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Keratinolytic Activity and Statistical Optimization of Keratinase Production by *Streptomyces matensis* PLS-1 and *Streptomyces malaysiensis* TMS-1a.

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ABSTRACT

Production of keratinase (EC 3.4.4.25) by *Streptomyces matensis* PLS-1, *Streptomyces malaysiensis* TMS-1a is optimized, which has importance in recycling wastes. In this, optimization of cultural conditions was performed using design of experiments for the maximum production of keratinase by *S. malaysiensis* TMS-1a, *S. matensis* PLS-1. The actinomycetes were identified earlier in our laboratory, used for the optimization of keratinase production. The factors such as feather powder, yeast extract, pH, and soybean meal were found significant in keratinase production in our previous experiments and used for optimization of extracellular production of keratinase at five coded levels by statistical approach employing Response Surface Methodology (Central Composite Design-CCD). The resulting data was subjected to Analysis of Variance (ANOVA) for model validation. By using this statistical optimization studies, a 2.65, 1.95-fold increase in yield of extracellular keratinase was achieved for *S. malaysiensis* TMS-1a and *S. matensis* PLS-1, respectively. The present study using CCD proved to be a valuable tool in optimizing keratinase production by *S. matensis* PLS-1 and *S. malaysiensis* TMS-1a. The results showed excellent correlation between predicted and experimental values. Scanning Electron Microscopy showed degradation of chicken feather after exposure to these isolates grown in basal medium. This study showed that these isolates were able to hydrolyze chicken feather with keratinase activity, hence these may be useful for future applications on pharmaceutical and cosmetic formulations.

Keywords: Response surface methodology, central composite design, keratinase, *Streptomyces*, analysis of variance, scanning electron microscopy.

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INTRODUCTION

Keratin is a fibrous, insoluble structural protein extensively cross-linked with disulphide, hydrogen and hydrophobic bonds resulting in mechanical stability and resistant to common proteolytic enzymes such as pepsin, trypsin and papain (Radha and Gunasekaran, 2007). It is the major constituent of skin and its appendages such as nails, hair, feathers, and wool. Until recent years, the keratin containing materials along with other animal wastes are treated at high temperatures and milled in order to produce the so called "animal flour" and used as "protein supplement" into the feed mixtures of domestic animals. However, it was established that this flour is the carrier of the enigmatic cause of some related diseases, for example, mad cow, swine fever, etc. Generally, keratinases are produced by microorganisms, when it acts on keratin substrate in nature (Tork et al., 2010). There are various studies have been reported including keratinases from fungi *Microsporum* (Essien et al., 2009), *Trichophyton* (Saritha and Neeraj, 2010) as well as from bacteria, *Bacillus*, *Fervidobacterium, Bacillus licheniformis* (Fakhfakh et al., 2009), *Bacillus pumilis*, *Chryseobacerium* sp., *Streptomyces* sp., (Cheng et al., 2010).

Response surface methodology, an experimental strategy for seeking optimum conditions for a multivariable system, is an efficient technique for optimization. This method has been successfully applied for medium optimization in different fermentation processes as well as for establishing the conditions of enzymatic hydrolysis and sulfuric acid production. Keratinase activity depends mainly on divalent ion (Ca^{+2}), PMSF and EDTA (Tatineni et al., 2008).

Using central composite design, optimization of medium components by the classical method involves changing one independent variable, at the same time keeping all other variables at constant. It is extremely time consuming, expensive for a large number of variables. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimum conditions for variables of desirable responses (Adinarayana et al., 2003). This method has been successfully applied in many areas of biotechnology such as bioconversion of cheese whey to mycelia of *Ganoderma lucidum* (Hwanyoung et al., 2003), optimization of neomycin production, enzyme production, enzyme kinetics and bacteriocin production.

Optimization of parameters by a statistical approach, such as central composite design and response surface methodology (RSM), has been well appreciated for a significant improvement in yield as well as a decrease in the production cost of the enzyme (Puri et al., 2002). Therefore, the present study mainly focused on statistical optimization of keratinase production using central composite design for maximum production with low cost.

MATERIALS AND METHODS

Isolation and identification of microorganisms

The keratinase producing strain of *S. malaysiensis* was isolated earlier from the termite mound soil, Visakhapatnam (A.P.), India (Pavani et al., 2014), *S. matensis* from poultry littered feather dump soil near Sujathanagar, Visakhapatnam (A.P.), India (Kalyani et al., 2014) and were subsequently identified at the Institute of Microbial Technology, Chandigarh, India and were given the codes TMS-1a, PLS-1, respectively.

Preparation of feather

Poultry feathers were collected, washed with water, and dried in oven at 45°C (Micro Scientific works Pvt. Ltd., Karnal Road, Delhi, India). To prepare feather powder, the feathers were cut into small fragments and milled in a ball mill, and then passed through a 100-mesh grid.

