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Optimization Of Exopolysaccharide Production By Marine Haloalkalotolerant *Psudomonas Aeroginosa* Using Response Surface Technique.

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Abstract :

Evaluating an optimal conditions for growth and exopolysaccharide production has been carried out using laboratory isolated marine by *Psudomonas aeroginosa* . The sequential statistical methods were used to maximize exopolysaccharide production. Initially, a Plackett-Burman design was used to optimize the variable i.e. nutritional components such as four carbon sources, four nitrogen sources ,four metal sources, out of which glucose ,ammonium sulfate, magnesium sulfate sodium hydrogen phosphate significant for exopolysaccharide production ($P < 0.05$). Further investigation of effect of selected four nutrients using a Response Surface Methodology (second –order central composite design (CCD) was done to optimize exopolysaccharide production, which was adequately approximated with a full quadratic equation obtained from a two-factor level design. The analysis of quadratic surfaces showed that glucose ,ammonium sulfate, magnesium sulfate sodium hydrogen phosphate has significant effect on exopolysaccharide production ($P < 0.05$).The determined (R2) value was 0.97 and adjusted R-squared 0.94 , indicating a good fitted model for EPS production. The F-test applied for analysis of variance (ANOVA), F value was found to be 34.73 explain the good correlation of independent variables . Validation of the experimental model was done where maximum exopolysaccharide production (4.52 g/l) was obtained with 1.14% glucose ,0.72% ammonium sulfate, 0.054 %magnesium sulfate,0.08 % sodium hydrogen phosphate . In these conditions, the maximum EPS yield was 4.52 g/l which was 2 fold in increase as compared to basal medium .

Key words: Exopolysaccharide, Response Surface Methodology, *Psudomonas aeroginosa*

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Introduction

Exopolysaccharide produced by a wide variety of microorganisms which are generally water soluble gums having novel and unique physical properties. Exoolysaccharides has found a wide range of applications in the food, pharmaceutical, textile ,dairy and other industries. Production of EPS by bacterial species in culture is greatly influenced by a number of factors, such as phases of growth, nutritional status and the environmental conditions. The nature and concentration of nutrients in particular, are necessary components for stimulation of growth and synthesis of EPS (Laws and Marshall, 2001; Pal and Paul, 2008). Therefore there is a need of medium optimization.

Medium optimization is still one of the most critically investigated phenomenon that are carried out before any large scale production process . It is part of biopharmaceutical as well as fermentation process development .Optimization is expected to increase the yield of the final product so many optimization techniques are available for optimization of fermentation medium and fermentation process such as one-factor-at-a-time, factorial design, Plackett and Burman design, central composite design, response surface methodology.Conventional or statistical methods are available for optimization of nutritional requirements such as C,N and metal sources which involves changing one independent variable at a time keeping the others at fixed level. In comparison, the statistical methods offer several advantages which is simple ,rapid and reliable which helps to shortlists significant nutrient, It also helps understanding the interactions among the nutrients at various concentrations and reduces the total number of experiments immensely resulting in saving time and also material.(Chauhan et al 2007).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, searching optimum conditions for desirable responses and evaluating the relative significance of several affecting factors even in the presence of complex interactions. Several researchers have been used response surface methodology for optimization of bioprocesses such as fermentation media, cultivation and process conditions (Yao et al., 2009; Ruchi et al., 2008), enzyme production (Mohana et al., 2008; Burkert et al., 2004) and extracellular polysaccharide production of *Oudemansiella radicata* (Zou, 2005).

We isolated EPS-producing marine bacteria from the coastal regions Anjerle beach, Dapoli, India which was a gram negative, rod-formed bacterium, requiring 3.5 % salt for growth. This strain was identified as *Psudomonas aeroginosa*and the EPS produced by this microorganism

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was designated as AB4- EPS. The aim of the present work is to screen and optimize the nutrients variables for EPS production from laboratory isolated haloalkalotolernt *Psudomonas aeroginosa* using statistical techniques.

