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Optimization of resistomycin production purified from *Streptomyces aurantiacus* AAA5 using response surface methodology

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Abstract

Response surface methodology was employed to optimize the composition of medium for the production of resistomycin by *Streptomyces aurantiacus* AAA5 in submerged fermentation. Cassava pulp, soybean meal and potassium nitrate were found to have significant effects on resistomycin production by Plackette Burman followed by Box Behnken design. A mathematical model was developed to identify the optimum concentration of key nutrients for higher resistomycin production. A quadratic model was found to fit the resistomycin production. The analysis revealed that the optimum values of the tested factors were 1.54g soybean meal, 10.39% cassava pulp and 1.3g potassium nitrate yielded 193.4 mg/L of resistomycin. A production of 194.3 mg/L which was in agreement with the prediction was observed in verification experiment. In comparison to the original production level, 3.7 fold increases had been obtained.

Key words: Resistomycin; *Streptomyces aurantiacus* AAA5; Optimization; Response surface analysis; Cassava pulp

Introduction

Resistomycin (3, 5, 7, 10-Tetrahydroxy-1, 1, 9-trimethyl-2*H*-benzo [*cd*] pyrene-2, 6(1*H*)-dione), an unusual (benzopyrenequinone) aromatic polyketide antibiotic with unique structure possess bactericidal including mycobacterial and vasoconstrictive activity. It inhibits RNA and protein synthesis (Arora 1985) and has also been implicated as a modulator of apoptosis that serves as a valuable tool in cell biology research. This yellow pigmented cytotoxic compound is first reported in *Streptomyces resistomycificus* (Brockmann and Schmidt-Kastner 1951). The biosynthesis gene cluster of resistomycin has been isolated and characterized from *S. resistomycificus,* that code for a type II polyketide synthase (Jakobi and Hertweck 2004). Resistoflavine, a derivative of resistomycin is produced by *S. chibaensis* AUBN(1)/7. It shows cytotoxicity against cell line HMO2 (gastric adenocarcinoma) and HepG2 (hepatic carcinoma) and possesses weak antibacterial activities against gram-positive and gram-negative bacteria (Gorajana et al. 2007). In previous study, resistomycin producing actinomycetes, *Streptomyces aurantiacus* AAA5 was isolated from Western Ghats, the largest and most biological diverse area of India. Taxonomy of producing strain, fermentation, physico chemical properties and structure of resistomycin was also determined. Hence, the present study is mainly focussed on the optimization of culture conditions using factorial design for resistomycin production from low-cost sources. Various environmental and fermentation parameters such as aeration, agitation, temperature, pH and concentrations of the medium components adversely affect the production in the bioprocess development. Optimization of the fermentation conditions is therefore very important for maximizing the yield and productivity and to minimize the production costs. Most of the recent optimization efforts have relied on statistical experimental design and response surface analysis (Haaland 1989). Statistical design is a powerful tool that can be used to account for the main as well as interactive influences of fermentation parameters on process performance. In this study, a combination of traditional non-statistical technology and statistical technology based experimental designs was used to optimize the medium for resistomycin production. One factor at a time experiments were used to optimize the physical parameters pH, temperature, incubation period. In the first step of statistical design, Plackett- Burman experiment was used to evaluate the impact of various factors viz., inexpensive carbon sources (cassava pulp and cassava starch), starch, nitrogen

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sources (soybean meal, potassium nitrate, yeast extract, peptone, urea) on production of resistomycin. A Box-Behnken design using response surface methodology (RSM) was then employed to build models to evaluate the effective factors and supply their interaction and select optimum conditions. In this paper we report on optimization of culture medium for resistomycin production with cheaper substrates for an economic antibiotic production.

Materials and methods

Materials

Cassava starch and cassava pulp (semi-solid liquid waste) were procured from sago factory (Salem, India). Soybean meal was obtained from local market. All the chemicals, salts and organic solvents used in the present study were of analytical grade (Merck, Mumbai, India). The growth media required for microbial analysis were procured from Hi Media laboratories, Mumbai, India.