Inoculum Preparation

Inoculum was prepared from 7 days old culture of PLS-1. 5 mL of sterile distilled water was added to the slant having optimum growth. Cells were then scrapped with sterilized inoculating loop and the tube was gently shaken and transferred at 10% level, aseptically into 250 mL Erlenmeyer (EM) flasks containing 45 mL of sterile inoculum medium containing (g/L): NH_4Cl 0.5, $CaCO_3$ 0.22, $MgSO_4.7H_2O$ 0.2, K_2HPO_4 0.3, Yeast Extract

10.0, NaCl 0.5, $FeSO_4$ 0.01, soyabean meal 0.05, chicken feather 20.0 and distilled water up to 1000, pH 7.5 ± 0.2 using the following modified medium (Bockle, 1995). The flasks were kept on rotary shaker at 150 rpm at 28°C for 2 days and used as inocula for subsequent experiments.

Shake flask fermentation

5 mL of cell suspension of the inocula was inoculated into 45 mL of sterile basal production medium which is same as that of the inoculum medium contained in 250 mL EM flask and incubated at 28°C on incubator shaker for 7 days at 150 rpm. At the end of fermentation, 5mL broth was centrifuged at 3000 rpm for 10 minutes and assayed for keratinase activity.

Keratinase assay

The keratinase activity was determined as described (Dozie et al., 1994). The assay mixture containing 1 mL of appropriately diluted enzyme, 4 mL glycine-NaOH buffer (50 mM, pH 9.0), and 20 mg of chicken feather was incubated at 70°C for 1 h. The reaction was terminated by adding 4 mL of 5% w/v trichloroacetic acid. Insoluble residues were removed by filtration through, and then, the filtrate was centrifuged at 5000 × *g* for 10 min, appropriate controls were also set up. Proteolytic products in the supernatant were determined by absorbance at 280 nm against controls (Thermo Scientific, UV-10, and UV-Vis). An increase in absorbance of 0.01 was considered as 1 U enzyme activity.

EXPERIMENTAL DESIGN

A central composite design with four variables at five coded levels (-2, -1, 0, +1, +2) was performed to investigate the effect of the variables on the response, *i.e.* production of keratinase. CCD was applied using the Design-Expert 9.0, trial version software. The independent variables studied were feather powder, yeast extract, pH and soybean meal. The total number of experiments carried out on the four variables was 30 (= 2^k + 2k + 6, where k is the number of independent variables).

The parameters with fixed central points of feather powder 2% w/v, pH 6.5, yeast extract 1.25% w/v and soybean meal 0.05% w/v were used in the present investigation. The treatment combinations were allocated into block. The first block contained the factorial design accompanied by sixteen runs. The second block contained the star points accompanied by eight runs and the third block contained the central points accompanied by six runs.

Statistical analysis and modeling

Data obtained from RSM on optimization of keratinase production were subjected to analysis of variance (ANOVA). The experimental data from RSM were then fitted *via* the response surface regression procedure by second order polynomial equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta_{23} BC + \beta_{24} BD + \beta_{34} BD$$

Data were processed using the software mentioned above as well as by means of ANOVA to determine the interactions between the process variables and the response. The quality of the fit of the polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked by the F-test in the same program.

Scanning electron microscopy (SEM) of feather

Samples of feather from inoculated and control (without microorganism) culture in basal medium were removed after 4 days of incubation at 37°C under shaking conditions (300 rev/min) and examined with SEM for observation of feather degradation.

The dried samples were mounted over the stubs with double-sided carbon conductivity tape dessicated in vaccum dessicator for 30 min and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model-JEOL JFC-1600) for 3 min and scanned under Scanning Electron

Microscope (SEM-Model: JOEL-JSM 5600) at required magnifications as per the standard procedures at RUSKA Lab's College of Veterinary Science, SVVU, Rajendranagar, Hyderabad, India (John and Lonnie 1998).

RESULTS

Statistical condition for optimization of keratinase production by *S. matensis* PLS-1, *S. malaysiensis* TMS-1a using RSM

Keratinase production of *S. matensis* PLS-1 and *S. malaysiensis* TMS-1a was optimized using CCD by varying concentrations of the medium components, especially carbon source (feather powder, soybean meal), nitrogen source (yeast extract) and other factor (pH). The range and levels of variables used in the central composite design were shown in the Table 1. RSM helps in evaluation of relationship between dependent (keratinase production) variable and independent variables (medium components such as yeast extract, soybean meal, feather powder and factor like pH) observed and predicted values of the keratinase production as shown in Table 3.1 (*S. malaysiensis* TMS-1a) and Table 3.2 (*S. matensis* PLS-1). The reliability of the model can be seen between observed and predicted values. The co-efficient and analysis of variance were shown in Table 2.1, 2.2 for *S. malaysiensis* TMS-1a and *S. matensis* PLS-1, respectively. The model F value of 19.98 and 23.24 indicated that the model is significant for both organisms used in this study. There is only a 1% chance that an F-value this large could occur due to noise. Values of Prob > F less than 0.05 indicated that model terms are significant. In this case A, B, C, D, BD, CD, A^2, B^2, C^2, D^2 were significant model terms. The fit of the model was checked by the co-efficient of determination R^2 was calculated to be 0.9491 and 0.9559 for *S. malaysiensis* TMS-1a and *S. matensis* PLS-1, respectively.