Materials and method :

Microorganism used :
Psudomonas aeroginosa (GenBank Accession No.MH 324730), a potent EPS producing moderately halophilic and alkalophilic bacterium isolated from Aniller beach , Dapoli ,India was used throughout this study. The isolate was maintained on Zubella marine agar medium by subculturing at a regular interval of one month and stored at 4 °C as and when required. Stock cultures were maintained at -4°C in MRS medium containing 20% glycerol.

Production of Exopolysaccharide :
The isolated AB4 strains was grown in YMG broth, pH10 seeded with 1% inoculum of overnight culture and maintained aerobically at 30°C for 72 hrs. Previously all these factors were analyzed by OATF was considered .

Extraction of Exopolysaccharide:
Modified method from Wu *et al*(2009) was used for the extraction of EPS .Inoculated YMG broth was centrifuged and one volume of Supernatant fluid was mixed with three volumes of 95 % ethanol, stirred vigorously, and incubated at 4 ° C for 18 h to precipitate the exopolysaccharide, which was separated by centrifugation at 14,000 rpm for 20 min at 4 °C and dried at 60 °C at constant volume . The EPS concentration was expressed as milligram per milliliter or gm /liter.

Optimization of process parametres by using response surface methodology (RSM)
The chemically defined standard medium for EPS production was first optimized by One Variable at a Time (OVAT) approach and Plackett-Burman design (data not included).Four nutrients were selected from parteo chart of placket burman design. This component was added in medium and used for further optimization by applying RSM of central composite design (CCD). Other components of the medium were (w/v) yeast extract 0.5%, NaCl 3.5 %, temperature 30 C ,pH 10, maintained as constant.

Experimental design

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The levels of four independent variables obtained previously i.e., glucose (A), ammonium sulfate (B),magnesium sulfate (C) and sodium hydrogen phosphate and (D) were optimized by RSM. The centralcomposite design (CCD) with four factors at four levels was employed to investigate the main effects of each factor and interactions amongst them. The four coded levels investigated in the current study were high (+1) and low (-1) coded value . The statisticalsoftware package "Design Expert®" version 11.0 (Stat Ease, Inc, Minneapolis, USA) was usedto generate polynomials and the contour plots. All experiments were carried out in triplicates.

Table No. :1 Actual values of the significant factors used in CCD

Sr.No.	Factors	Code	Minimum	Maximum	Coded Low	Coded High
1	Glucose	A	7.10	24.30	(-1) 11.40	(+1) 20.00
2	ammonium sulfate	B	5.40	7.8	(-1) 6.00	(+1) 7.20
3	Magnesium Sulfate	C	0.46	0.78	(-1) 0.62	(+1) 0.70
4	sodium hydrogen phosphate	D	0.53	0.89	(-1) 0.54	(+1) 0.80

Analysis of variance (ANOVA)

A second-order polynomial equation was established on the basis of analysis ofvariance. The optimum ratio of the medium components was found using the Design- Expert11.0 software optimization toolbox. Standard deviation, F-value, R^2 values were also analyzed. Lack of fit was also checked .

Result and discussion

Media optimization using central composite design

The final medium optimization and interaction amongst the screened factors were considered using CCD. All the experiments were carried out in triplicate , and average yield of EPS given in Table 2was subjected to multiple linear regression analysis. The effect of glucose , ammonium sulfate ,magnesium sulfate and sodium hydrogen phosphate for EPS production was described in the form of second-order polynomial model in coded units (Eq. 1).