Microorganism

In our previous study of pigmented actinomycetes, an orange – yellow pigmented strain was isolated from Annaikatti hills, Western Ghats, India, an unexplored area for actinomycetes population. Starch casein agar was used for isolation and was sub-cultured periodically (soluble starch 1.0%, casein 0.03%, KNO_3 0.2%, NaCl 0.2%, K_2HPO_4 0.005%, $CaCO_3$ 0.002%, $FeSO_4$. $7H_2O$ 0.001% and agar 2.0%), incubated at 37˚C for 7 days to achieve good sporulation and pigmentation and was then preserved in 20% glycerol at -80˚C. The strain was identified as *Streptomyces aurantiacus* AAA5 on the basis of partial 16s rRNA sequencing.

Medium and culture conditions

The strain *Streptomyces aurantiacus* AAA5 was grown in Erlenmeyer flasks at 37˚C for 2 days containing 50 mL of seed medium (starch-casein broth) on a rotary shaker (220 rpm). Erlenmeyer flasks containing 100 mL of production medium (modified starch –casein broth with 1% soybean meal) was inoculated with 5% of pre-culture and incubated on a shaker (220 rpm) at 37˚C for 9 days. The concentration of various carbon and nitrogen sources and culture conditions varied according to the experimental design described below. Unless otherwise stated, the cultivation was carried out using 250 mL flasks on a 220 rpm shaker at 37˚C for 9 days. All experiments were performed in duplicate.

Analytical method

Biomass was harvested and the cells were washed twice with distilled water. A 5 mL quantity of methanol was added to the biomass and ground using mortar and pestle and methanol extract (orange-yellow) was then separated from the cells by centrifugation at 10,000 rpm for 20 min. This extraction procedure was repeated until the cells were completely bleached and all the supernatants were collected for measuring the concentration (Wang et al. 2009). The crude extract was subjected to LC-MS analysis and both total ion chromatogram (TIC) and contour plot from diode array detection (DAD). The crude extract is dominated by a compound eluted at 15.6 min. This compound displays several peak maxima and the dominating mass/ charge in the corresponding mass spectrum was 377.102. Dictionary of Natural Products (http://www.chemnetbase.com/) was searched using the accurate mass of 376.094 with a 5 ppm window. The search returned ten hits of which resistomycin, the most likely identity was based on corresponding UV / Vis absorbance information. Purified compound was scanned by using an ultraviolet spectrophotometer and the maximum absorbance of resistomycin was found to be at 457 nm. Hence the absorbance of crude extract was measured at 457 nm and concentration of resistomycin pigment was calculated according to the following formula (Wen et al. 1993).

$$Resistomycin(mg/L) = \frac{ADV_1}{15400V_2}$$

Where A the absorbance of methanol extract solution at 457 nm, D the dilution ratio, V_1 the volume of methanol added, 15400 is extinction coefficient of resistomycin and V_2 is the volume of methanol extract. Further, purification of the crude extract by silica gel column chromatography yields a pure yellow active compound. This compound was characterized through UV-vis, FT-IR, NMR analyses and was found to be a quinone-related antibiotic resistomycin (Vijayabharathi et al. 2011).

Optimization of physical parameters using one factor at a time approach

The effect of pH on biomass and resistomycin production was studied by varying the pH of the medium from pH 6.0-11.0. The pH was adjusted using 0.1N hydrochloric acid or 0.1N sodium hydroxide. The effect of temperature on biomass and resistomycin production was studied by incubating the production medium at varying temperature from 25 ˚C to 50 ˚C. 72h Culture was inoculated with an inoculum concentration of 5 % (v/v) and incubated at 37 ˚C for 9 days for these optimization. To optimize incubation period culture was inoculated with an inoculum concentration of 5 % (v/v) and incubated at 37 ˚C for 15 days to determine the influence of incubation period. In all three parameters after incubation, biomass was harvested from 100 mL of culture at an interval of 3 days, filtered through Whatman filter paper no. 1, washed with distilled water and dried them at 105 ˚C to a constant weight and resistomycin concentration was determined with the methanol extract of biomass.

Cassava pulp extract

Cassava pulp is a semi-solid material obtained as waste during processing of tapioca but rich in starch content. It was dried in sunlight, powdered and used on dry weight basis. According to the experimental design, cassava pulp was weighed and boiled with appropriate amount of distilled water for 30 minutes to obtain 100 mL of extract which was further filtered through muslin cloth and centrifuged at 12,000 rpm for 15 minutes. This extract was used for preparation of fermentation broth.

