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Research Article

Optimization of fermentation media constituents for higher production of naringinase by *Paenibacillus stellifer* RAMCM-44 using Response Surface Methodology

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Abstract
Naringin is a flavonoid in citrus fruits and contributes to the bitterness of extracted citrus fruit juices. Naringinase has potential to hydrolyse the naringin into tasteless compounds and, therefore, reduces the bitterness of citrus juices. Majority of studies focused on naringinase production from fungal sources. The present study aimed to use *Paenibacillus stellifer* RAMCM-44 for the production of naringinase by optimization of media constituents using Response Surface Methodology (RSM) at the shake-flask level. Five media components were taken as variables with different concentrations to determine the optimal concentrations of media components. The five variables included peptone (0.5-1%, w/v), yeast extract (0.4-0.8%, w/v), NaCl (0.5-1%, w/v), naringin (0.1-0.25%, w/v) and KH_2PO_4 (0.5-1%, w/v), while $MgSO_4$ (0.05%, w/v), $MnSO_4$ (0.001%, w/v) and initial pH (6.5±0.2) during shake-flask fermentation, media were kept constant. The experimental data was analysed (RSM) and optimal levels of peptone (0.75%), yeast extract (0.6%), NaCl (0.75%), naringin (0.17%) and KH_2PO_4 (0.75%) were determined. The experimental values of naringinase activity (8.55 IU/ml) and biomass (0.826 OD_{600}) closely matched the predicted values. The criteria, including analysis of variance (ANOVA), R values, coefficient of variance and model significance were assessed. The R-value for naringinase production was found to be 0.9976, while the model F-value was 228.74 and the lack of fit F-value was 109.77 for naringinase activity, revealing the current study's significance. This is the first report on optimising media components and applying RSM for the production of naringinase by *P. stellifer* RAMCM-44, which could benefit studies involving scale-up.

Keywords: Analysis of variance (ANOVA), Media optimization, Naringin, Naringinase, Response surface methodology (RSM)

INTRODUCTION

Naringin is an important flavonoid found in citrus fruits and contributes to the bitterness of citrus juices. Naringinase catalyses the hydrolysis of naringin and produces the tasteless compound naringenin, which is non-bitter and tasteless. This have two subunits, i.e.α-L-rhamnosidase and a β-D-glucosidase, which sequen-

tially hydrolyse the naringin in two steps. In the first step, the naringin is hydrolysed with the help of α-L-rhamnosidase, leading to the formation of prunin accompanied by release of rhamnose. While in the second step, purnin is hydrolysed by β-D-glucosidase and production of naringenin (a tasteless flavanone) with the release of glucose takes place (Chandler and Nicol, 1975; Habelt and Pittner, 1980; Pavithra *et al*., 2012; Puri *et al.,* 2008; Yadav *et al.,* 2018; Nara *et al.,* 2024). Various industrial uses of microbial naringinase have been reported (Puri and Banerjee, 2000; Ribeiro, 2011). Naringinase has found its main application in industries dealing with the processing of citrus fruits, where naringin-mediated bitterness needs to be removed (Busto *et al.,* 2007; Puri *et al.,* 2010a, 2010b; Srikantha *et al.,* 2016). Microbes are the major sources of naringinase. Though bacterial and fungal strains including yeast have been reported for production of naringinase, bacterial sources are still very limited compared to fungal sources. Bacteria are well known for some obvious advantages over fungal cultures for production of industrial enzymes and metabolites. The bacterial sources have not been widely explored for this valuable enzyme's production (Yadav *et al.,* 2018; Nara *et al.,* 2024; Tripathi *et al.,* 2024). Response surface methodology (RSM), is a statistical tool that is now frequently used to optimize the media components for the optimal production of microbial enzymes and metabolites (Singh and Yadav, 2013). The interaction among the variables and responses that affect enzyme production can also be determined. Response surface methodology (RSM) has emerged as a crucial statistical method for optimizing media to achieve high enzyme yields. Among its designs, the Central Composite Rotatable Design (CCRD) is the most prevalent, allowing for studying various factors and their interactions influencing the common response. The present study aimed for the production of naringinase by optimization of media components through RSM in the bacterial strain *Paenibacillus stellifer* RAMCM-44, an isolate of the present lab (Nara *et al.,* 2024) and also to see the regulatory effects of observations in media constituents at varying concentrations.