Table 1: The range and levels of variables used in the central composite design for *Streptomyces matensis* PLS-1 *and* *Streptomyces malaysiensis* TMS-1a

Variable	Range and levels				
	-2	-1	0	+1	+2
Feather (A)	1.0	1.5	2.0	2.5	3.0
Yeast-extract (B)	0.75	1.0	1.25	1.5	1.75
pH (C)	4.5	5.5	6.5	7.5	8.5
Soybean meal (D)	0	0.03	0.05	0.07	0.09

Table 2: ANOVA

Table 2.1: ANOVA for model used in keratinase production by *Streptomyces malaysiensis* TMS-1a

Terms	*Streptomyces malaysiensis* TMS-1a
F-value	19.98
P>F*	0.01
Mean	30.48
R^2	0.9491
Adjusted R^2	0.9016
Coefficient variance %	14.45
Adequate precision	14.803

Values of *"Prob > F" less than 0.0500 indicate model terms are significant.

Table 2.2: ANOVA for model used in the keratinase production by *Streptomyces matensis* PLS-1

Terms	*Streptomyces matensis* (PLS-1)
F-value	23.24
P>F*	0.01
Mean	40.46
R^2	0.9559
Adjusted R^2	0.9148
Coefficient variance %	13.62
Adequate precision	16.377

Values of *"Prob > F" less than 0.0500 indicate model terms are significant.

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Regression equation for the level of keratinase production in terms of coded factor for *S. malaysiensis* TMS-1a

$$Y = +45.56 + 5.21*A + 6.35*B + 3.06*C + 7.14*D + 0.18*AB + 1.37*AC + 1.54*AD - 1.48*BC + 4.38*BD + 2.40*CD - 6.10*A^2 - 5.40*B^2 - 4.04*C^2 - 3.31*D^2$$

By optimizing the above equation, the maximum keratinase production predicted by the model was 56.87 IU/mL, whereas experimental value 53.05 IU/mL (Table 3.1). Response surface plot figures obtained by the analysis of the experimental data of CCD showed a relationship between two variables at a time while maintaining third and fourth variable at fixed levels. These figures are helpful in understanding both linear and interaction effect of two variables. The 3D response surface plots described by the regression model were drawn to illustrate the combined effects of the independent variables and combined effects of each independent variable upon the response variable. Fig. 1a showed the interaction of yeast extract and soybean meal with fixed coded values of pH and feather powder as mentioned earlier, the activity of 54.15 IU/mL with yeast extract 1.49% w/v and soybean meal 0.07% w/v was obtained. The interaction of yeast extract and pH keeping soybean meal and feather powder constant as shown in Fig. 1b, the activity of 47.71 IU/mL was obtained with yeast extract 1.47% w/v and at pH 7.04. Fig. 1c showed the interaction of feather powder and soybean meal with the fixed coded values of pH and yeast extract, the activity obtained was 51.11 IU/mL with feather powder 2.22% w/v and soybean meal 0.07% w/v. RS plot of Fig. 1d illustrated the effect of feather powder and pH at constant soybean meal and yeast extract concentrations, activity obtained was 47.44 IU/mL with feather powder 2.24% w/v and at pH 7.07. The interaction of feather powder and yeast extract (Fig. 1e) keeping pH and soybean meal constant, the activity of 48.57 IU/mL was obtained with feather powder 2.24% w/v and yeast extract 1.39% w/v. The interaction of pH and soybean meal with the fixed coded values of yeast extract and feather powder as shown in Fig. 1f, the activity of 51.12 IU/mL was obtained at pH 7.10 and with soybean meal 0.07% w/v.

Table 3: Central composite design

Table 3.1: Central composite design for keratinase production by *Streptomyces malaysiensis* TMS-1a

Run no.	Feather powder (w/v)	Yeast extract (w/v)	pH	Soybean meal (w/v)	Keratinase activity (IU/mL)	
					Observed response	Predicted response
1	1.50	1.50	5.50	0.03	11.29	13.35
2	2.50	1.50	5.50	0.03	14.36	14.56
3	1.50	1.00	7.50	0.03	16.58	19.88
4	2.50	1.00	7.50	0.03	23.31	24.84
5	1.50	1.50	7.50	0.03	17.12	18.45
6	2.50	1.50	7.50	0.03	19.74	24.59
7	1.50	1.00	5.50	0.03	12.38	15.48
8	2.50	1.00	5.50	0.03	23.32	25.93
9	1.50	1.50	5.50	0.07	11.71	10.99
10	2.50	1.50	5.50	0.07	18.61	15.61
11	1.50	1.00	7.50	0.07	34.02	35.04
12	2.50	1.00	7.50	0.07	42.61	46.16
13	1.50	1.50	7.50	0.07	17.79	22.13
14	2.50	1.50	7.50	0.07	39.48	38.02
15	1.00	1.25	6.50	0.07	41.54	40.25
16	3.00	1.25	6.50	0.07	53.05	56.87
17	2.00	0.75	6.50	0.05	11.74	11.50
18	2.00	1.75	6.50	0.05	38.46	31.59
19	2.00	1.25	4.50	0.05	13.79	11.26
20	2.00	1.25	8.50	0.05	41.94	40.24
21	2.00	1.25	6.50	0.05	28.09	23.27
22	2.00	1.25	6.50	0.05	38.53	35.50
23	2.00	1.25	6.50	0.01	23.42	46.60
24	2.00	1.25	6.50	0.09	49.06	46.62
25	2.00	1.25	6.50	0.05	43.75	45.55
26	2.00	1.25	6.50	0.05	46.08	45.55
27	2.00	1.25	6.50	0.05	45.91	45.55
28	2.00	1.25	6.50	0.05	45.62	45.55
29	1.50	1.50	5.50	0.05	46.04	45.55
30	2.50	1.50	5.50	0.05	46.03	45.55