Experimental design

Optimization of media components for maximum resistomycin production was carried out following the two statistical approaches. Plackett-Burman design was employed for initial screening of the most significant factors which potentially influences the responses. To describe the nature of the response surface in the optimum region, a Box Behnken design and the response surface

methodology was performed.

Plackett- Burman design

The Plackett- Burman design is a two-factorial/ design based on the first–order polynomial model

$$Y = \beta_o + \Sigma \beta_i X_i$$

Where Y is the response (resistomycin concentration), β_0 is the model intercepts, β_i is the linear coefficient and X_i is the level of the independent variables (Plackett and Burman 1946). In the present study, a set of twelve experiments was constructed using the Design Expert software version 8.0.2 for eight components: soybean meal, cassava starch, cassava pulp, starch, potassium nitrate, urea, peptone, yeast extract. These factors were selected based on our previous preliminary experimental results. Each factor was tested at two levels, low and high and statistical designs are always expressed in coded values as -1(low level) and +1 (high level). The experimental design for screening the variables is shown in Table 1. Three dummy variables were studied in 12 experiments to calculate the standard error. Resistomycin production was carried out in duplication and the average value was taken as response. Effect of medium components on resistomycin production was determined by p-values obtained by analysis of variance (ANOVA) using Design Expert software and p-value (Prob> F) less than 0.050 indicate that factors are significant.

Table 1: Plackett-Burman design variables in coded levels with resistomycin as response

Run	A	B	C	D	E	F	G	H	I D1	J D2	K D3	Resistomycin (mg/L)
1	1.	1.	0.	3	0.	0.	0.	0.	+1.	-	+1.	68.9
2	1.	0.	1.	1	0.	0.	0.	0.	-	-	-	128.6
3	1.	0.	0.	3	0.	0.	0.	0.	-	+1.	+1.	92.5
4	0.	0.	1.	3	0.	0.	0.	0.	+1.	+1.	-	33.2
5	1.	1.	0.	1	0.	0.	0.	0.	-	+1.	-	101.4
6	0.	0.	0.	1	0.	0.	0.	0.	+1.	+1.	+1.	82.8
7	0.	1.	0.	1	0.	0.	0.	0.	+1.	-	-	42.6
8	0.	1.	1.	1	0.	0.	0.	0.	-	+1.	+1.	39.8
9	1.	0.	1.	1	0.	0.	0.	0.	+1.	-	+1.	72.5
10	1.	1.	1.	3	0.	0.	0.	0.	+1.	+1.	-	96.7
11	0.	1.	1.	3	0.	0.	0.	0.	-	-	+1.	28.4
12	0.	0.	0.	3	0.	0.	0.	0.	-	-	-	22.5

A- Soybean meal (g); B- Cassava starch (g); C- Starch (g); D- Cassava pulp(%); E- Peptone (g); F- Urea (g); G- Potassium nitrate (g); H- Yeast extract (g); D1-D3- Dummy variables

Box-Behnken design

Based on Plackett-Burman design, the significant parameters were selected and their concentrations were arranged at 17 levels with five replicates of the centre point. The experimental design was shown in the Table 2. Using this design, factors of highest confidence level percentage are prescribed into three levels, coded -1, 0 and +1 for low, middle and high concentration. For predicting the optimal point, a second order polynomial function was fitted to correlate relationship between variables and response (resistomycin production). For the three factors the equation is

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Where Y is the predicted response, β_0 model constant; X_1, X_2 and X_3 independent variables; β_1, $\beta_2$and β_3 are linear coefficients; β_{12}, $\beta_{13}$and β_{23} are cross product coefficients and β_{11}, $\beta_{22}$and β_{33} are the quadratic coefficients (Box and Behnken 1960). The validity of the model was determined based on Student's t test. Data from the Box-Behnken design were subjected to first and second order multiple regression analysis using least-squares regression methodology to obtain the parameters of the mathematical models. Model co-efficients, R^2 values, F values and significance probabilities generated by the software were used to justify the significance of each experimental variable. The optimal fermentation conditions for enhanced yield of resistomycin production were obtained by solving the regression equation and by analysing the response surface contour plots using the same software.

