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Optimization of culture conditions for Keratinase production in *Streptomyces* sp. JRS19 for Chick feather wastes degradation

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ABSTRACT

The aim of this study was to find the optimal conditions of a newly isolated Streptomyces strain JRS 19. Screening for keratinase producing bacteria and their keratinase production were investigated. The keratinase produced by the isolate was purified by chromatographic techniques and the optimum pH maintained was 7.0 and maintained at different temperatures Viz., 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. Besides temperature and pH, the enzyme is active upon different sources and its effect on carbon, nitrogen, aminoacids were determined and recorded the protein content and enzyme activity.The carbon sources revealed that sucrose was found highly significant, whereas peptone was found highly significant in nitrogen source. The effect of keratinase on amino acid shows that valine was found highly significant. Based on this study, the physio-chemical properties of keratinase were analysed and the results were interpreted.

Key words: Enzyme, *Streptomyces sp*., feather, Keratinase.

INTRODUCTION

Chicken feather mainly contains keratin, which is an insoluble protein with high stability and indigestible by common proteases [2, 8]. Actinomycetes have the ability to breakdown many different varieties of organic compounds and are crucial in the mineralization of organic matter [12]. Carbohydrate inhibition of keratinase production was observed, indicating that this strain has a catabolite repression regulatory mechanism, a common control mechanism for biosynthesis of bacterial proteases [3], including keratinases [17, 14].Proteases constitute an important fraction of the global enzyme sales, and a relevant part of this market is accounted by bacterial

proteases [11]. Bacterial keratinases are of particular interest because of their action on insoluble keratin substrates and generally on a broad range of protein substrates [7].These enzymes have been studied for de-hairing processes in the leather industry [10].Cultivation conditions are essential in successful production of enzyme, and optimization of parameters such as pH, temperature and media composition is important in developing the cultivation process. Despite all the work that has been done on production of proteolytic enzymes, relatively little information is available on keratinases [18]. Upto now, a limited number of studies have been reported on the isolation of thermophiles, in particular thermophilic actinomycetes with the ability to hydrolyse wool and other keratinous wastes [5, 6]. Natural products have been our single most successful source of medicines [13] and the use of microorganisms to produce natural products and processes that benefit and improve our socio-economic lifestyles had been a part of our human history [9]. Hence it was thought interesting to carry out an analysis of the physico-chemical parameters such as temperature; pH etc., [16] During the last decade, the immobilization of enzymes has been often used in the production of pharmaceuticals, food and other biological products, and it is also essential for their application to industrial processes[14].

EXPERIMENTAL SECTION

1) Effect of different temperatures on Keratinase production:

Fifty mL production medium was prepared and amended with 0.4% Keratin, pH 7.0 and inoculated with 2mL of the spore's suspension and incubated at different temperatures viz., 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. The culture filtrate was harvested for every 3 days of interval for a period of 15 days and recorded the protein content and enzyme activity.

2) Effect of different pH on Keratinase production:

The production medium was prepared with various pH such as 5.5, 6.0, 6.5, 7, 7.5 and 8.0 and inoculated with 2 mL spore suspension of *Streptomyces* sp. JRS19 and kept in the rotary shaker at 120 rpm at room temperature. The protein content and Keratinase activity were recorded for every 3 days for a period of 15 days. The uninoculated medium served as a blank for the Keratinase assay. Among the different pH investigated, the bacterium showed maximum Keratinase activity at pH 7.0. Therefore, in the following experiments the initial pH of of the medium was adjusted to 7.0.

3) Effect of different carbon sources on Keratinase production

Different carbon sources like dextrose, maltose, sucrose, galactose, and mannitol were added separately at 2% in the production medium and inoculated the spore's suspension of *Streptomyces* sp. JRS19. The pH of the medium was adjusted to 7.0 before sterilization. Every three days of interval the culture Filtrate were obtained and recorded the protein content and enzyme activity.

4) Effect of different nitrogen sources on Keratinase production

In order to find out a suitable nitrogen source for the maximum production of Keratinase from *Streptomyces* sp. JRS19, the following organic nitrogen sources namely, beef extract, peptone, casein, yeast extract and gelatin at 0.1% along with 80 mM sucrose as a carbon source were amended separately in the production medium, pH 7.0 and inoculated 2 mL of spores

suspension, incubated for 15 days at 30ºC. The culture filterate was collected at every three days of interval for protein content estimation and Keratinase activity.

5) Effect of different amino acids on Keratinase production

Fifty mL of production medium was amended with different amino acids like alanine, valine, asparagine, methionine and histidine separately at 5 mM and inoculated with 2 mL of spore's suspension and incubated for a period of 15 days. Culture filtrate was obtained and studied for protein content and Keratinase activity.

