation of chemotype analysis of the strain by LC-Q-TOF-MS analysis.

MATERIALS AND METHODS

Chemicals and reagents

The medium components of Starch casein nitrate agar medium and nutritional requirements such as sucrose and biopeptone were obtained from Merck, India, while the solvent, ethyl acetate, and other chemicals/reagents of analytical grade were obtained commercially.

Isolation and identification

The marine silt specimens were gathered from the Bay of Bengal of north coastal Andhra Pradesh, India, and were dried at ambient temperature. The atmospheric dried silt samples were exposed to (6% w/v) peptone pre-treatment and (0.05 %) sodium lauryl sulfate at a temperature of 50°C and for 10 min (Kyung and Yong, 1996) to confine the unsolicited impurities such as molds and microbes. The example of pre-pickled sediment (1 g) was dissolved in a solution of 100 ml of quarter force ringer's solution, homogenized by vortexing. Starch casein nitrate agar solution composed of 1% soluble Starch, 0.03% Casein, 0.2% K_2HPO_4, 0.2% KNO_3, 0.2% NaCl, 0.005% $MgSO_4$, 0.002% $CaCO_3$, 0.001% $FeSO_4$, and 2% agar supplemented with Nalidixic acid (50 µgml^{-1}) and Secnidazole (50 µg ml^{-1}), was used for serial (100 µl of 10^{-4}) dilution and incubated at 30ºC for three weeks. Morphologically particular strain was specifically isolated and kept up by subcultured on yeast extract malt extract dextrose (YMD) agar medium at 4ºC for further examination. The strain was identified as *S. flava* VSM-3 by polyphasic taxonomy and deposited in GenBank (Accession number: KU507600).

RSM experimental design

RSM designs are connected for displaying and investigation of the issues in which the reaction of premium is impacted by a few factors and their association (Sandrina *et al*., 2016) to produce the antimicrobial metabolites by VSM-3. From the preliminary experiments, time of incubation, pH, temperature of medium, concentrations of sucrose, and biopeptone were selected due to their influence for the bioactive metabolite production, and its antimicrobial activity against five responses, namely (measured as Zone of inhibition) *Xanthomonas campestris* (MTCC 2286), *Pseudomonas solanacearum* (NCIM 5103), *Botrytis cinerea* (MTCC 2104), *Curvularia maculans*, and *Penicillium expansum*. The most significant factors (*A*, *B*, *C*, *D*, and *E*) at their ideal levels were identified for maximal response (inhibition zone against the responses). Low and high scopes of all factors are utilized in RSM in genuine and the coded structure was given in Table 1. In total, 50 (32 factorial points, 10 axial points, and 8 replicates) experiments were performed (Table 2). All the variables' central-coded value was considered as zero. The data obtained from experiments were fitted into the second-order polynomial model through the RSM.

Analysis of variance (ANOVA)

Statistical analysis of the model was attempted to estimate the ANOVA. Model adequacy of the responses was verified by calculating the determination coefficient (R^2), adjusted

Table 1. Coded and actual factors and their experimental ranges.

Factors	Notation	Coded factors actual levels		
		−1 (Low)	*0* (Middle)	*+1* (High)
Incubation time (days)	*A*	5	6	7
pH	*B*	6	7	8
Temperature (°C)	*C*	30	35	40
Concentration of sucrose (%w/v)	*D*	0.5	1	1.5
Concentration of bio peptone (%w/v)	*E*	0.25	0.5	0.75

Table 2. Central composite factor experimental design along with experimental and predicted values.