MATERIALS AND METHODS

Bacterial strain, maintenance and inoculum preparation

The bacterial strain *Paenibacillus stellifer* RAMCM-44, an isolate of the present lab (Nara *et al.,* 2024), was used in the present study to produce naringinase and optimise media components using RSM. The strain was kept on nutrient agar slants containing naringin (0.1%; w/v). Inoculum was prepared in nutrient broth containing citrus peel powder (Nara *et al.,* 2024). Inoculum was grown for 24 hrs at 37ºC (150 rpm), assessed for purity by microscopic examination and used for the inoculation of naringin production media.

Experimental design and analysis of naringinase production

Five media components (Nara *et al.,* 2024) were used as independent variables to obtain optimal values for higher production of naringinase. Central Composite Design (CCD), incorporating five variables, was used to determine the combination (optimal) of independent variables (media components) for two responses: naringinase activity and biomass yield. The five variables that were optimized included : peptone (0.5-1%, w/v), yeast extract (0.4-0.8%, w/v), NaCl (0.5-1%, w/v), naringin (0.1-0.25%, w/v) and KH_2PO_4 (0.5-1%, w/v) while $MgSO_4$ (0.05%, w/v), $MnSO_4$ (0.001%, w/v) and initial pH (6.5±0.2) and the media were kept constant throughout the production of naringinase. Each variable was examined at five coded levels, as illustrated in Table 1. The designed experiments evaluated the responses, specifically biomass yield and naringinase activity. The fermentation conditions for the production of naringinase at shake-flask level were similar as reported previously (Nara *et al.,* 2024). A total of 32 experimental runs were designed using Design-Expert (Stat-Ease Inc., Minneapolis, USA). Statistical analysis of results (responses) of 32 experimental runs was done with the help of Design-Expert (Stat-Ease Inc., Minneapolis, USA). Moreover, the responses acquired underwent analysis of variance (ANOVA) to investigate the impacts of the independent variables. The optimal variable combination was identified through graphical and numerical analysis to enhance naringinase production.

Naringinase assay

Naringinase activity was measured based on naringin hydrolysis as described by Davis (1947) with minor modifications (Puri *et al.,* 2010a; 2010b; Pavithra *et al.,* 2012; Nara *et al.,* 2024). Briefly, the fermented broth was centrifuged (7,000 rpm, 10 min, 4°C), and the cell-free broth was used as a crude enzyme to determine enzyme activity. The typical assay mixture consisting 900 µl of naringin (0.05%, w/v) dissolved in sodium acetate buffer (0.1 M; pH 4.5) and 100 µl of crude enzyme (supernatant/cell-free broth) was incubated for 1 hour at 50°C. After completion of incubation time, an aliquot of 100 µl from the reaction mixture was taken and added to the 5 mL of diethylene glycol (90%, v/v), followed by the addition of 100 µl NaOH (4 N) solution. The resultant reaction mixture was incubated for 10 minutes at room temperature. The intensity of the developed yellow color was measured at 420 nm in a UV-visible spectrophotometer. A unit of naringinase was defined as the quantity of enzyme that hydrolysed 1 µmol of naringin per minute under the assay's standard

conditions.

Biomass determination

The growth of bacteria was measured and expressed as Biomass (OD_{600}). The biomass was measured by recording the optical density of the harvested/ cultured broth at 600 nm using a UV-visible spectrophotometer (Singh and Yadav, 2012), with the harvested cell-free broth serving as the blank. Before determining the biomass, the cultured broth from each run was diluted to the same level.

RESULTS AND DISCUSSION

A total of 32 experiments were conducted, each with specific combinations of media components i.e. peptone, yeast extract, NaCl, naringin and KH_2PO_4 (Nara *et al.,* 2024) were derived with help of RSM and experiments were performed.