RESULTS AND DISCUSSION

Effect of different concentrations of keratin on extracellular protein and keratinase activity

Among the different concentrations of keratin tested *Streptomyces* sp. JRS19 produced maximum extracellular protein of 46.20 µg/mL in 0.4 % concentration on 9th day and maximum enzyme activity of 1.84 U/mL on 6th day when compared to the rest of concentrations.

One way analysis of variance calculated for protein and keratinase production revealed that the concentration of 0.4% keratin showed keratinase activity and protein content at $P<0.05$ level.

1) Effect of different temperatures on extracellular protein and keratinase activity

The data presented showed that *Streptomyces* sp. JRS19 produced a maximum protein content of 52.11 µg/mL on 9th day at 30ºC and keratinase activity of 3.17 U/mL on 6th day at 30ºC.

One way analysis of variance for the extracellular protein and keratinase activity revealed that the temperature 30°C was highly significant at $P<0.05$ level than other temperatures (**Table 1)**. Thus 30°C was selected for further studies.

2) Effect of pH on extracellular protein and keratinase activity

The results showed that the pH significantly influenced the extracellular protein content and keratinase activity in *Streptomyces* sp. JRS19. It was able to release a maximum protein content of 50.19 µg/mL at pH 7.0 after nine days of incubation and keratinase of 3.08 U/mL at pH 7.0 on 6th day (**Table 2**)

One way analysis of variance for the extracellular protein and keratinase activity production in different pH revealed that at pH 7.0 [4] keratinase was highly significant at $P<0.05$ level than other pH . Therefore, pH 7.0 was selected for further studies. Maximum biomass and keratinase activity were observed from pH 6.0 to 8.0, which agrees with those described for most feather-degrading Bacillus [15, 18].The same was observed for alkaline protease production by *B. licheniformis* [1]

3) Effect of different carbon sources on extracellular protein and keratinase activity

Five different carbon sources such as dextrose, maltose, sucrose, galactose and mannitol when tested at 2% for keratinase production in *Streptomyces* sp. JRS19 showed that sucrose it enhanced maximum protein content of 53.0 µg/mL on 9th day and also sucrose induced a maximum keratinase activity of 11.06 U/mL on 6th day. Whereas maximum enzyme activity of

8.38 U/mL on sixth day in galactose and 4.51 U/mL in maltose recorded on the same day. Dextrose was not significantly induced the keratinase production.

One way analysis of variance calculated between extracellular protein and keratinase production in different carbon sources revealed that sucrose was found highly significant at $P<0.05$ level than other carbon sources (**Table 3**)

Table 1. Effect of pH on extracellular Keratinase production in *Streptomyces* sp. JRS19

Initial pH	Keratinase activity (U/mL)				
	3rd day	6th day	9th day	12th day	15th day
5.5	0.04±0.02 a	0.23±0.03 a	0.32±0.03 a	0.95±0.03 a	0.61±0.03 a
6.0	0.13±0.03 b	0.95±0.03 b	0.45±0.03 b	1.22±0.05 b	0.71±0.04 b
6.5	0.14±0.04 cb	2.46±0.01 c	1.5 ±0.03 c	1.30±0.04 cb	0.64±0.04 ab
7.0	0.31±0.04 d	3.08±0.05 d	1.97±0.09 d	1.10±0.08 d	0.82±0.08 c
7.5	0.06±0.02 a	1.8±0.03 e	1.0±0.03e	0.33±0.04 e	0.21±0.03 d
8.0	0.06±0.02 a	0.50±0.02 f	0.83±0.04 f	0.54±0.04 f	0.25±0.04 ed

Different superscripts in the same column are significantly different at P< 0.05 level (Least Significance Difference)
Means followed by ±S.D

Table 2. Effect of different temperatures on extracellular Keratinase production in *Streptomyces* sp. JRS19

Temperature	Keratinase activity (U/mL)				
	3rd day	6th day	9th day	12th day	15th day
20ºC	0.05±0.02 a	0.32±0.04 a	0.83±0.04 a	0.53±0.07 a	0.44±0.03 a
25ºC	0.07±0.02 a	1.30±0.36 b	0.82±0.03 a	0.54±0.04 a	0.62±0.05 b
30ºC	0.15±0.04 b	3.17±0.30 c	1.69±0.11 b	0.74±0.11 b	0.43±0.06 a
35ºC	0.32±0.06 c	1.21±0.12 db	0.89±0.09 a	0.76±0.07 cb	0.52±0.06 ad
40ºC	0.06±0.02 a	0.23±0.05 a	0.35±0.05 c	0.58±0.08 a	0.17±0.03 c

Different superscripts in the same column are significantly different at P< 0.05 level (Least Significance Difference)
Means followed by ±S.D

Table 3. Effect of different carbon sources on the Keratinase production in *Streptomyces* sp. JRS19