Run	*A*-Time (days)	*B*-pH	*C*-Temperature (°C)	*D*-[Sucrose] (%w/v)	*E*- [biopeptone] (%w/v)	*X. campestris*		*P. solanacearum*		*B. cinerea*		*C. maculans*		*P. expansum*	
						Actual	RSM	Actual	RSM	Actual	RSM	Actual	RSM	Actual	RSM
1	5.00	8.00	30.00	0.50	0.25	17.00	17.18	17.00	16.84	17.00	16.65	16.00	16.25	15.00	14.51
2	7.00	8.00	40.00	0.50	0.75	17.00	16.89	16.00	16.30	16.00	16.16	16.00	16.25	15.00	14.51
3	5.00	8.00	40.00	0.50	0.25	17.00	16.89	16.00	16.07	17.00	16.65	16.00	15.77	14.00	14.28
4	7.00	7.00	35.00	1.00	0.50	17.00	17.09	16.00	15.78	16.00	16.16	16.00	15.77	14.00	14.28
5	5.00	8.00	40.00	1.50	0.75	17.00	16.92	16.00	16.13	16.00	16.37	15.00	14.97	14.00	14.22
6	5.00	7.00	35.00	1.00	0.50	16.00	16.12	16.00	15.84	16.00	15.88	15.00	14.97	14.00	14.22
7	7.00	8.00	30.00	0.50	0.25	16.00	16.12	15.00	15.11	16.00	16.37	15.00	14.99	14.00	13.98
8	6.00	7.00	35.00	1.00	0.50	16.00	15.83	15.00	15.07	16.00	15.88	15.00	14.99	14.00	13.98
9	5.00	8.00	40.00	0.50	0.75	17.00	17.18	17.00	16.84	16.00	16.16	16.00	15.77	14.00	14.28
10	5.00	8.00	30.00	1.50	0.25	17.00	16.89	16.00	16.30	16.00	16.18	16.00	15.77	14.00	14.28
11	7.00	8.00	30.00	1.50	0.25	17.00	16.89	16.00	16.07	16.00	16.16	15.00	15.28	14.00	14.04
12	6.00	7.00	35.00	1.00	0.50	17.00	17.09	16.00	15.78	16.00	16.18	15.00	15.28	14.00	14.04
13	5.00	6.00	40.00	1.50	0.75	17.00	16.92	16.00	16.13	16.00	15.88	15.00	14.99	14.00	13.98
14	6.00	7.00	35.00	1.00	0.25	16.00	16.12	16.00	15.84	16.00	15.90	15.00	14.99	14.00	13.98
15	5.00	8.00	30.00	0.50	0.75	16.00	16.12	15.00	15.11	16.00	15.88	15.00	15.00	14.00	13.75
16	6.00	8.00	35.00	1.00	0.50	16.00	15.83	15.00	15.07	16.00	15.90	15.00	15.00	14.00	13.75
17	7.00	8.00	30.00	1.50	0.75	18.00	18.18	17.00	17.30	17.00	17.37	17.00	16.77	15.00	15.28
18	5.00	8.00	40.00	1.50	0.25	18.00	17.89	17.00	16.52	17.00	16.88	17.00	16.77	15.00	15.28
19	6.00	7.00	35.00	1.00	0.50	18.00	17.89	17.00	16.78	17.00	17.37	16.00	16.28	15.00	15.04
20	6.00	7.00	35.00	1.50	0.50	18.00	18.09	16.00	16.25	17.00	16.88	16.00	16.28	15.00	15.04
21	7.00	6.00	30.00	1.50	0.75	18.00	17.92	17.00	16.84	17.00	16.59	16.00	15.99	15.00	14.98
22	5.00	6.00	40.00	1.50	0.25	17.00	17.12	16.00	16.30	16.00	16.10	16.00	15.99	15.00	14.98
23	5.00	6.00	30.00	0.50	0.75	17.00	17.12	16.00	16.07	17.00	16.59	16.00	16.00	15.00	14.75
24	7.00	6.00	30.00	0.50	0.25	17.00	16.83	16.00	15.78	16.00	16.10	16.00	16.00	15.00	14.75
25	5.00	6.00	40.00	0.50	0.25	18.00	18.18	17.00	17.30	17.00	16.88	16.00	16.28	15.00	15.04
26	5.00	6.00	40.00	0.50	0.75	18.00	17.89	17.00	16.52	17.00	16.90	16.00	16.28	15.00	15.04
27	5.00	6.00	30.00	1.50	0.25	18.00	17.89	17.00	16.78	17.00	16.88	16.00	15.80	15.00	14.80
28	6.00	7.00	35.00	1.00	0.50	18.00	18.09	16.00	16.25	17.00	16.90	16.00	15.80	15.00	14.80
29	6.00	7.00	35.00	0.50	0.50	18.00	17.92	17.00	16.84	16.00	16.10	16.00	16.00	15.00	14.75
30	7.00	8.00	40.00	1.50	0.25	17.00	17.12	16.00	16.30	16.00	16.12	16.00	16.00	15.00	14.75
31	6.00	7.00	30.00	1.00	0.50	17.00	17.12	16.00	16.07	16.00	16.10	16.00	16.02	14.00	14.51
32	6.00	7.00	35.00	1.00	0.50	17.00	16.83	16.00	15.78	16.00	16.12	16.00	16.02	14.00	14.51
33	6.00	7.00	35.00	1.00	0.50	20.00	19.55	19.00	18.73	19.00	18.99	18.00	17.86	17.00	16.83
34	7.00	8.00	30.00	0.50	0.75	19.00	19.26	18.00	18.31	19.00	18.76	18.00	17.86	17.00	16.83
35	6.00	7.00	40.00	1.00	0.50	20.00	19.55	19.00	18.84	19.00	18.88	18.00	17.98	17.00	16.95
36	6.00	7.00	35.00	1.00	0.50	19.00	19.26	18.00	18.20	19.00	18.88	18.00	17.74	17.00	16.72
37	7.00	8.00	40.00	1.50	0.75	20.00	19.79	18.00	18.31	19.00	18.64	18.00	17.63	17.00	16.48
38	5.00	8.00	30.00	1.50	0.75	19.00	19.02	18.00	17.73	18.00	18.11	17.00	17.10	16.00	16.19
39	5.00	6.00	30.00	1.50	0.75	20.00	19.90	19.00	19.02	19.00	18.99	18.00	17.98	17.00	16.95
40	6.00	7.00	35.00	1.00	0.50	20.00	19.90	19.00	19.02	19.00	18.76	18.00	17.74	17.00	16.72
41	7.00	6.00	30.00	0.50	0.75	19.00	18.90	18.00	17.73	18.00	17.64	17.00	16.98	16.00	15.95
42	7.00	6.00	40.00	1.50	0.75	20.00	19.90	18.00	18.31	18.00	18.11	18.00	17.74	17.00	16.72
43	5.00	6.00	30.00	0.50	0.25	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
44	6.00	7.00	35.00	1.00	0.75	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
45	7.00	6.00	40.00	1.50	0.25	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
46	6.00	6.00	35.00	1.00	0.50	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
47	7.00	6.00	40.00	0.50	0.25	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
48	7.00	8.00	40.00	0.50	0.25	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
49	7.00	6.00	40.00	0.50	0.75	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17
50	7.00	6.00	30.00	1.50	0.25	20.00	20.10	19.00	18.98	19.00	19.12	18.00	18.14	17.00	17.17