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EPS (g/l) = 3.53 -0.060 A +0.1617 B + 0.1017- 0.1425 D -0.1571 A2 -0.1196 B2 -0.0058 C2 -0.1046 D2 -0.1625 AB -0.0687 AC 0.2813AD 0.0175 BC – 0.1075 BD -0.2587 CD (Eq. 1)

Table 2 : CCD matrix of independent variables used in RSM with corresponding experimental values of EPS production

Std	Run	Glucose (g/l)	Ammonium sulfate (g/l)	Na2HPO4 (g/l)	MgSo4	experimental values EPS yield g/l
23	1	15.7	6.6	0.71	0.46	3.5
14	2	20	6	0.8	0.7	3.21
10	3	20	6	0.62	0.7	3.59
9	4	11.4	6	0.62	0.7	2.77
30	5	15.7	66	0.71	0.62	3.56
5	6	11.4	6	0.8	0.54	3.61
6	7	20	6	0.8	0.54	3.12
25	8	15.7	6.6	0.71	0.62	3.57
22	9	15.7	6.6	0.89	0.62	3.62
26	10	15.7	6.6	0.71	0.62	3.65
18	11	24.3	6.6	0.71	0.62	2.72
24	12	15.7	6.6	0.71	0.78	2.63
8	13	20	7.2	0.8	0.54	3.4
29	14	15.7	6.6	0.71	0.62	3.53
7	15	11.4	7.2	0.8	0.7	4.53
20	16	15.7	7.8	0.71	0.62	3.32
16	17	20	7.2	0.8	0.7	2.97
17	18	7.1	6.6	0.71	0.62	2.99
19	19	15.7	5.4	0.71	0.62	2.69
4	20	20	7.2	0.62	0.54	2.7
1	21	11.4	6	0.62	0.54	2.76
3	22	11.4	7.2	0.62	0.54	3.63
15	23	11.4	7.2	0.8	0.7	3.03
12	24	20	7.2	0.62	0.7	3.46
21	25	15.7	6.6	0.53	0.62	3.3
2	26	20	6	0.62	0.54	2.6
11	27	11.4	7.2	0.62	0.7	3.1
28	28	15.7	6.6	0.71	0.62	3.57
13	29	11.4	6	0.8	0.7	2.54

27	30	15.7	6.6	0.71	0.62	3.39

Table 3Analysis of variance of second-order Polynomial model for effect of variable on EPS production

Source	Sum of square	Df	Mean Square	F value	P-Value
Model	5.60	14	0.3997	34.73	<0.0001
A-Glucose	0.0888	1	0.0888	7.72	0.0141
B-Ammonium sulfate	0.6273	1	0.6273	54.50	<0.0001
C-Na2HPO4	0.2481	1	0.2481	21.55	0.0003
D-MgSo4	0.4874	1	0.4874	42.35	<0.0001
AB	0.4225	1	0.4225	36.71	<0.0001
AC	0.0756	1	0.0756	6.57	0.0216
AD	1.27	1	1.27	109.97	<0.0001
BC	0.0049	1	0.0049	0.4258	0.5240
BD	0.1849	1	0.1849	16.07	0.0011
CD	1.07	1	1.07	93.08	<0.0001
A2	0.6768	1	0.6768	58.81	<0.0001
B2	0.3922	1	0.3922	34.08	<0.0001
C2	0.0009	1	0.0009	0.0811	0.7797
D2	0.3000	1	0.3000	26.07	0.0001
Residual	0.1726	15	0.0115		
Lack of fit	0.1359	10	0.0136	1.85	0.2581
Pure Error	0.0367	5	0.0073		
Cor Total	5.77	29			

$R^2 = 0.9701$ Adjusted $R^2 = 0.9421$,Predicted $R^2 = 0.8551$

The P-value and F-test was performed to determine the significance of the model. The residuals analysis was performed to validate the model at 95 % confidence level. In this model, the R2 value of 0.9701 indicated that the response model can explain 97.01 % of the total variations. In general, a regression model having an R2 value higher than 0.9 is considered to have a very high correlation (Haaland 1989). The value of the adjusted determination coefficient (R adj 2 = 94.21%) was also high enough to indicate the significance of the model. The model fitted well with EPS production, and the optimal values from the model were justified (p >0.001). The P-Value less than 0.05 indicates the significance of the model terms. The ANOVA results given in

