Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Special Issue [1]2022 : 1188-1197

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Optimization, Application and Some Properties of Extracellular Keratinase from Chicken Feathers-Degrading Bacillus Tequilensis S-5

Narendra Kumar Dabi, Ajaj Ahmed, Gautam Kumar Meghwanshi*
Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Raj. India.
*Corresponding author : E-mail: drgkm_biotech@yahoo.com

ABSTRACT
Optimization of keratinase production was performed using "one variable at a time" approach. Results of the optimization study showed that the Bacillus tequilensis S-5 strain produced 43.8 U/ml of keratinase at pH-7.0, 200 rpm, 1.5% feather concentration and 2% inoculum level after 48 h of incubation at 30ºC. This is an increase of more than3-fold when compared to the unoptimized production of 13.9 U/ml. Enzyme keratinase finds industrial application in many biotechnological processes such as conversion of waste feathers to biofertilizers, animal feed, and leather processing etc.Bacillus tequilensis S-5 keratinase was used to transform raw feathers to keratin powder. This keratin powder was used to prepare bioplastic. The bioplastic was molded into thin films. These films showed biodegradability that is they degraded into fine particles when buried under soil for 7 days. The Bacillus tequilensis S-5 keratinase was also used to degrade raw feathers into feather meal.
Key Words: -Keratinase, optimization, feather degradation, bioplastic.

Received 15.02.2022 Revised 11.03.2022 Accepted 10.04.2022

INTRODUCTION

Keratinase comes under serine hydrolyses and are a sub-class of proteolytic enzymes. They are produced by prokaryotic and eukaryotic microbes such as bacteria, actinomycetes and fungi. These enzymes are extracellularly secreted and are specifically induced in environments rich in keratinacious wastes viz. poultry farms, slaughter houses, etc. Keratin being protein, its sources like waste feathers, nails, hairs [1] can be processed into protein rich animal/ bird feed or biofertilizers [2] by controlled bioremediation through keratinolytic microbes or isolated keratinases. Bikaner is a hub to many poultry farms spread in and around the city. These farms produced high quantity of feather wastes which are dumped in dumping zones. These unregulated dumping results in foul smell and environmental pollution. So, transforming the waste feathers to some useful product like feed, biofertilizer, bioplastic etc [2] is an environmentally benign and ecofriendly process, which would not only solve the problem of pollution but also reduce the health hazards and generates wealth from waste. Other than waste remediation keratinasesfind application in other important industrial sectors like detergent, cosmetics, medicine & leather processing/ manufacturing [3,4]. Keratinases sourced from GRAS (Generally Regarded as Safe) organisms can be used as nutraceuticals, which can enhance the weight gains and overall health and productivity of broilers [1,3]. They are also implicated in the field of medical for the treatment of prion mediated disease in cattle, specifically the mad cow disease and they also have promising application in the manufacturing of bioplastic and biocomposites [5,6,7].In the present paper result of optimization study for maximal keratinase production from a strain of *Bacillus tequilensis*S-5 is presented. To attain the maximum production of keratinase from the selected Bacterial strain"one variable at a time" method was followed. "One variable at a time" method is a conventional method for optimization studies for enhancement of the production of any bioactive molecule. In this approach, different physiological and nutritional factors are evaluated and optimized one at a time for the maximum production of a biomolecule. However, precautions are taken that once a factor is optimized, it is incorporated before optimizing the subsequent factor. This sequence of incorporation is followed till a final medium composition and production conditions are obtained. In the present study, this approach was followed, where in, different physiological parameters such as pH, temperature, agitation rate, incubation period and nutritional parameters such as type of carbon and nitrogen sources and their concentrations were evaluated and

optimized for maximum keratinase production by the selected isolate. The keratin produced through enzymatic hydrolysis of raw feathers was used for the production of bioplastic. The keratinase enzyme was also used for feathers degradation.

MATERIAL AND METHODS

Keratin Powder: - Prepared as per the method of Dabi *et al* [8].

Media: -Nutrient agar (NA) & Nutrient Broth (NB), (Composition g/l:Peptone- 5, Sodium chloride-5, Meat Extract-1.5, Yeast Extract- 1.5, pH-7.4 Distilled water-1000ml for Nutrient Broth (NA) 1% agar-agar was added to this medium. Production Medium-I was prepared as described in Dabi et al.[8](Composition g/l: NaCl – 0.5, KH_2PO_4-0.7, K_2HPO_4-1.4, $MgSO_4$-0.1, Yeast Extract-2.0, Keratin Powder-1.0 (Prepared from chicken feather as described in Dabi *et al*. [8], pH-7.0,Keratin powder - prepared as per the method of Dabi et al[8].

Other Reagent: Polyvinyl alcohol, Acetone, Dimethyl sulphoxide (DMSO), Trichloro acetic acid (TCA)**.**

Quantitative Assay of Keratinase

Quantitative assay of extracellular keratinase was performed using the assay method as described in Gradisar et al[9].

Enzyme Unit

One unit (U) of keratinolytic activity is defined as an increase of corrected $A_{280}$of 0.100 under assay conditions as described in Gradisar et al[9].