determination coefficient (adj. R^2), variation coefficient (CV), and the Fisher's test value (*F*-value). The 3-D surface reaction plots were utilized to break down the connection between independent variables and the responses, and these reaction plots address the dependent variable in capacity of two independent variables, where the third variable is kept steady (Coded terms).

Unstructured model development

Production of the bioactive metabolites by strain VSM-3 is influenced by the growth rate of biomass and limiting substrate concentration utilization. To simulate the cell (*S. flava* VSM-3) proliferation and bioactive metabolite synthesis, Logistic (*L*) and Luedeking–Piret models (*LP*) were applied. Since the rate of specific bioactive metabolite production and cell growth can be calculated from kinetic expressions. As per the experimental data obtained from batch shake-flask for bioactive metabolite production, our previously developed models (*L*, Logistic Incorporated *LP* (*LILP*) and Logistic incorporated modified *LP* (*LIMLP*) were used to determine the process parameters (Ushakiranmayi *et al*., 2016).

Fermentation and extraction

Spores of potential strain *S. flava* VSM-3 were scrapped from Starch casein nitrate agar and transferred into 100 ml of YMD seed medium consists of yeast extract 0.4%, malt extract 1%, and dextrose 0.4% and kept in an orbital shaker at 120 rpm, 48h at 35°C. 100 ml of optimized production medium (composed of 0.5 % Sucrose, 0.25 % Biopeptone, 3% NaCl at pH 7.0) was inoculated with 10% inoculum and incubated on an orbital shaker at 120 rpm, 6 days at 35°C. The fermented broth collected at the end of the incubation period was centrifuged, and the culture filtrate obtained was extracted with ethyl acetate. Separating funnel was used to separate the ethyl acetate phase containing bioactive metabolite from the aqueous phase. The pooled solvent extracts were concentrated in a vacuum drier and the crude extract was subjected to LC-Q-TOF-MS for the elucidation of chemical composition analysis for *S. flava,* as per the method described by Maya and Benjamin (2016).

RESULTS AND DISCUSSION

RSM optimization studies and model adequacy for the bioactive metabolite production

Central Composite Design (CCD) module of RSM was involved to assess the interaction among significant variables and to determine their optimal values. Using CCD reduces the experimental run numbers and escalates the resulting efficiency to be obtained. It has been considered as an efficient statistical experimental tool used to design the experiments (Bipasha *et al*., 2015). Table 2 tabulates the comparative responses of predicted and experimental runs values. Optimized concentration of the bioactive compound synthesis by *S. flava* VSM-3 and its effect against experimental responses (growth inhibition as the response) were found to be 20, 19, 19, 18, and 17 mm for *X. campestris*, *P. solanacearum*, *B. cinerea*, *C. maculans*, and *P. expansum*, respectively, that was identified at 6 days incubation time, 7 pH, 35°C temperature, 0.5% sucrose concentration, and 0.25% biopeptone concentration.

Table 3 tabulates the best outcome quadratic model with the sequential model sum of squares (suggested) and lack of fit tests (showing degrees of freedom; mean square, F-value, and p-value). The results show 99% of confidence (since the Prob > *F* value of the model was found to be <0.00001, for all the five responses), which implies that the model is significant. ANOVA statistics have shown a 99% confidence level, when models were checked for their adequacy. Furthermore, ANOVA results based on the bioactive metabolite produced by *S. flava* VSM-3 confirm that the model has a satisfactory level coefficient of determination (R^2) against all the responses (Table 4).

Independent variables interaction and their impact on bioactive metabolite response

Figures 1–5 illustrate the 3-D plots for significant interactive effects of the independent variables such as incubation time, pH, temperature, sucrose, and biopeptone concentrations against the bioactive metabolite responses. 3-D plots (showing response (on z-axis against any two independent variables by

Table 3. Sequential model fitting for all the responses (in terms of inhibition zone from the bioactive metabolite production).

Model parameter	*X. campestris*	*P. solanacearum*	*B. cinerea*	*C. maculans*	*P. expansum*
Sequential model sum of squares—Quadratic versus 2FI (suggested)					
Sum of squares	82.01	73.80	71.34	53.14	66.02
df[a]	5	5	5	5	5
Mean square	16.40	14.76	14.27	10.63	13.20
F-value	387.42	240.13	199.56	198.74	134.39
p-value (Prob > *F*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lack of fit tests—Quadratic (suggested)					
Sum of squares	1.23	2.25	2.07	1.55	2.85
df[a]	22	22	22	22	22
Mean square	0.056	0.10	0.094	0.070	0.13
F-value	—	—	—	—	—
p-value (Prob > *F*)	—	—	—	—	—

df = degrees of freedom.