Table 2 presents the experimental design and results for naringinase production and biomass. The experimental outcome of the Central Composite Rotatable Design (CCRD) was modelled using a second-order polynomial equation. Further, regression coefficients were also calculated, and the resulting equations (in coded values) for predicting naringinase production (X) and biomass yield (Y) are provided below, irrespective of the significance of the coefficients:

$$X = +7.22 + 0.1508 * A + 0.0433 * B - 0.1192 * C + 0.0125 * D + 0.3483 * E - 0.1525 * A * B + 0.0050 * A * C + 0.5288 * A * D - 0.2100 * A * E - 0.2250 * B * C + 0.1488 * B * D + 0.3175 * B * E - 0.1937 * C * D + 0.0675 * C * E + 0.0162 * D * E + 0.1123 * A^2 - 0.0215 * B^2 - 0.0577 * C^2 - 0.3965 * D^2 + 0.0710 * E^2 \quad (1)$$

$$Y = + 0.7451 + 0.0111 * A + 0.0098 * B + 0.0007 * C + 0.0051 * D + 0.0371 * E - 0.0129 * A * B + 0.0058 * A * C + 0.0410 * A * D - 0.0166 * A * E - 0.0055 * B * C + 0.0193 * B * D - 0.0229 * B * E - 0.0226 * C * D + 0.0020 * C * E - 0.0073 * D * E + 0.0064 * A^2 - 0.0038 * B^2 - 0.0021 * C^2 - 0.0337 * D^2 - 0.0003 * E^2 \quad (2)$$

In the equation, “A” represents peptone concentration; “B”, yeast extract concentration; “C”, NaCl concentration; “D”, naringin concentration; and “E”, KH_2PO_4 concentration. The statistical significance of Equations 1 and 2 was assessed using analysis of variance (ANOVA) for the response surface quadratic model, with the summarized results presented in Table 3. The model's significance is demonstrated by the extremely low probability values for both naringinase production and biomass yield, as shown in Table 3. The P values indicate the significance of each coefficient, potentially revealing the interaction patterns between them. A smaller P value signifies a more significant corresponding coefficient (Rao *et al.,* 2000). The multiple correlation coefficients (R) for naringinase activity and biomass yield were 0.9976 and 0.9979, respectively. Being very close to one, these values indicated an excellent correlation between the predicted and experimental results. The coefficient estimates for Equations 1 and 2 are provided in Table 4. The model F-values observed were 228.74 for naringinase production and 263.08 for biomass yield. Table 3 shows the lack of fit sum of squares values for naringinase activity and biomass yield relative to the pure error. The lack of fit F-values was 109.77 for naringinase activity and 284.61 for biomass yield, suggesting a good fit of the experimental data with the model. Table 5 displays the production and biomass yield predicted by the final quadratic model alongside the corresponding observed values. A comparison of these values revealed an excellent agreement between the predicted and experimental data.

By differentiating the quadratic model, the ideal concentrations (%; w/v) for attaining maximal naringinase production and biomass output were as follows: Peptone (A) = 0.75 (%; w/v), Yeast extract (B) = 0.60 (%; w/v), NaCl (C) = 0.75 (%; w/v), Naringin (D) = 0.17 (%; w/v) and KH_2PO_4 (E) = 0.75 (%; w/v). Additional experiments were conducted in triplicate using the optimal medium components to validate the model's accuracy in predicting maximum naringinase production and biomass yield. The experiments yielded an average maximum naringinase and biomass yield of 8.55 IU/ml and biomass yield 0.826 OD_{600}, respectively. These were in good agreement with the predicted values for confirmation of model (Table 5). Various concentrations of different media components had a significant effect on naringinase production. Naringin is observed as one of the potent constituents of media affecting the production of naringinase. Higher naringinase activity (8.18IU/ml) and biomass (0.814 OD_{600}) productivity were observed in experimental run number 4as shown in Table

Table 1. Experimental range and levels of independent variables in terms of coded and actual factors

Factors	Symbols	Actual Levels of Coded Factors				
		Min.	-1.000	0	+1.000	Max.
Peptone	A	0.25	0. 50	0.75	1.0	1.25
Yeast Extract	B	0.20	0.40	0.60	0.80	1.0
NaCl	C	0.25	0. 50	0.75	1.0	1.25
Naringin	D	0.025	0.10	0.175	0.250	0.325
KH_2PO_4	E	0.25	0. 50	0.75	1.0	1.25