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Fig. 1: Response surface plots showing interaction between variables on the production of keratinase by *Streptomyces malaysiensis* TMS-1a

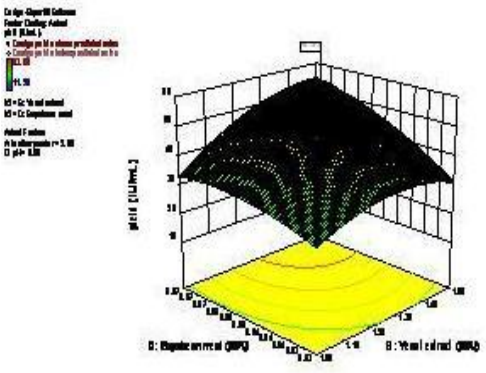

Fig. 1a: Interaction between soybean meal and yeast extract

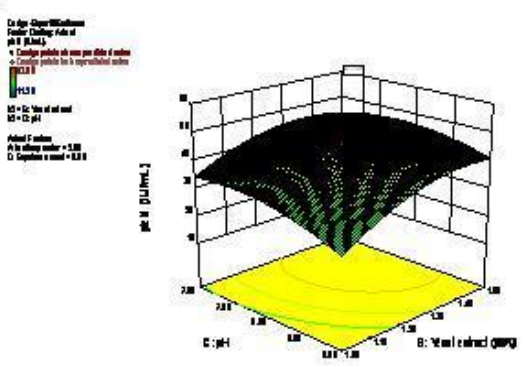

Fig. 1b: Interaction between pH and yeast extract

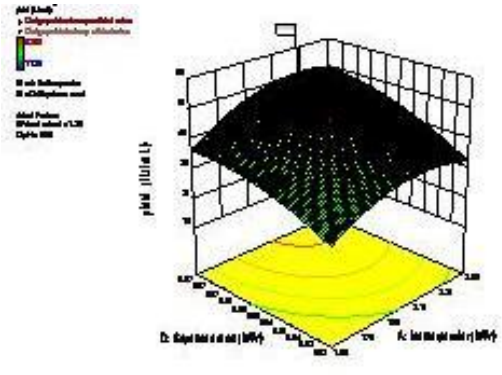

Fig. 1c: Interaction between soybean meal and feather powder

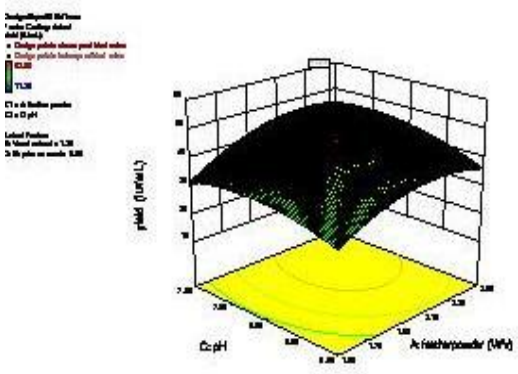

Fig. 1d: Interaction between pH and feather powder

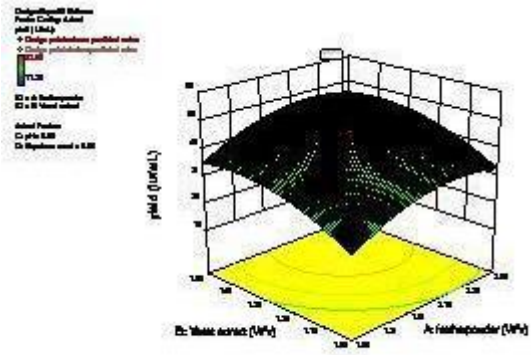

Fig. 1e: Interaction between yeast extract and feather powder and pH

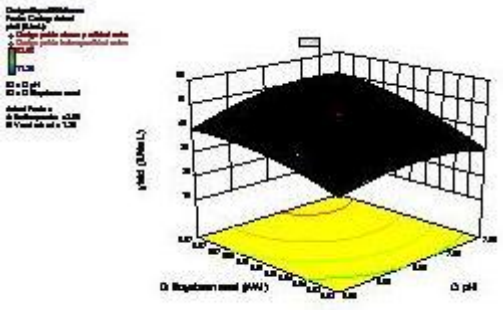

Fig 1f: Interaction of soybean meal

Regression equation for the level of keratinase production in terms of coded factor for *S. matensis* PLS-1

$$Y = +60.50 + 7.55*A + 9.10*B + 4.12*C + 9.46*D + 0.34*AB + 2.86*AC + 1.07*AD - 0.99*BC + 4.77*BD + 3.10*CD - 8.10*A^2 - 7.18*B^2 - 5.37*C^2 - 4.40*D^2$$