Table 4. Results of Analysis of variance for predicting the model adequacy.

Statistics	Response				
	Xanthomonas campestris	*Pseudomonas solanacearum*	*Botrytis cinerea*	*Curvularia maculans*	*Penicillium expansum*
R^2	0.9877	0.9743	0.9740	0.9760	0.9623
Adj-R^2	0.9792	0.9566	0.9560	0.9594	0.9362
Pred-R^2	0.9560	0.9028	0.9051	0.9151	0.8643
Adequate precession	31.999	21.891	18.705	21.127	16.835
CV%	1.14	1.63	1.55	1.40	2.04

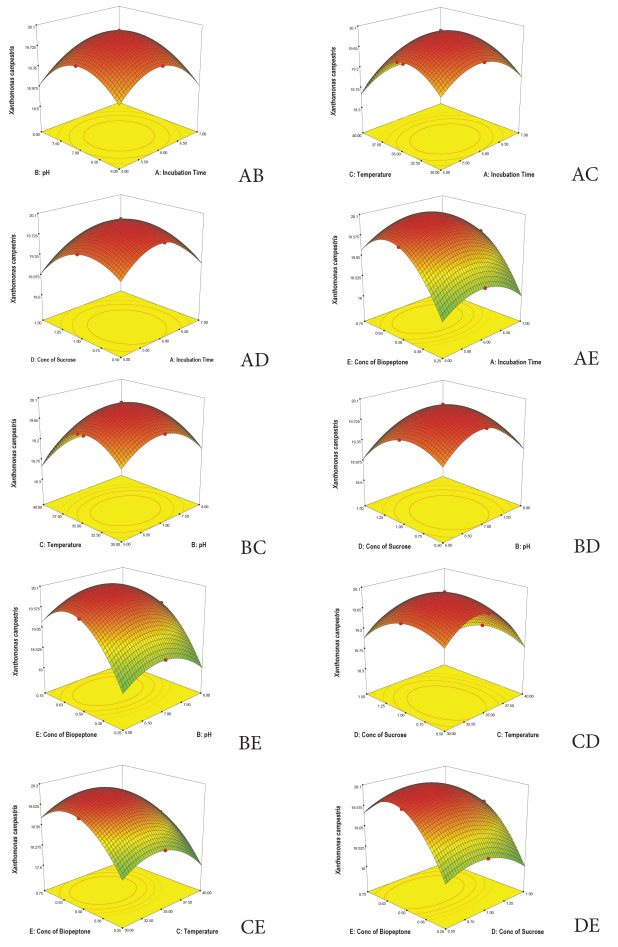

Figure 1. Response surface plots comprised of 3-D views and contours showing interactive effects of selective variables on Zone of Inhibition (mm) of the bioactive compound production by *S. flava* VSM 3 against *X. campestris.*

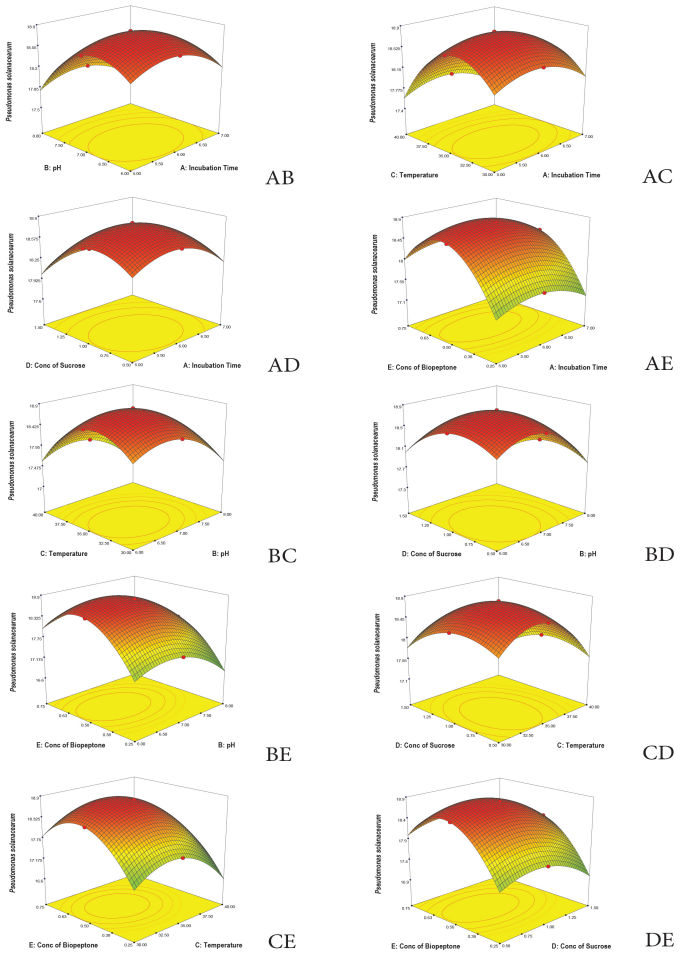

Figure 2. Response surface plots comprised of 3-D views and contours showing interactive effects of selective variables on Zone of Inhibition (mm) of the bioactive compound production by *S. flava* VSM 3 against *P. solanacearum.*

keeping the third variable constant) were generated from CCD of RSM to analyze the effect of these variables' interactions.