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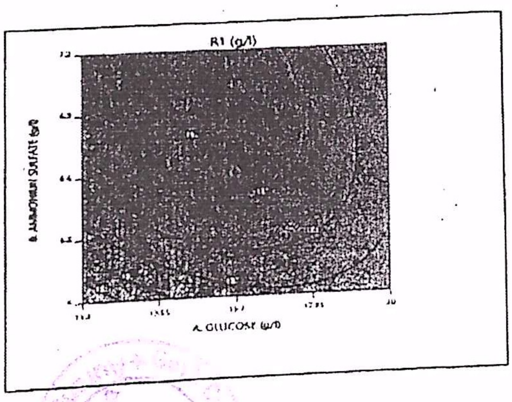
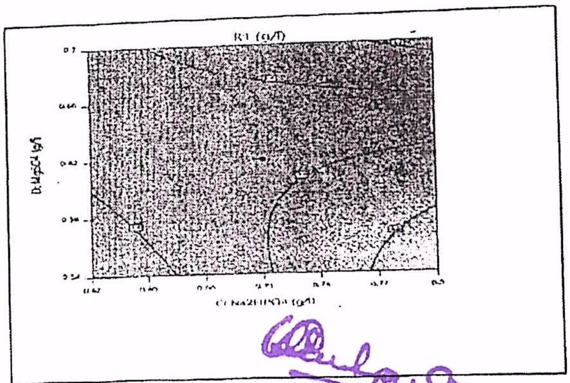
Table 3indicate that the linear and square terms in second-order polynomial model were highly significant (p>0.005) and adequate to represent the relationship between EPS. 2 D contour plots were chosen to determine the interaction of the fermentation conditions and the optimum levels for EPS production. The shape of the contour plot is used to identify theinteraction of variables. Strong interaction exists if contour lines are elliptical in shape, and no interaction is observed if contour lines are circular. The same result was accordance with Sethuraman *et al* 2015.

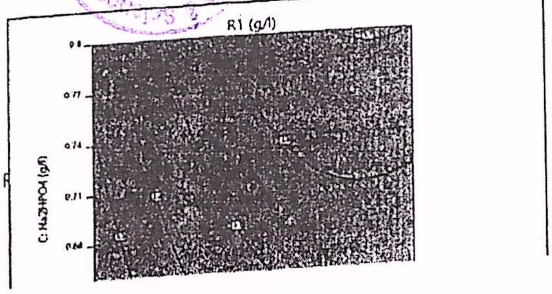
Conclusion

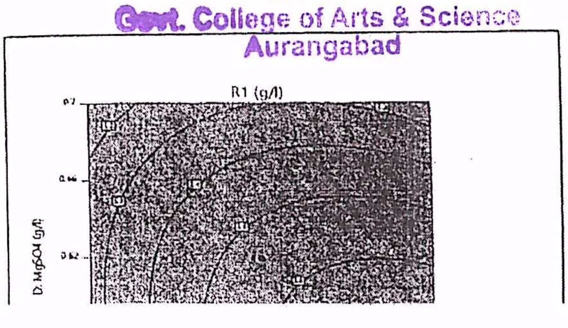
Statistical optimization was confirmed to be a powerful method for the optimization of the EPS production by *Psudomonas aeroginosa*. The four medium components were already screened to be the most significant components for EPS production by PB experiment. CCD was proposed to study the interaction effects of fermentation condition. Maximum EPS production 4.6 g/L was obtained using the optimized condition of glucose 1.14 (%),ammonium sulfate 0.72 (%),magnesium sulfate 0.054 (%), and sodium hydrogen phosphate 0.08 (%) at 10 pH, and time 72 h. By applying this conditions, the maximum EPS yield was 4.52 g/l which was 2 fold in increase as compared to basal medium .