Table 2. Central composite design matrix for the experimental design for naringinase production and biomass yield

STD (Standard)	Run	Factor 1 A: Peptone % (w/v)	Factor 2 B: Yeast Extract % (w/v)	Factor 3 C: NaCl % (w/v)	Factor 4 D: Naringin % (w/v)	Factor 5 E: KH_2PO_4 % (w/v)	Response 1 Naringinase Activity (IU/ml)	Response 2 Biomass $OD_{(600nm)}$
27	1	0.75	0.6	0.75	0.175	0.75	7.23	0.745
19	2	0.75	0.2	0.75	0.175	0.75	7.02	0.709
31	3	0.75	0.6	0.75	0.175	0.75	7.23	0.745
26	4	0.75	0.6	0.75	0.175	1.25	8.18	0.814
21	5	0.75	0.6	0.25	0.175	0.75	7.27	0.744
22	6	0.75	0.6	1.25	0.175	0.75	6.63	0.73
3	7	0.5	0.8	0.5	0.1	0.5	6.71	0.642
25	8	0.75	0.6	0.75	0.175	0.25	6.75	0.674
12	9	1	0.8	0.5	0.25	0.5	7.78	0.769
16	10	1	0.8	1	0.25	1	7.69	0.804
10	11	1	0.4	0.5	0.25	1	7.41	0.751
29	12	0.75	0.6	0.75	0.175	0.75	7.23	0.745
5	13	0.5	0.4	1	0.1	0.5	7.29	0.717
32	14	0.75	0.6	0.75	0.175	0.75	7.23	0.745
14	15	1	0.4	1	0.25	0.5	7.62	0.751
11	16	0.5	0.8	0.5	0.25	1	7.95	0.806
2	17	1	0.4	0.5	0.1	0.5	6.76	0.658
13	18	0.5	0.4	1	0.25	1	6.16	0.63
8	19	1	0.8	1	0.1	0.5	5.68	0.625
1	20	0.5	0.4	0.5	0.1	1	7.1	0.745
24	21	0.75	0.6	0.75	0.325	0.75	5.66	0.619
9	22	0.5	0.4	0.5	0.25	0.5	5.8	0.621
30	23	0.75	0.6	0.75	0.175	0.75	7.23	0.745
4	24	1	0.8	0.5	0.1	1	6.8	0.677
17	25	0.25	0.6	0.75	0.175	0.75	7.34	0.748
7	26	0.5	0.8	1	0.1	1	8.15	0.84
18	27	1.25	0.6	0.75	0.175	0.75	7.92	0.794
15	28	0.5	0.8	1	0.25	0.5	5.13	0.603
23	29	0.75	0.6	0.75	0.025	0.75	5.53	0.602
20	30	0.75	1	0.75	0.175	0.75	7.17	0.751
28	31	0.75	0.6	0.75	0.175	0.75	7.21	0.745
6	32	1	0.4	1	0.1	1	7.01	0.743

2. In this run, concentrations of peptone, yeast extract, NaCl, naringin and KH_2PO_4were 0.75 (%;), 0.60 (%; w/v), 0.75(%; w/v), 0.17(%; w/v) and 1.25(%; w/v), respectively. Fig. 1 and 2 demonstrate a positive interaction among these constituents, wherein variations in their concentrations significantly impact naringinase activity. It as found that naringin concentration beyond a level leads to reduced production of naringin and this may attributed to the inhibitory action of naringin on bacterial strain. As depicted in Table 2, yeast extract and peptone supported the growth of bacterial strain *Paenibacillus stellifer* RAMCM-44, while naringin served as a potent inducer for the naringinase production. Naringin not only acts as an inducer but also

Table 3. Analysis of variance (ANOVA) for response surface quadratic model obtained from experimental design