The least square method of nonlinear regression (in Microsoft Excel solver 2010) was attempted to fit the experimental data with unstructured models. Experimental versus model-predicted inhibition zones were compared in terms of the bioactive metabolite responses by Kirby Bauer disk diffusion method against the test pathogens *X. campestris*, *P. solanacearum*, *B. cinerea*, *C. maculans*, and *P. expansums* strains against the time, were shown in Figure 6 (a)–(e). Figure 6(f) shows the experimental and model-predicted comparative profiles of *S. flava* VSM-3 substrate utilization. All the profiles show very good fit between model and experiment values. Table 5 lists the evaluated biokinetic parameters used in the mathematical model equations, which indicate high R^2 values *L*, *LILP*, and *LIMLP* models fitting to the experimental data revealing good precision of these models. Inhibition zones of agar diffusion tests were also found to be matching with experimental and model data (Table 6). In this way, these developed mathematical models found to be a good representation of bioactive metabolite production kinetics of *S. flava* VSM-3 in submerged shake-flask fermentations (Ushakiranmayi *et al*., 2016).

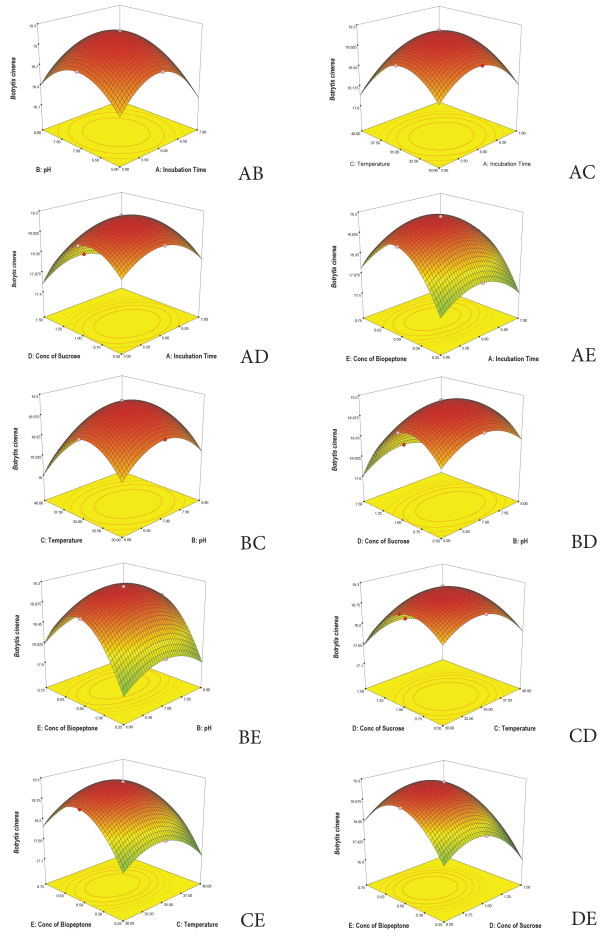

Figure 3. Response surface plots comprised of 3-D views and contours showing interactive effects of selective variables on Zone of Inhibition (mm) of the bioactive compound production by *S. flava* VSM 3 against *B. cinerea.*

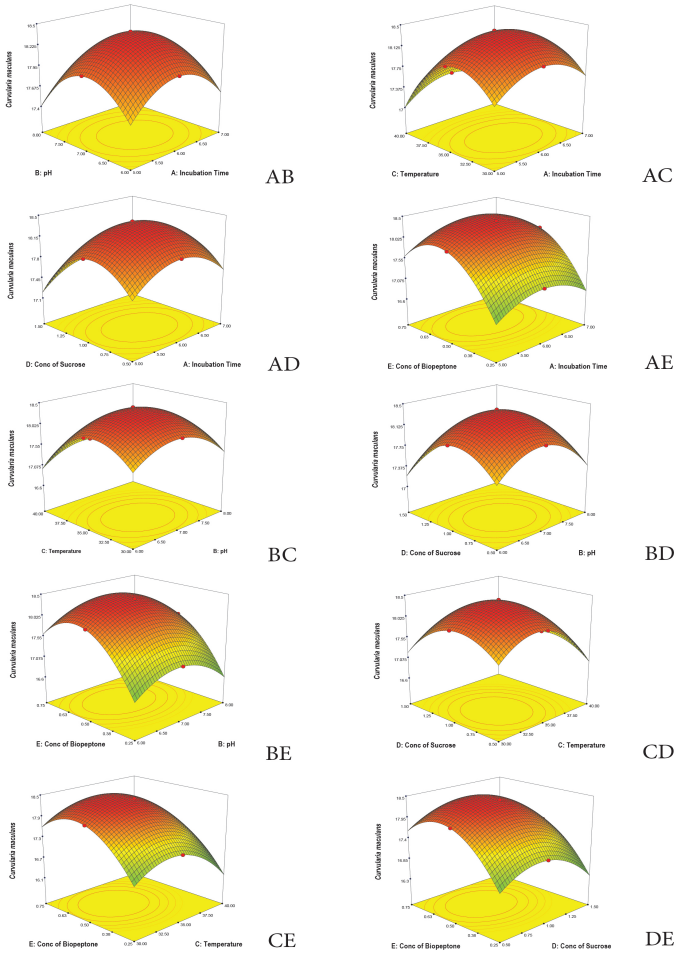

Figure 4. Response surface plots comprised of 3-D views and contours showing interactive effects of selective variables on Zone of Inhibition (mm) of the bioactive compound production by *S. flava* VSM 3 against *C. maculans.*