By optimizing the above equation, the maximum keratinase production predicted by the model was 76.82 IU/mL, whereas experimental value 70.34 IU/mL (Table 3.2). The interaction between yeast extract and pH (Fig. 2a) with the fixed coded values of soybean meal and feather powder, the activity of 63.95 IU/mL was obtained with yeast extract 1.40% w/v and at pH 6.89. The interaction of yeast extract and soybean meal with the fixed coded values of pH and feather powder as shown in Fig. 2b, the keratinase activity was 71.80 IU/mL

with yeast extract 1.49% w/v and soybean meal 0.07% w/v. Fig. 2c showed the interaction of feather powder and soybean meal keeping pH and yeast extract constant, the activity of 67.26 IU/mL was found with feather powder 2.35% w/v and soybean meal 0.07% w/v. The effect of feather powder and pH (Fig. 2d) keeping soybean meal, yeast extract constant and the interaction effect of pH and soybean meal (Fig. 2e) with constant yeast extract and feather powder, the activity was 63.65 IU/mL with feather powder 2.32% w/v and at pH 7.07 as well as 67.49 IU/mL at pH 7.35 and soybean meal 0.07% w/v obtained, respectively. Fig. 2f showed the interaction of feather powder and yeast extract with the fixed coded values of pH and soybean meal, the activity of 65.23 IU/mL was obtained with feather powder 2.26% w/v and yeast extract 1.41% w/v.

Table 3.2: Central composite design for keratinase production by *Streptomyces matensis* PLS-1.

Run no.	Feather powder (w/v)	Yeast extract (w/v)	pH	Soybean meal (w/v)	Keratinase activity (IU/mL)	
					Observed response	Predicted response
1	1.50	1.00	5.50	0.03	14.97	16.35
2	2.50	1.00	5.50	0.03	18.56	22.93
3	1.50	1.50	5.50	0.03	21.89	26.33
4	2.50	1.50	5.50	0.03	30.91	34.25
5	1.50	1.00	7.50	0.03	15.15	14.66
6	2.50	1.00	7.50	0.03	26.14	32.66
7	1.50	1.50	7.50	0.03	16.42	20.69
8	2.50	1.50	7.50	0.03	39.41	40.03
9	1.50	1.00	5.50	0.07	15.55	17.40
10	2.50	1.00	5.50	0.07	24.67	28.25
11	1.50	1.50	5.50	0.07	45.11	46.44
12	2.50	1.50	5.50	0.07	55.69	58.64
13	1.50	1.00	7.50	0.07	23.59	28.10
14	2.50	1.00	7.50	0.07	52.35	50.38
15	1.50	1.50	7.50	0.07	55.09	53.19
16	2.50	1.50	7.50	0.07	70.34	76.82
17	1.00	1.25	6.50	0.05	15.55	13.00
18	3.00	1.25	6.50	0.05	51.00	43.20
19	2.00	0.75	6.50	0.05	18.29	13.57
20	2.00	1.75	6.50	0.05	55.61	49.99
21	2.00	1.25	4.50	0.05	37.25	30.78
22	2.00	1.25	8.50	0.05	51.13	47.26
23	2.00	1.25	6.50	0.01	31.05	23.97
24	2.00	1.25	6.50	0.09	65.06	61.80
25	2.00	1.25	6.50	0.05	58.12	60.50
26	2.00	1.25	6.50	0.05	61.113	60.50
27	2.00	1.25	6.50	0.05	60.9	60.50
28	2.00	1.25	6.50	0.05	60.75	60.50
29	2.00	1.25	6.50	0.05	61.08	60.50
30	2.00	1.25	6.50	0.05	61.044	61.05

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Fig. 2: Response surface plots showing interaction between variables on the production of keratinase by *Streptomyces matensis* PLS-1