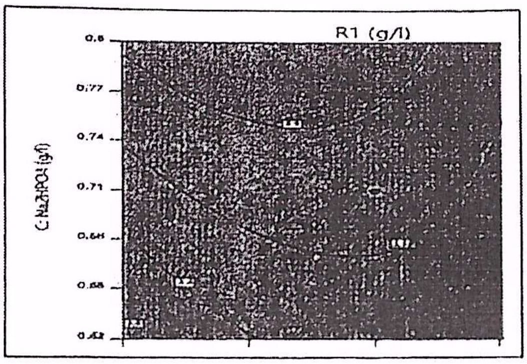
Fig. 1 Contour plots showing the combined effect of the medium variables (glucose , ammonium sulfate ,magnesium sulfate and sodium hydrogen phosphate) on EPS production by AB4 *Psudomonas aeroginosa*

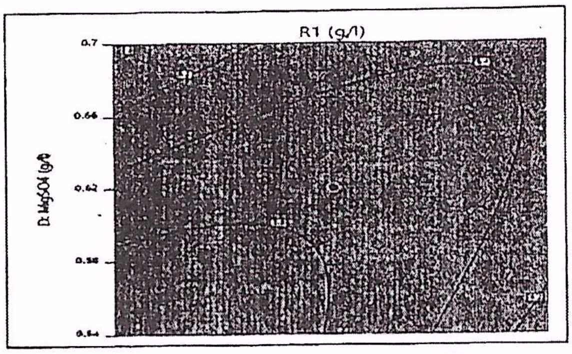












References :

1. Burkert JFM ., Maugeri F., Rodrigues MI .(2004). Optimization of extracellular lipase production by *Geotrichum* sp. Using factorial design. Bioresour. Technol., 91: 77-84.

2. Chauhan K, Trivedi U, Patel KC. Statistical screening of medium components by Plackett-Burman design for lactic acid production by Lactobacillus sp. KCP01 using date juice. Biores Technol 2007; 98: 98-103.

3. Haaland PD (1989) Experimental design in biotechnology. Marcel Dekker, New York

4. Laws, A.P. and Marshall,V.M. 2001. The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. Int. Dairy J. 11: 709-721. DOI: 10.1016/S0958-6946(01)00115-7

5. Mohana S, Shah A, Divecha J, Madamwar D (2008). Xylanase production by *Burkholderia* sp. DMAX strain under solid state fermentation using distillery spent wash. Bioresour. Technol., 99:7553-7564.

6. Pal, A. and. Paul A.K. (2008). Microbial extracellular polymeric substances: Central elements in heavy metal bioremediation. Indian J. Microbiol., 48: 49-64.DOI: 10.1007/s12088-008-0006-5

7. Ruchi G, Anshu G, Khare K .(2008). Lipase from solvent tolerant *Pseudomonas aeruginosa*strain: production optimization by response surface methodology and application. Bioresour. Technol., 99: 4796-4802.

8. Sethuraman Padmanaban ,Nagarajan Balaji , Chandrasekaran Muthukumaran ,Krishnamurthi Tamilarasan1 (2015)Statistical optimization of process parametersfor exopolysaccharide production by Aureobasidium pullulansusing sweet potato based medium 3 Biotech (2015) 5:1067–1073DOI 10.1007/s13205-015-0308-3

9. Yao Y., Lv Z ., Min H ., Lv Z ., Jiao H. (2009). Isolation, identification and characterization of a novel *Rhodococcus* sp. Strain in biodegradation of tetrahydrofuran and its medium optimization using sequential statistic-based experimental designs. Bioresour. Technol., 100: 2762- 2769.

10. Zou X. (2005). Optimization of nutritional factors for exopolysaccharide production by submerged cultivation of the medicinal mushroom *Oudemansiella radicata.* World J. Microbiol. Biotechnol., 21: 1267- 1271.