Chemotype analysis by ESI-LC-MS/MS

The chemical composition of *S. flava* VSM-3 was elucidated for the first time by liquid chromatography quadrupole time-of-flight mass spectrometry analysis and the compounds' spectra was shown in Figure 7. Mass fragmentation analysis confirms the identity of the compounds. Positive ion mode detected a total of seven active metabolites from *Saccharothrix flava* ethyl acetate extract. The compounds identified were 7-Deazaadenosine, 5-Hydroxy-9-Methylstreptimidone, Amiclenomycin, Dihydroabikoviromycin, Epopromycin A, OAP Silane 55, and MKN-003B. Negative ion mode did not yield any known compounds (Fig. 8).

RSM designs are applied for modeling and analysis of the problems in which the response of interest is influenced by several variables and their interaction (Sandrina *et al*., 2016) to produce the antimicrobial metabolite by VSM-3. The model's variability suggested that R^2 should be close to 1, or at least 0.80, to be a good fit model (Xiangli *et al*., 2008). Adjusted R^2 is used to determine the model adequacy (in number of model terms) (Onsekizoglua *et al*., 2010). The desirable Coefficient of variation (CV) should be below 10% and adequate precision must be above 4. Non-significance of the lack fit indicates that the model is a good fit (Mojtaba *et al*., 2014).

Logistic (L) model-based parameters, μ_{max}, X_0, and X_{max} were calculated from the growth kinetic profile of the data obtained from the shake-flask culture of *S. flava* VSM-3 used in this study and *LILP* model yields α and β (non-growth associated product parameters). Comparatively, α value greater than β concludes bioactive metabolite synthesis associated by *S. flava* VSM-3 growth (Table 5). In addition, the simulated *LIMLP* model parameters, γ and η, have good fit with experimental data confirms that this model well represents metabolite synthesis is done from substrate consumption in *S. flava* VSM-3 fermentation (Ushakiranmayi *et al*., 2016).

Seven potent bioactive compounds were identified in the VSM 3 strain's LC-Q-TOF-MS analysis. Among the active compounds, 7-Deazaadenosine (Tubercidin), a nucleoside antibiotic, exhibited potent cytotoxic activity (Biabani *et al*., 2002), while 5-Hydroxy-9-Methylstreptimidone, a glutarimide metabolite, was reported for its herbicidal activity (Chatterjee *et al*., 1995). Amiclenomycin, a free amino acid, was reported bioactive against *Mycobacteria* and has part of di- and tri-peptides that show antibiotic activities (Kern *et al*., 1985). The compound Dihydroabikoviromycin was formerly mentioned for its genotoxic activity (Holmalahti *et al*., 1998), while Epopromycin A, an

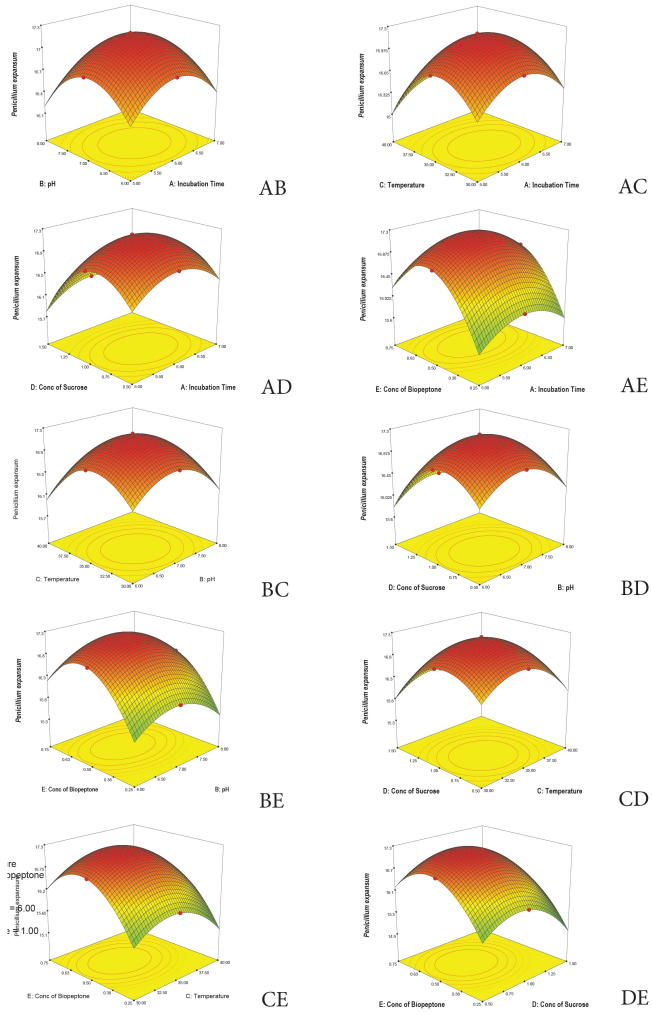

Figure 5. Response surface plots comprised of 3-D views and contours showing interactive effects of selective variables on Zone of Inhibition (mm) of the bioactive compound production by *S. flava* VSM 3 against *P. expansum*.