Source	Naringinase Activity			Biomass		
	Sum of Squares	**DF**	**Prob.>F**	**Sum of Squares**	**DF**	**Prob.>F**
Model	18.40	20	0.0001	0.1333	20	0.0001
A-Peptone	0.5460	1	0.0001	0.0029	1	0.0001
B-Yeast Extract	0.0451	1	0.0065	0.0023	1	0.0001
C-NaCl	0.3408	1	0.0001	0.0000	1	0.5297
D-Naringin	0.0038	1	0.3550	0.0006	1	0.0004
E-KH2PO4	2.91	1	0.0001	0.0330	1	0.0001
AB	0.3721	1	0.0001	0.0027	1	0.0001
AC	0.0004	1	0.7584	0.0005	1	0.0008
AD	4.47	1	0.0001	0.0269	1	0.0001
AE	0.7056	1	0.0001	0.0044	1	0.0001
BC	0.8100	1	0.0001	0.0005	1	0.0011
BD	0.3540	1	0.0001	0.0059	1	0.0001
BE	1.61	1	0.0001	0.0084	1	0.0001
CD	0.6006	1	0.0001	0.0082	1	0.0001
CE	0.0729	1	0.0013	0.0001	1	0.1403
DE	0.0042	1	0.3274	0.0008	1	0.0001
A^2	0.3698	1	0.0001	0.0012	1	0.0001
B^2	0.0135	1	0.0938	0.0004	1	0.0017
C^2	0.0978	1	0.0004	0.0001	1	0.0459
D^2	4.61	1	0.0001	0.0333	1	0.0001
E^2	0.1480	1	0.0001	3.409E-06	1	0.7207
Residual	0.0442	11		0.0003	11	
Lack of Fit	0.0439	6	0.0001	0.0003	6	
Pure Error	0.0003	5		0.0000	5	
Cor Total	18.44	31		0.1336	31	

serves as a carbon source. Its different concentrations, along with yeast extract and peptone lead to higher biomass and naringinase activity . The interactive effect of KH_2PO_4 and naringin is revealed from experimental run number 4 where naringin concentration is (0.17 %) but high concentration of KH_2PO_4 (1.25%) along with peptone (0.75%), yeast extract (0.60%) and NaCl (0.75%) resulted in a significant increase in naringinase activity. Interaction among peptone, yeast extract NaCl, KH_2PO_4 and naringin influenced both naringinase activity and biomass yield (Fig. 1 and Fig. 2). A higher yield of naringinase was observed at 0.75% for peptone and 0.60% for yeast extract. These factors also contributed to increased biomass yield.

Optimizing media constituents led to enhanced naringinase activity and intensified growth. The application of RSM enabled the consideration of interactive effects of media components, which would be unattainable without a statistical model. The increased production of naringinase underscores the applicability of statistical

Table 4. Regression coefficients and significance of quadratic model for naringinase activity and biomass yield

Model	Naringinase Activity			Biomass		
	Coefficient Estimate	Standard Error	F-value	Coefficient Estimate	Standard Error	F-value
Intercept	7.22	0.0253	228.74	0.7451	0.0020	263.08
A-Peptone	0.1508	0.0129	135.76	0.0111	0.0010	116.37
B-Yeast Extract	0.0433	0.0129	11.21	0.0098	0.0010	90.06
C-NaCl	-0.1192	0.0129	84.74	0.0007	0.0010	0.4210
D-Naringin	0.0125	0.0129	0.9324	0.0051	0.0010	24.48
E-KH2PO4	0.3483	0.0129	724.06	0.0371	0.0010	1302.76
AB	-0.1525	0.0159	92.52	-0.0129	0.0013	104.69
AC	0.0050	0.0159	0.0995	0.0058	0.0013	20.88
AD	0.5288	0.0159	1112.24	0.0410	0.0013	1061.66
AE	-0.2100	0.0159	175.44	-0.0166	0.0013	174.56
BC	-0.2250	0.0159	201.40	-0.0055	0.0013	19.10
BD	0.1488	0.0159	88.03	0.0193	0.0013	234.03
BE	0.3175	0.0159	401.04	0.0229	0.0013	330.47
CD	-0.1937	0.0159	149.34	-0.0226	0.0013	323.29
CE	0.0675	0.0159	18.13	0.0020	0.0013	2.53
DE	0.0162	0.0159	1.05	-0.0073	0.0013	33.20
A^2	0.1123	0.0117	91.94	0.0064	0.0009	47.56
B^2	-0.0215	0.0117	3.36	-0.0038	0.0009	17.08
C^2	-0.0577	0.0117	24.31	-0.0021	0.0009	5.06
D^2	-0.3965	0.0117	1146.50	-0.0337	0.0009	1316.22
E^2	0.0710	0.0117	36.79	-0.0003	0.0009	0.1346