Table 2: The design and result for Box-Behnken design

Run	A Soybean meal	B Cassava pulp	C Potassium nitrate	Resistomycin (mg/L)	
				Actual	Predicte
1	3	10	1.5	123.6	124.4
2	4.5	12.5	0.3	125.4	127.3
3	1.5	10	0.9	187.6	188.7
4	1.5	12.5	1.5	172.4	170.6
5	3	12.5	0.9	99.8	100.3
6	1.5	12.5	0.3	100.2	98.7
7	1.5	15	0.9	152.6	154.9
8	4.5	10	0.9	110.4	108.1
9	4.5	15	0.9	156.3	155.2
10	3	12.5	0.9	100.8	100.3
11	3	15	0.3	128.6	127.8
12	3	12.5	0.9	100.1	100.3
13	3	10	0.3	132.5	132.9
14	3	15	1.5	143.2	142.8
15	3	12.5	0.9	102.4	100.3
16	3	12.5	0.9	98.6	100.3
17	4.5	12.5	1.5	60.3	61.8

Statistical analysis

The statistical and regression analyses of experimental data obtained from the Plackett- Burman factorial design and Box-Behnken design were done by using commercial software Design Expert version 8.0.2 (STAT- EASE Inc., Minneapolis, MN, USA) to determine the significant differences ($p \leq 0.05$) in response under different conditions. The response surface graphs were plotted using this software.

Results and discussion

In order to obtain the suitability of fermentation conditions for biomass and bioactive metabolite production on fermentative medium, the effect of pH, temperature and incubation period on biomass and metabolite production was necessary to investigate.

Effect of pH

pH is a significant factor that influences the physiology of a microorganism by affecting nutrient solubility and uptake, enzyme activity, cell membrane morphology, by product formation and oxidative reduction reactions (Bajaj et al. 2009). Evaluation of data at different pH conditions indicated that pH 7.0 is suitable for growth and maximum production (51.56 g/L) was also noticed at pH 7.0 (Fig.1).

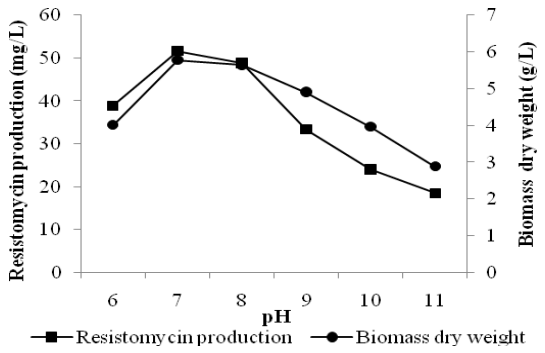

Fig. 1: Effect of pH in resistomycin production and biomass of *S. aurantiacus* AAA5

Higher or lower pH showed in reduced production values. Most of the antibiotics were observed to be in higher production in pH range 6.5-7.0. Cephamycin C was reported to be higher at pH 6.0 (Kagliwal et al. 2009).

Effect of Temperature

Temperature is an important factor as it influences metabolic activities and microbial growth. From the results it was evident that maximum resistomycin production was obtained at 40°C, clearly indicates that the strain might be a mesophilic organism. The yield of the temperature increases from 25 °C till 40 °C and began to decrease. The maximum yield was 49.99 mg/L at 40 °C (Fig. 2). This result indicates that the temperature influences resistomycin production.

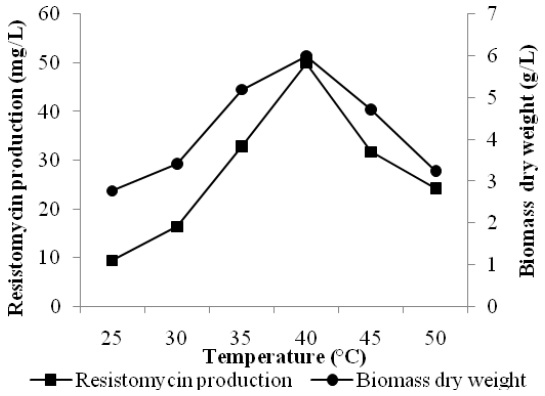

Fig. 2: Effect of temperature in resistomycin production and biomass of *S. aurantiacus* AAA5

Effect of incubation period

The maximum biomass production of 5.83 g biomass/L was reached during 9th day of incubation after which a decline phase was observed. Resistomycin also increased with increase in biomass production and reached maximum of 52.5 mg/L at 9th day of incubation (Fig. 3) and on further incubation there was no increase in resistomycin production.