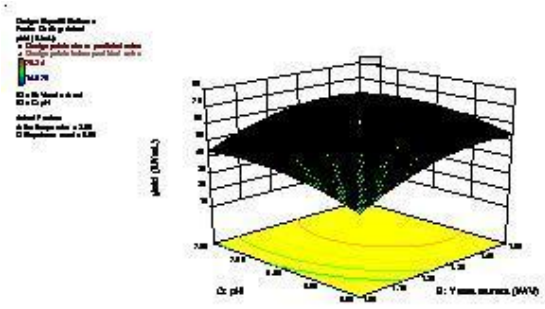

Fig. 2a: Interaction of pH and yeast extract

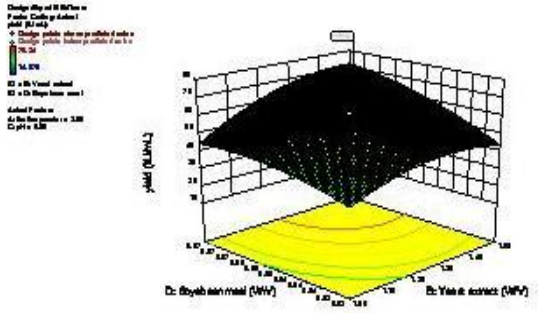

Fig. 2b: Interaction of soybean meal and yeast extract

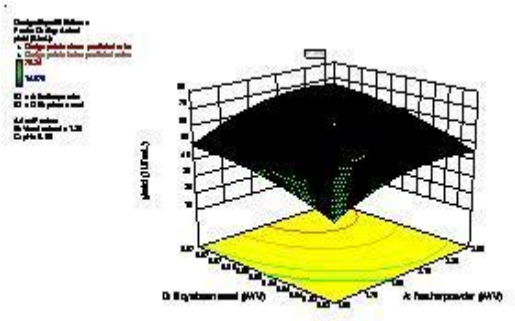

Fig. 2c: Interaction of soybean meal and feather powder

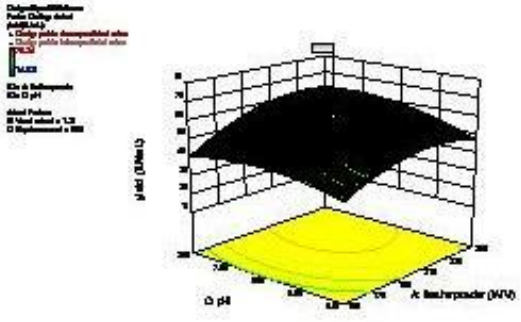

Fig. 2d: Interaction of pH and feather powder

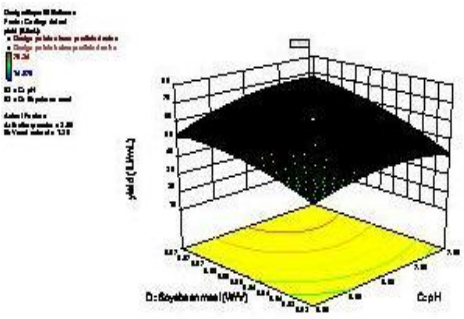

Fig. 2e: Interaction of soybean meal and pH

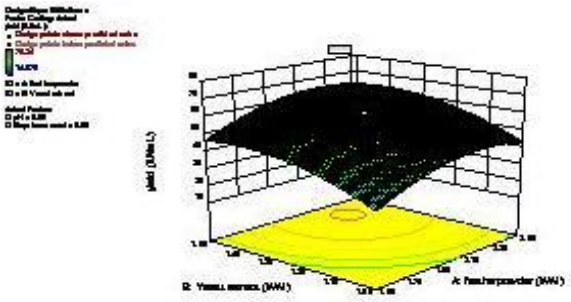

Fig. 2f: Interaction of yeast extract and feather powder

Validation of the model

The adequacy of the response surface equations were indicated by a comparison between the experimental value and the predicted data. The comparison is done by generating a fitted-line plot (experimental runs on X-axis, experimental values and predicted data on Y-axis) for the results obtained, showing how close it is to or how far it deviates from the fitted line. As shown in Fig. 3.1, 3.2, high R^2 (≥ 0.95) for fitted line plots and overall closeness of these variables, indicating that the response surface model was adequate for predicting optimization conditions for the production of keratinase.

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Fig.3: Fitted line plot indicating the closeness between predicted values and experimental values for keratinase activity by taking experimental runs on X-axis and predicted and experimental data on Y-axis.

Fig. 3.1: Fitted line plot for *S. malaysiensis* TMS-1a.