Carbon source (2%)	Keratinase activity (U/mL)				
	3rd day	6th day	9th day	12th day	15th day
Dextrose	0.62±0.05 a	1.23±0.08 a	0.93±0.08 a	0.32±0.06 a	0.32±0.06 a
Maltose	0.44±0.06 b	4.51±0.41 b	3.33±0.10 b	0.62±0.04 a	0.48±0.05 a
Sucrose	1.30±0.11 c	11.06±0.48 c	7.50±0.49 c	3.63±0.40 b	1.63±0.30 b
Galactose	0.33±0.06 dbe	8.38±0.51 d	4.21±0.27 d	1.45±0.27 c	0.64±0.10 ca
Mannitol	0.24±0.08 e	0.70±0.08 a	3.22±0.26 eb	1.60±0.51 dc	1.32±0.22 db

Different superscripts in the same column are significantly different at P< 0.05 level (Least Significance Difference)
Means followed by ±S.D

4) Effect of different nitrogen sources on extracellular protein and Keratinase production

Five different nitrogen sources such as beef extract, peptone, casein, yeast extract and gelatin were tested for extracellular protein and keratinase production in *Streptomyces* sp. JRS19. Among them, peptone influenced maximum extracellular protein content of 54.67 µg/mL on 9th

day and keratinase production of 16.53 U/mL on 6th day. However maximum extracellular protein content was recorded on 9th day and then it decreased gradually.

One way analysis of variance calculated between extracellular protein and keratinase production in different nitrogen sources revealed that peptone was found highly significant at $P<0.05$ level than other nitrogen sources (**Table 4**)

5) Effect on different amino acids on extracellular protein and keratinase production

Five different amino acids like Alanine, Valine, Asparagine, methionine and Histidine at 5 mM concentration when tested on the organism Histidine induced maximum protein content 44.33 µg/mL on 12th day followed by Valine induced 40.67 µg/mL on 9th day. At this condition the amount of extracellular enzyme induced certain extent in valine at 4.94 U/mL on 9th day incubation. The rest of the other amino acids were inhibited the keratinase production (**Table 5)**

Table 4. Effect of different nitrogen sources on the Keratinase production in *Streptomyces* sp. JRS19

Nitrogen source (0.1%)	Keratinase activity (U/mL)				
	3rd day	6th day	9th day	12th day	15th day
Beef extract	2.37±0.36 [a]	1.42±0.43 [a]	1.49±0.41 [a]	0.82±0.18 [a]	0.38±0.17 [a]
Peptone	7.31±0.31 [b]	16.53±0.50 [b]	1.41±0.43 [a]	2.62±0.37 [b]	3.37±0.38 [b]
Casein	10.31±0.41 [c]	9.63±0.36 [c]	5.53±0.48 [b]	1.39±0.41 [c]	1.44±0.45 [c]
Yeast extract	3.30±0.36 [d]	2.42±0.42 [d]	1.62±0.24 [a]	1.12±0.18 [ac]	0.55±0.09 [a]
Gelatin	2.60±0.31 [e]	2.60±0.31 [ed]	0.64±0.11 [c]	1.20±0.29 [ac]	1.09±0.21 [dc]

Different superscripts in the same column are significantly different at P< 0.05 level (Least Significance Difference)
Means followed by ±S.D

Table 5. Effect of different amino acids on extracellular Keratinase production in *Streptomyces* sp. JRS19

Amino acid 5 mM	Keratinase activity (U/mL)				
	3rd day	6th day	9th day	12th day	15th day
Alanine	0.06±0.01 [a]	0.11±0.01 [a]	0.14±0.01 [a]	0.08±0.03 [a]	0.10±0.01 [a]
Valine	0.35±0.03 [b]	2.20±0.18 [b]	4.94±0.40 [b]	2.32±0.19 [b]	4.91±0.40 [b]
Asparagine	0.05±0.01 [a]	0.09±0.01 [a]	0.08±0.01 [a]	0.07±0.01 [a]	0.15±0.02 [a]
Methionine	0.04±0.01 [a]	0.06±0.01 [a]	0.05±0.01 [a]	0.39±0.53 [a]	0.05±0.01 [a]
Histidine	1.05±0.09 [c]	0.80±0.07 [c]	0.36±0.03 [ac]	0.34±0.03 [a]	0.23±0.02 [a]

Different superscripts in the same column are significantly different at P< 0.05 level (Least Significance Difference)
Means followed by ±S.D.

CONCLUSION

The Physico-chemical properties of Keratinase were analysed, the optimum pH and stability of the purified Keratinase was determined. The pH stability was assayed after pre-incubation of enzyme with the pH ranging from 3.5-9.5. The optimum temperature and thermal stability were determined at different temperatures viz., 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70°C. The thermal stability was investigated by incubating the enzyme at different temperature from 25°C-70°C at 120 minutes. The data obtained from the optimization studies, further work is focused on biocontrol studies has to be analyzed using a computer statistical package SPSS. (Statistical Package for social science) University.

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