This could be because the organism might have reached death phase. Usually synthesis of the secondary metabolites occurs after the growth ceases but in our case resistomycin production reaches maximum at initial idiophase itself as this production may be growth associated. Hence 9th day of incubation was found to be optimum and fixed for further optimization process.

Evaluation of factors affecting resistomycin production

The primary purpose of screening experiments is to select the main effects from less important nutritional factors. Nutritional requirements of *Streptomyces* play an important role during metabolite synthesis process. Amongst various nutritional requirements, carbon source and nitrogen source are generally regarded as important factors of metabolism, and several examples of the production of metabolites in media with optimized contents of these components are also described in the literature (Yuan et al. 2008).

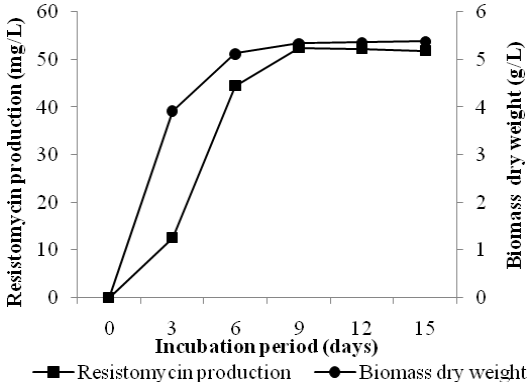

Fig. 3: Effect of incubation period in resistomycin production and biomass of *S. aurantiacus* AAA5

For the majority of *Streptomyces,* the preferred carbon source is starch, especially for the production of secondary metabolites (Syed et al. 2009). Thus, cassava pulp was found to be a significant factor, as shown by PBD results. Just as microorganisms have preferred carbon energy sources, they also have preferred sources of nitrogen. It was reported that complex nitrogen sources could increase the production of antibiotic by *Streptomyces*. These sources could sustain high antibiotic titre and this property is supposed to be linked to the slow release of nitrogenous components during the course of the fermentation. More generally, several studies have shown that nitrogen assimilation is crucial for the regulation of antibiotic production but the mechanisms involved is not clearly understood (Voelker and Altaba 2001).

In this study, Plackett-Burman design was used to detect the influence of eight factors on resistomycin production in submerged fermentation. The data in table 1 indicated there was a wide variation from 22.5 to 128.6 mg/L of the resistomycin in the 12 runs and were subjected to regression analysis and the analysis of variance (ANOVA). This variation reflected the importance of medium optimization to attain higher production. First order models were fitted to the data to evaluate the main effects of influencing factors. The statistic test factor, *F*, was used to evaluate the significance of the models and factors at 95 % confidence level. On application of ANOVA, it was found that the first order model for

resistomycin production was satisfactory. The following model was established by regression:

Resistomycin = 67.492 + 25.942 * A + 10.458 *D + 11.108 * G

where A is soybean meal, D is cassava pulp and G is potassium nitrate. The p-value is the probability that the magnitude of a contrast coefficient is due to random process variability and serves as a tool for checking the significance of each of the coefficients. A low p-value indicates a real or significant effect (Levin et al. 2005). According to ANOVA, the model of regression was significant (p-value 0.0011), and p-value for soybean meal, cassava pulp and potassium nitrate are 0.0004, 0.0453 and 0.0360 respectively. Values of prob>*F* less than 0.0500 indicate model terms are significant and in this case A, D and G are significant model terms.

The pareto chart, which has been described as a useful tool for identifying the most important effects (Haaland 1989) was also applied to determine the significant factors and graph was shown in Fig. 4. This chart shows the ranking of the factors influencing the production and is based on the coefficient estimate of factors. The factors A (soybean meal), G (potassium nitrate), D (cassava pulp), F (urea) had positive effects and factors E (peptone), B (cassava starch), C (starch) and H (yeast extract) had negative effects whereas I, J and K were assigned as dummy variables. The factors A, G and D ranked first three with coefficient estimate of 25.94, 11.11 and 10.46 respectively and were selected to determine the optimum levels. The other carbon and nitrogen sources were excluded for further optimization process and the selected factors were added in the production medium along with other inorganic salts as same. The variables temperature, incubation period and pH were used as fixed using one factor at a time approach. It is reported that actinomycetes are able to grow better in starch amended medium (Dharmaraj et al. 2009).