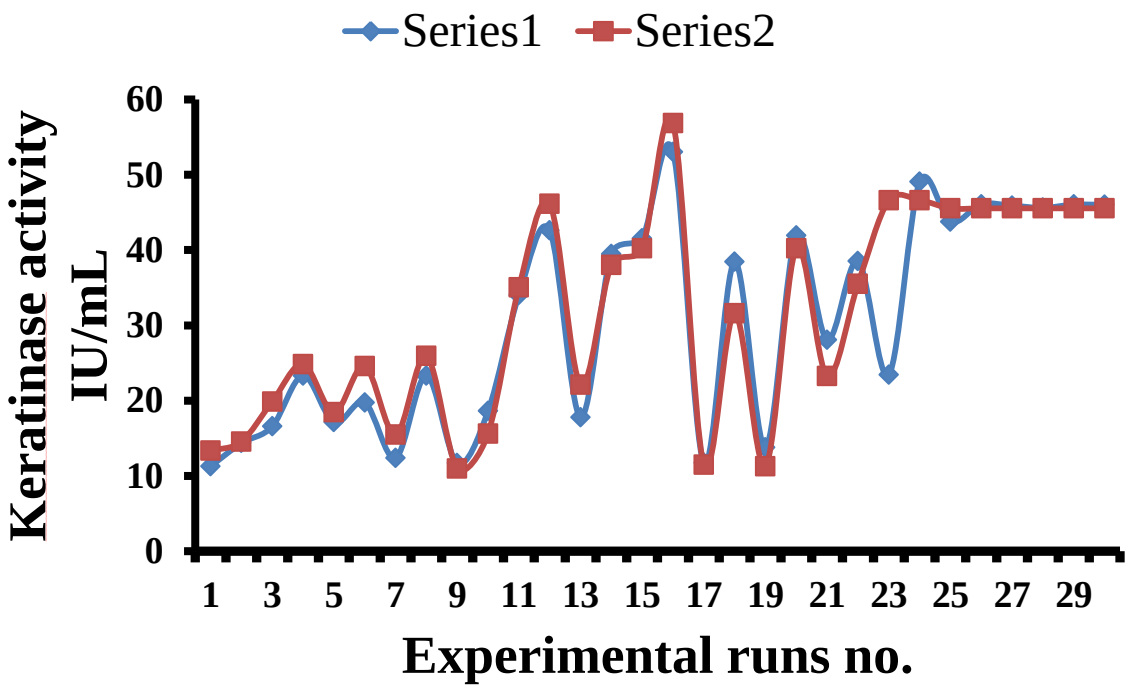
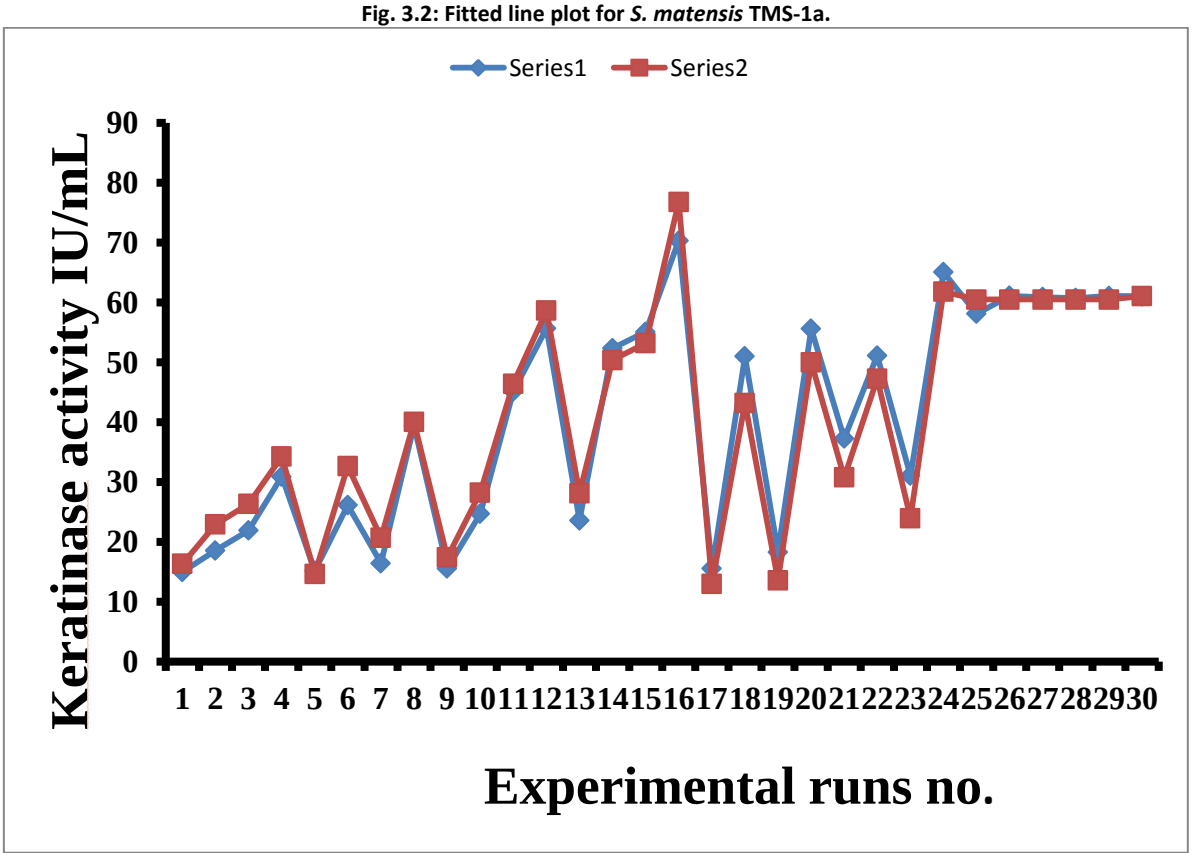


Fig. 3.2: Fitted line plot for *S. matensis* TMS-1a.