Table 5. Predicted values vs experimental values for maximum naringinase activity and biomass yield

Factors	Concentration (%; w/v)	Naringinase Activity (IU/ml)		Biomass (OD_{600})	
		Predicted Value	Experimental Value	Predicted Value	Experimental Value
Peptone (A)	0.75	8.48	8.55	0.840	0.826
Yeast extract (B)	0.60				
NaCl (C)	0.75				
Naringin (D)	0.17				
KH_2PO_4 (E)	0.75				

and mathematical models in controlling biological processes. Response surface methodology was earlier investigated for higher production of naringinase from *Staphylococcus xylosus* (Puri *et al.,* 2010a). Reportedly, the production of the enzyme naringinase was greatly influenced by different media components and their interactions. Puri *et al.* (2010a) studied effect of sodium nitrate, naringin, and sucrose. The production of naringinase is expressed as 8.45 U/mL. They obtained higher naringinase production at naringin concentration of 0.50 % (w/v). The present results also corroborate with the study of Pavithra *et al.* (2012), who reported

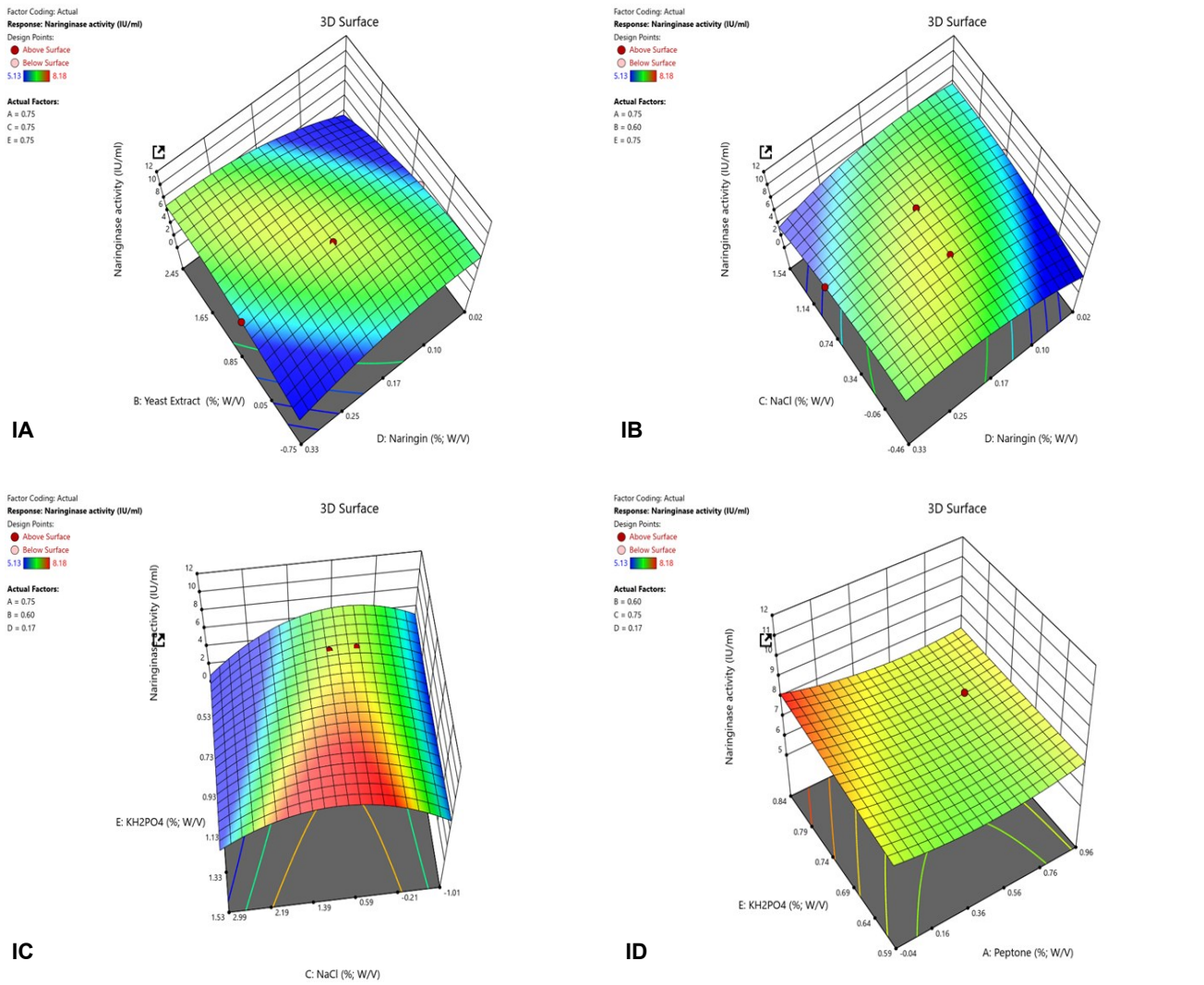

Fig. 1. *Response surfaces showing the effect of varying concentrations of yeast extract and naringin (1A); NaCl and naringin (1B); KH_2PO_4 and NaCl (1C); Peptone and KH_2PO_4 (1D) on naringinase production. The interaction among two variables has a significant effect on naringinase production*

using similar media components to produce naringinase from bacterial strain (*Serratia* sp.) isolated from soil. The media components included (g/L): KH_2PO_4 0.4g, NH_4NO_3 5g, $ZnSO_4$ 0.01g; KCl 0.2g; $FeSO_4.7H_2O$ 0.01g; $MnSO_4$ 0.01g; Agar 15g; $MgSO_4.7H_2O$ 0.2g; and naringin 1g. In a similar study, Pegu *et al.