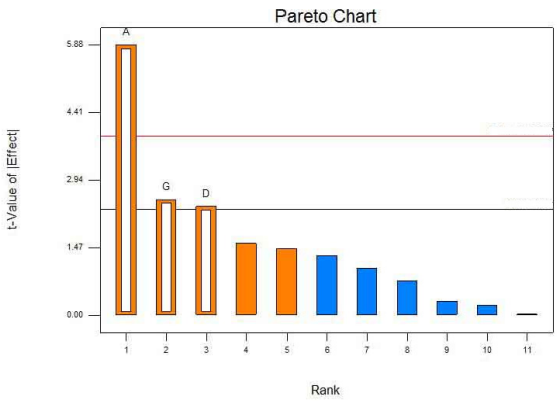

Fig. 4: Pareto chart for Plackett-Burman design showing the ranking of the medium components on the basis of coefficient estimate

Cassava pulp, a waste from sago industries which is rich in starch with 50 % is hence used for an economic cheap carbon source. Soy bean meal highly influenced the resistomycin production at +1 level. Mostly for antibiotic production soybean meal is added to the production medium which supplies adequate amount of nitrogen sources for organism growth and production. Potassium nitrate an inorganic nitrogen source was also found to have a positive influence in resistomycin production as reported by Mahalaxmi et al. (2010) for rifamycin production. More than 70% contribution on antibiotic production was observed with nitrogen sources (Potassium nitrate and Soybean meal).

Optimization of culture conditions by Box-Behnken design

The Box-Behnken design for the three factors (soybean meal, cassava pulp and potassium nitrate) was used for optimizing resistomycin production in submerged fermentation and Table 2 shows the design matrix and experimental responses and these runs produced substantially improved results for resistomycin production over those obtained from PBD experiments. The models were then submitted to statistical analysis to neglect the insignificant terms (P>0.05). Consequently, the polynomial model describing the correlation between the resistomycin production and the three variables as follows:

$$\text{Resistomycin} = +100.34 - 20.05 * A + 3.33 * B + 1.60 * C + 20.22 * A * B - 34.33 * A * C + 5.87 * B * C + 16.99 * A^2 + 34.39 * B^2 - 2.76 * C^2$$

In this experiment, the obtained model was significant (P <0.0001) according to ANOVA (Table 3). The model *F*- value of 379.21 implies the model is significant and is calculated as ratio of mean square regression and mean square residual. It is evident from the data presented that lack-of-fit was not significant (P>0.05) for this model. The value of adjusted R^2, being a measure of fitness of the regressed model, was 0.9953, which suggested that the experimental data be in good agreement with predicted responses. The interaction between soybean meal, cassava pulp and potassium nitrate (P< 0.0001, 0.0001 and 0.0010 respectively) were highly significant since smaller the p value the more significant the corresponding coefficient values (Hamedi et al. 2002).

Table 3: ANOVA of regression model

Source	Sum of squares	DF	Mean square	F value	p-value Prob > F	
Model	16285.19	9	1809.465	379.207	< 0.0001	*
A	3216.02	1	3216.02	673.976	< 0.0001	
B	88.445	1	88.445	18.535	0.004	
C	20.48	1	20.48	4.292	0.077	
AB	1636.203	1	1636.203	342.896	< 0.0001	
AC	4712.823	1	4712.823	987.658	< 0.0001	
BC	138.063	1	138.063	28.934	0.001	
A^2	1215.769	1	1215.769	254.787	< 0.0001	
B^2	4980.396	1	4980.396	1043.733	< 0.0001	
C^2	32.016	1	32.016	6.710	0.036	
Residual	33.402	7	4.772			
Lack of Fit	25.57	3	8.523	4.353	0.095	**
Pure Error	7.832	4	1.958			
Cor Total	16318.59	16				

A-Soybean meal; B-Cassava pulp; C-Potassium nitrate; *- Significant; **- not significant.

The regression models were used to construct the contour plots to explain the effects of medium components on production of resistomycin. Each figure presents the effect of two variables on the production of active substances, while other two variables were held at zero level. The circular contour plots of response surfaces suggest that the interaction is negligible between the corresponding variables. Figure 5a contour plot shows that soybean meal and cassava pulp had a remarkable interaction. An elliptical or saddle nature of the contour plots indicates the significance of the interactions between the

corresponding variables (Muralidhar et al. 2010). It is clear from figure 5b that, there was a significant interaction between cassava pulp and potassium nitrate but was not as significant as soybean meal. This implies that these carbon and nitrogen ratio was not critical and similarly there was only a slight interaction found between the 2 nitrogen sources potassium nitrate and soybean meal (Fig. 5c).