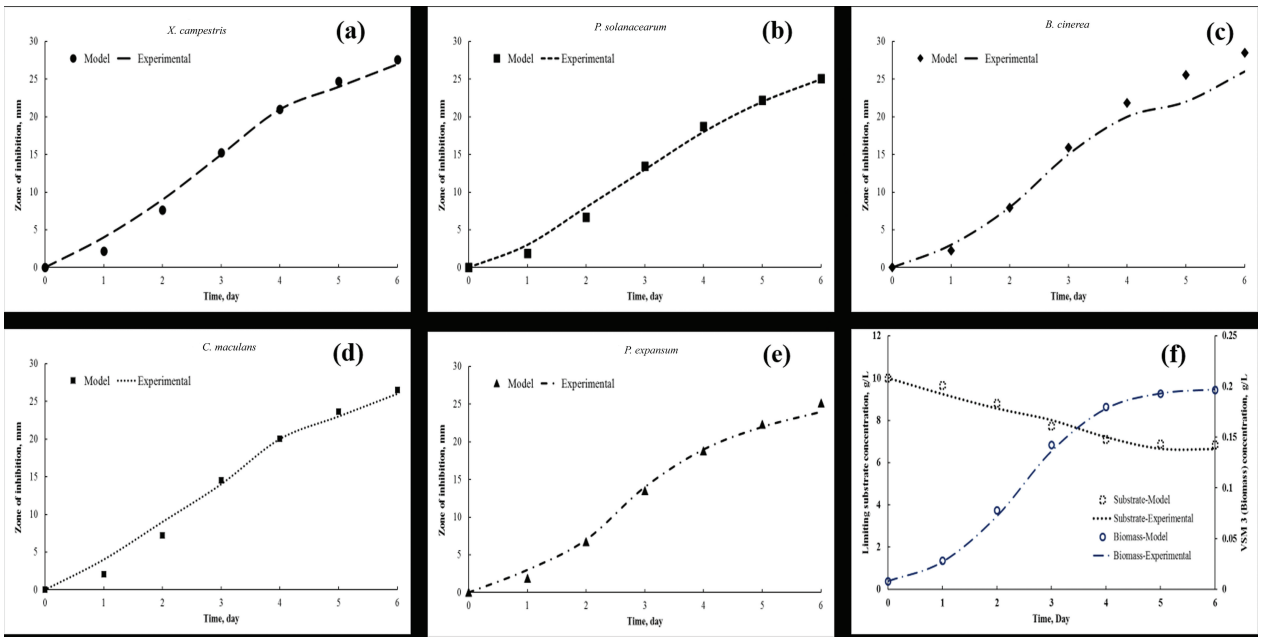

Figure 6. Comparison of experimental and model predicted kinetics (a)–(e): for zone of inhibition (mm); (f): for biomass growth (g/l), substrate utilization (g/l).

Table 5. Estimated kinetic parameters using L, LILP, and LIMLP model equations.

Kinetic parameters	*X. campestris*	*P. solanacearum*	*B. cinerea*	*C. maculans*	*P. expansum*
Logistic (L) model parameters					
μ_{max} (d^{-1})	1.37				
R^2	0.998				
X_0 (g/l)	0.008				
X_m (g/l)	0.198				
Logistics incorporated modified Luedeking–Piret (LIMLP) model parameters					
γ (g.S/g.X)	17.18				
R^2	0.959				
η (g.S/(g.X.d))	0.101				
Logistics incorporated Luedeking–Piret (LILP) model parameters					
α (mm/g.X)	96.290	82.946	101.080	90.675	83.322
R^2	0.970	0.977	0.988	0.959	0.979
β (mm/(g.X.d))	12.987	12.987	17.316	12.987	8.658

Table 6. Maximum zones of inhibition (mm) comparison for experimental with model results.

Maximum Zone of Inhibition (mm)	*X. campestris*	*P. solanacearum*	*B. cinerea*	*C. maculans*	*P. expansum*
Experimental	27	25	26	26	24
Model fitted	27.586	25.066	28.49	26.525	25.138

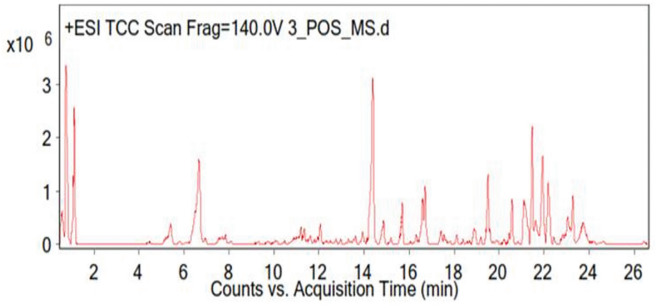

Figure 7. Positive ion (+) ESI-MS/MS analysis of ethyl acetate extract of strain VSM-3.