* (2019) reported the production of naringinase from *Bacillus cereus*. They optimized the various parameters for higher production of naringinase including pH, incubation time, temperature, and carbon-nitrogen source. Recently, Balaraman *et al.* (2022) produced naringinase from procured culture (MTCC, India) *Bacillus amyloliquifaciens* using liquid nutrient media containing naringin (1%). Kumar *et al.* (2015) reported the production of naringinase from *Micrococcus* sp. using media constituents including NH_4NO_3, KCl, KH_2PO_4, $FeSO_4.7H_2O$, $ZnSO_4$, $MnSO_4$, $MgSO_4.7H_2O$ and naringin as inducer.

Recently, *Bacillus subtilis* strain BSnari, an isolate from the Red Sea has been reported for naringinase production with activity of 7.09 U/ml (Selim *et al.,* 2023). The media majorly included sucrose (1.5%), citrus peel powder (0.6%) and soybean meal (1%) with pH of 7. Mukund *et al.* (2014) reported the production of naringinase from an isolated strain *Bacillus methylotrophicus.* They optimized the production of naringinase and found an increase in enzyme production (7.46 U/L) by using sucrose-yeast extract compared to the basic medium. Statistical optimization was used further for higher production of the enzyme and a level of higher production of naringinase (12.05 U/L) was obtained. The present study contributes to the existing knowledge about the production of naringinase from bacterial sources. Most studies have reported the production of naringinase from fungal sources while bacterial sources have been studied to a lesser extent. Though, in recent times, naringinase production from bacterial sources has gained some pace, it still requires extensive re-