Series1: Observed response
Series2: Predicted response

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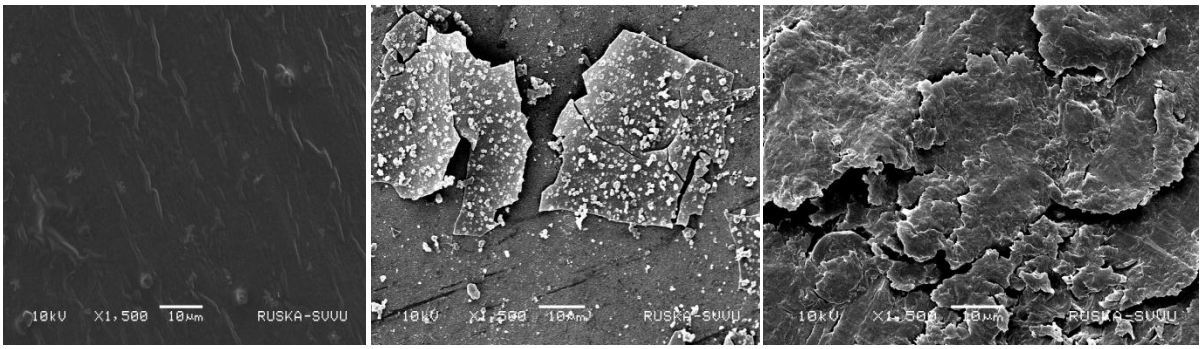
Scanning Electron Microscopy

The degradation of the feather by TMS-1a (*S. malaysiensis*) and PLS-1 (*S. matensis*) were observed in different parts of feather. Thus, in contrast to the control feather samples degradation was observed in the treated samples (Fig. 4).

Fig. 4: Scanning Electron Microscopy (SEM) of feather.



a) Control B) PLS-1 C) TMS-1a

DISCUSSION

Keratinases from micro-organisms have attracted a great deal of attention in the last decades, particularly because of their multitude of industrial applications (Gupta and Ramnani, 2006). In our investigation, we describe keratinolytic *S. malaysiensis* (TMS1a) and *S. matensis* (PLS-1) strains able to use chicken feather, a keratin as substrate. The ability of the strains to produce keratinase capable of degrading chicken feather can offer a great potential for development of biotechnological methods for the hydrolysis of keratinaceous substrates. The use of statistical model to optimize culture medium components and other factors is to enhance the enzyme production in industrial scale due to its easy applicability, reliability and validity. In the present study, the significant factors necessary for the enhancement of keratinolytic enzyme production were optimized using CCD, it also suggested that the importance of various factors at different levels. The central composite design exploited in the present study enabled to study and explore the culture conditions, which had supported increase in keratinase production to 53.05 IU/mL (predicted value 56.85 IU/mL) for *S. malaysiensis* TMS-1a, which is 2.65-fold higher than production from basal medium (20 IU/mL), 70.34 IU/mL (predicted value 76.82 IU/mL) for *S. matensis* PLS-1, which is 1.95-fold higher than production from basal medium (36 IU/mL). From the Fig. 1b and Fig. 2b, it was observed that with increase in yeast extract and soybean meal concentrations, the keratinase activity gradually increased but later on slightly decreased at constant pH and feather powder. CCD maximizes the amount of information that can be obtained, while limiting the numbers of individual experiments (Kunamneni & Singh, 2005). Thus, smaller and less time consuming experimental designs could generally suffice for the optimization of many fermentation processes. The result of study endorses this point of view. The high degree of similarity was observed between the predicted and experimental values (shown in Table 3.1, 3.2), which reflected the accuracy and applicability of RSM to optimize the culture conditions for maximum enzyme production. RSM was successfully applied to the production of keratinase by *Streptomyces* Sp7 (Tatineni et al., 2007), where the maximum production was 84.75 U/mL. In the present study, the four factors namely feather powder, yeast extract, pH, and soybean meal were used for optimization of keratinase production. The factors like feather meal, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride and other factors (pH, temperature, rpm) were used for optimization of keratinase production in *Streptomyces* sp7 (Tatineni et al., 2007). Thirty run experimental set up was used in the RSM study for the production of keratinase enzyme in the present study, whereas twenty seven experimental set up was used in RSM study for the production of protease and the factors utilized were whole chicken feather, peptone, NaCl and Na_2CO_3 (Panchanathan et al., 2013). The medium components play an important role in protease beta keratinase production by bacteria (Rai et al., 2010).

As shown in the results, the model was adequate for prediction of keratinase with optimized production within the range of experimental variables because the determination coefficient $R^2 = 0.9491$ (*S.*

malaysiensis TMS-1a), R^2 = 0.9559 (*S. matensis* PLS-1) and only 5.09%, 4.41% of the total variations were not explained by the model. The R-squared value provided a measure of how much of the variability in the observed response values could be explained by the experimental factors and their interactions. SEM analysis of feather after incubation with *S. malaysiensis* (TMS-1a) and *S. matensis* (PLS-1) showed that these organisms were capable of degrading feather more completely; hence they may be used for cosmetic and pharmaceutical purposes.

CONCLUSION

The optimum medium established for keratinase production by actinomycetes using response surface methodology in this work might result in the cost for the maximum production of keratinase and would thus offer advantages for large-scale fermentation.

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