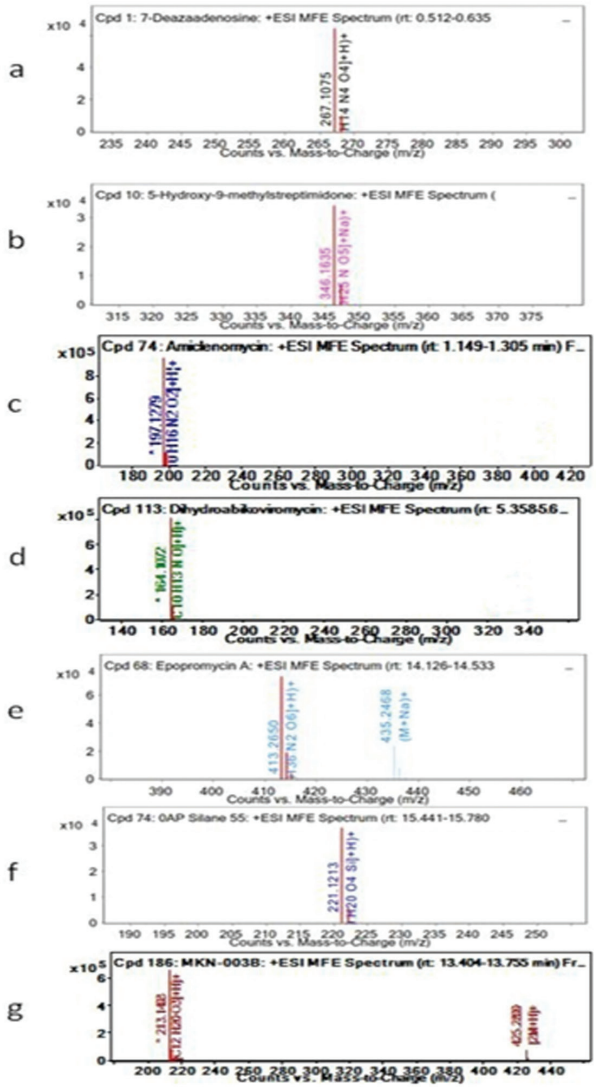

Figure 8. Negative ion (-) ESI-MS/MS analysis of ethyl acetate extract of strain VSM-3.

analog of eponeomycin showed herbicidal activity and potent cytotoxicity (Tsuchiya *et al*., 1997). The compound OAP Silane55 (S-520) was reported for its antibacterial activity, especially in opposition to gram positive bacteria (Shoji *et al*., 1970), while the compound MKN-003B, a butenolide confirmed moderate to weak antifungal activity (Wang *et al*., 2014).

Till date, Madumycin antibiotic was synthesized by *Actinomadura flava* (later renamed as *Nocardiopsis flava*) INA 2171 strain with maximum quantities of madumycin I and II by fifth day of cultivation (Gauze and Sveshnikova, 1985; Gauze *et al*., 1974; Kochetkova *et al*., 1976). *Nocardiopsis flava* has been mentioned to be transferred to the genus *Saccharothri*x on chemotaxonomic traits basis (Labeda and Lechevalier, 1989). The current work also found that only after 6th day of fermentation yielded maximum bioactive metabolite from *S. flava*. Zitouni *et al*. (2005) have reported that *Saccharothrix* and *Nocardiopsis* species were active against Gram positive bacteria and yeast. In the present study, bioactive compounds synthesized from *S. flava* VSM-3 exhibited good antimicrobial activity against gram-positive bacteria and Gram-negative plant pathogenic bacteria like *P. solanacearum*, agriculturally important fungi like *B. cinerea*. Hence, the strain *S. flava* VSM-3 collected from shore samples of Bay of Bengal, located in Andhra Pradesh, India, is novel actinomycete which enabled the production of antibacterial and antifungal compounds.

CONCLUSION

Central composite design (of RSM) was successfully employed to determine the optimizing conditions and to analyze the independent and interactive effects of the process variables, namely, incubation time, pH, temperature, concentrations of

sucrose, and bio peptone. The results indicated that the process variables have a significant effect on bioactive metabolites production by *S. flava* VSM-3 and their effects counter to the five responses (inhibition zones). Statistical summary of the model explained that developed model was adequate and precise with the experimental data that were in near concurrence with the predicted values. Further unstructured models, applied in the study, had given a superior guess of kinetic profiles of bioactive metabolite synthesis by *S. flava* VSM-3 in submerged shake flask fermentations. The values of predicted (model) and experimental responses (zones of inhibition) for five responses were almost similar, which concludes that the developed kinetic model fits the data accurately. The bioactive compounds obtained from strain VSM-3 exhibited significant bioactivity especially against agriculturally important bacteria and fungi, and reveals that the strain is a promising producer of antimicrobial compounds. The actinobacterium's ethyl acetate extract was subjected to LC-QTOF-MS, which revealed the presence of significant bioactive compounds also supported that the strain VSM 3, and it was most potent for further pharmacological studies. The study also supports that north coastal Bay of Bengal of Andhra Pradesh, a potent ecological niche with inimitable strain diversity remained to be explored for bioactive compounds.

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ABBREVIATIONS

RSM Response surface methodology

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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