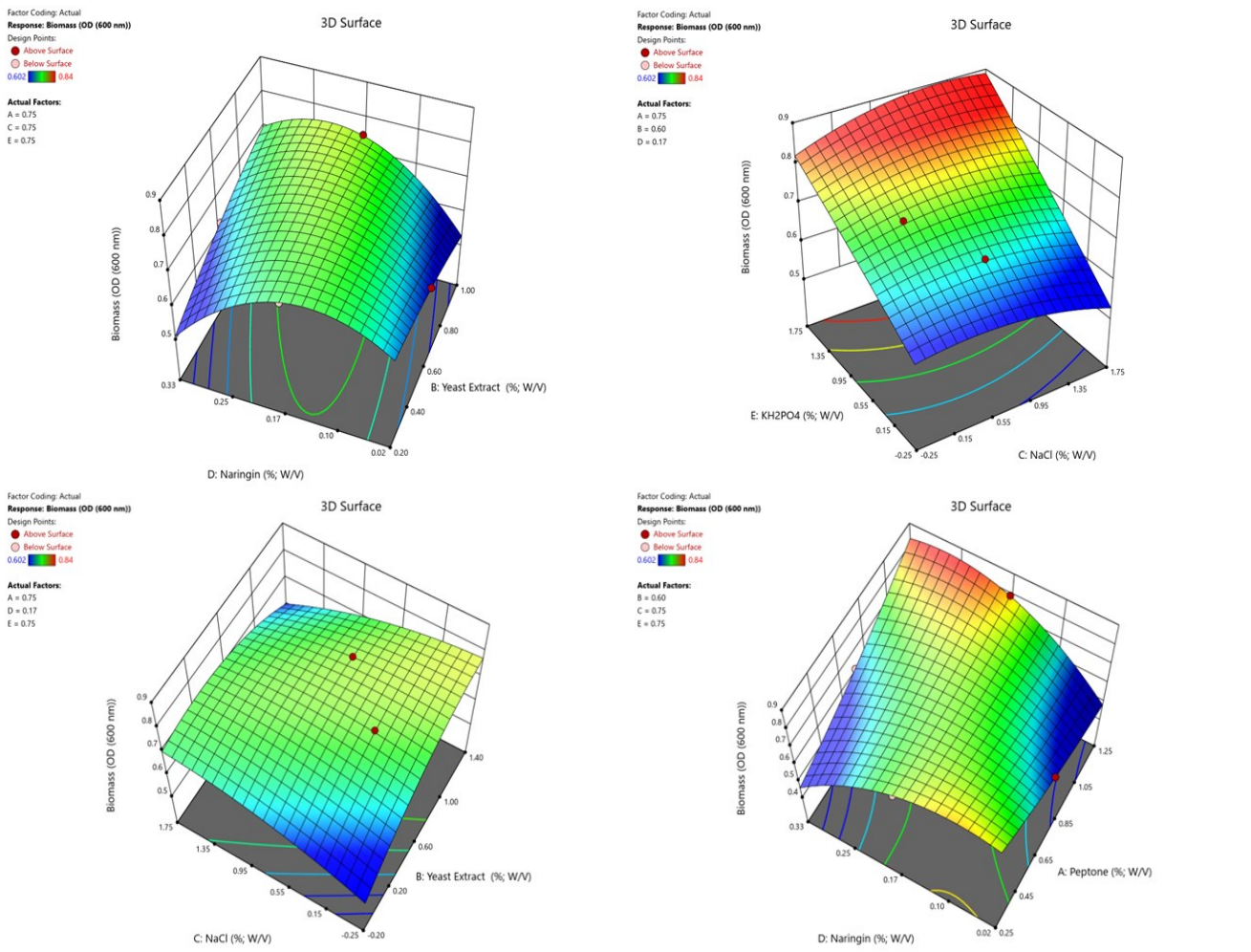

Fig. 2. *Response surfaces showing the effect of varying concentrations of naringin and yeast extract (2A); KH_2PO_4 and NaCl (2B); yeast extract and NaCl (2C); naringin and peptone (2D) on biomass yield*

search and focus on naringinase production from bacteria.

Conclusion

In the present study, optimal levels of media components viz. peptone (0.75%; w/v), yeast extract (0.60%; w/v), NaCl (0.75%; w/v), naringin (0.17%; w/v) and KH_2PO_4 (0.75%; w/v) naringinase production was determined and validated through experimentation. Among the five variables, naringin was the highly effective constituent for naringinase production from *Paenibacillus stellifer* RAMCM-44. Naringin acts as an activation factor and inducer for naringinase production. This emerged as a potent regulator of naringinase expression, with interactions among various media components peptone, yeast extract, NaCl, KH_2PO_4, and naringin impacting both naringinase activity and biomass yield. Modulating the concentration of media constituents can stimulate the production of naringinase by *P. stellifer* . Whole cells with higher naringinase production potential can be biocatalysts for industrial applications. This study represents the first application of RSM for naringinase production from *P. stellifer* AMCM-44. It may give important insights into naringinase production from bacterial sources and the role of media optimisation in bioprocess technology, particularly industrial enzymes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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