An explanation for this phenomenon maybe soybean meal and potassium nitrate had no complementary effects in promoting biomass and resistomycin production. Use of cheap nitrogen sources along with cassava pulp could lead to design low cost fermentation media for economic production of resistomycin.

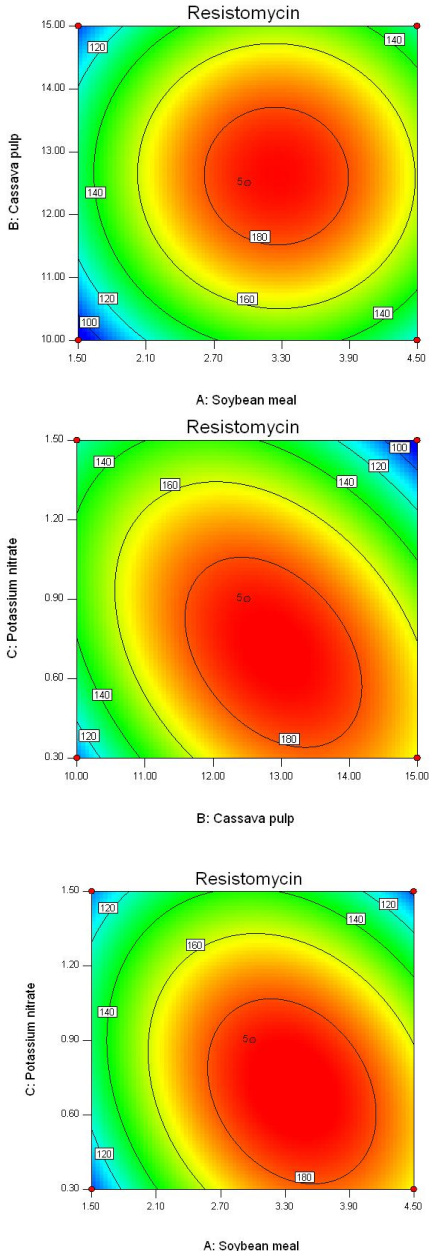

Fig. 5: Contour plot described by the model on resistomycin production. (5A) Effect of soybean meal and cassava pulp (5B) Effect of cassava pulp and potassium nitrate (5C) Effect of soybean meal and potassium nitrate

Validation of the second-order polynomial equation

In order to confirm the predicted results of the second order polynomial equation, experimental rechecking was carried out using the conditions representing the optimal factors described in table 4.

Table 4: Design and results of validation experiment

Run	Soybean meal (g)	Cassava pulp (%)	Potassium nitrate (g)	Resistomycin (mg/L)	
				Experimental	Predicted
1	1.5	10.94	1.5	189.34	190.69
2	1.51	10.14	1.01	187.56	188.86
3	1.53	10.01	1.0	190.34	191.62
4	1.54	10.39	1.3	194.23	193.04
5	1.68	10.70	1.33	180.69	177.39

Under the optimal conditions, the maximum resistomycin production of 194.23 mg/L was obtained, with agreed predicted value of 193.04 mg/L and suggested that the model was valid for predicting the resistomycin production. The optimal condition for maximum resistomycin production was reached with 1.54 g of soybean meal, 10.39 % of cassava pulp and 1.3 g of potassium nitrate. Initially, the production was 52.5 mg/L and on optimization of the medium, the production was 194.23 mg/L with 3.7 fold increase in production. This validation reflected the necessity of optimization process

Conclusion

In the present study, sequential optimization strategy was used to optimize the culture conditions for resistomycin production by *Streptomyces aurantiacus* AAA5. Maximum production of 194.23 mg/L was obtained with 1.54 g soybean meal, 10.39% cassava pulp and 1.3 g potassium nitrate, and production was increased by 3.7 fold after optimization process. Current study indicated that the substrates are economical and also avoids inconvenience of cassava waste in sago industries. The results also give a basis for further study with large scale fermentation for production of resistomycin.

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