Optimization of cultural condition for keratinase production:-

Different physical & nutritional parameter were studied for optimization of Keratinase production from *Bacillus tequilensis*S-5

1. **Effect of different percentage of feathers:-**Keratinase production was studied in presence of different percentage of feather viz 1%, 1.5%, 2%, 3% and 5%. Keratinase production was determined as per the method of Gradisar et al[9].
2. **Effect of temperature**:-Keratinase production was carried out at different temperature viz 20ºC, 30 ºC, 40 ºC, 50 ºC and 60 ºC. All other experimental conditions were same as previously described.
3. **Effect of pH:-**The production of keratinase was examined at different pH values ranging from 5.0 to 10.0. The initial pHs of production media were adjusted to different values using 1N NaOH or 1N HCl. Keratinase production was carried out for 48h as stated earlier.
4. **Effect of shaking rate:**-The production of keratinase was examined at different shaking rates ranging from 100 to 250 rpm. Fermentation was carried out for 48h as stated earlier.
5. **Effect of different carbon sources:-** Different carbon sources like glucose fructose, lactose sucrose maltose, starch, and glycerol at 0.2% (w/v) concentration were used as a carbon source in the production medium and their effect on keratinase production was evaluated.
6. **Effect of different nitrogen sources:-** Different organic and inorganic nitrogen sources at 1% concentration were used in the medium to achieve maximum keratinase production. The organic and inorganic nitrogen sources such as casein hydrolysate, peptone,meat extract, yeast extract, casein, ammonium chloride, ammonium nitrate, ammonium dihydrogen orthophosphate and ammonium sulphate were used in the medium.
7. **Effect of incubation period :-** To determine the optimal incubation period for maximum keratinase production, the selected organism was grown under optimized conditions for keratinase production for 24h, 48h and 72h.
8. **Effect of inoculum level :-** Different inoculum levels that is 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0% were used in the medium to determine the appropriate inoculum levels required for maximum keratinase production.

Application of keratinase in feather degradation and Bioplastic production

Application in feather degradation

To 100 ml ofkeratinase enzyme contained in 250 ml conical flask 5 g of raw chicken feathers were added and the mixture was incubated at 37ºC for 12h. The flask was observed for feathers degradation after 12h**.**

Production of bioplastic and its degradation

10 gm of poly vinyl alcohol was dissolved in 100 ml of distilled water by stirring at 80 º C for 1 hr. After one hour 2 % of keratin powder was added to it and the mixture was again stirred at 60º C for 15 min. Then the mixture was poured in glass petri plates having a diameter of 20 cm and the plates were placed in hot air oven at 60º C for overnight. Next day the bioplastic films were separated from glass petri plates and stored for further analysis. Plastic films werecut into small pieces of 5×5 cm which were buried under garden soil in a ground. The bioplastic film were observed from time to time for degradation.

Note:- All the experiment were conducted in triplicate. The results presented are average from triplicates and the standard deviation and coefficient of variants were calculated.

Results and Discussion:-

Table 1:- Effect of feather concentration on keratinase production after 48 h of incubation at 37ºC & 150 rpm.

Feather %	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
0.5%	8.3	±0.5	6.5
1%	13.2	±0.4	3.6
1.5%	24.3	±0.6	2.4
2%	13.7	±0.3	2.7
3%	10.8	±0.2	1.8
5%	7.5	±0.5	6.6

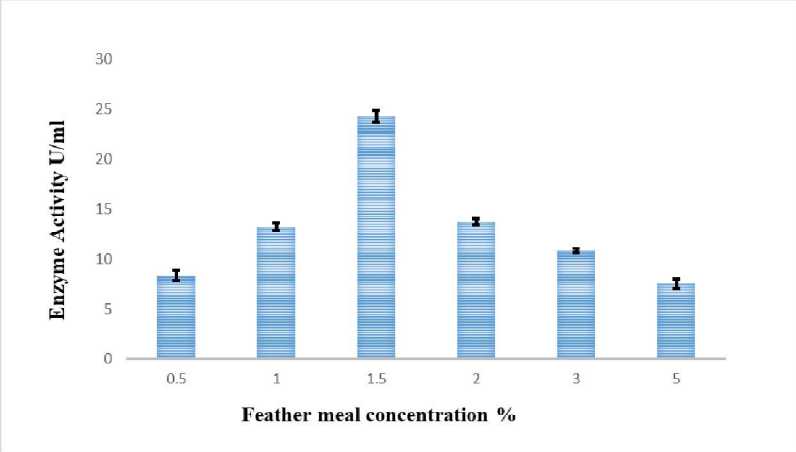


Figure 1:- Effect of different concentration of raw chicken feathers on keratinase production By *Bacillus tequilensis* S-5.

Before inoculation of *Bacillus tequilensis* S-5.After inoculation of *Bacillus tequilensis*S-5.

Figure 2:- Effect of different concentration of raw chicken feathers on keratinase production By *Bacillus tequilensis* S-5.

Feather being rich source of keratin and are readily available, so they act as a good alternative to pure keratin for evaluating the inducible nature of keratinase by any given keratinase producing microorganism. Realizing this different feather concentration were evaluated to see its their effect on keratinase production by *Bacillus tequilensis* S-5. Results presented in table 1 & figure 1 shows that maximum keratinase production by *Bacillus tequilensis* S-5 occurred at feather concentration of 1.5%. However, on further increasing the feather concentration no increase in keratinase production was observed, but a decline in production was observed. Optimal keratinase production has been reported at 0.5% feather concentration for *Alcaligenes* sp. ACL 05-001[11] and *Bacillus cereus*[12]. Whereas another study reported feather concentration of 1% as optimal for keratinase production from *Bacillus* sp. Okoh-K1[13]. Whereas, while optimal keratinase from *Arthrobacter* sp. KFS-1 occurred at 0.25% feather concentration[14].

Table 2:- Effect of different incubation temperature on keratinase production by *Bacillus tequilensis* S-5:-

Temperature	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
20ºC	6.8	±0.4	7.3
30ºC	32.4	±0.4	1.2
40ºC	25.2	±0.65	2.5
50ºC	9.6	±0.48	5
60ºC	0.7	±0.07	10

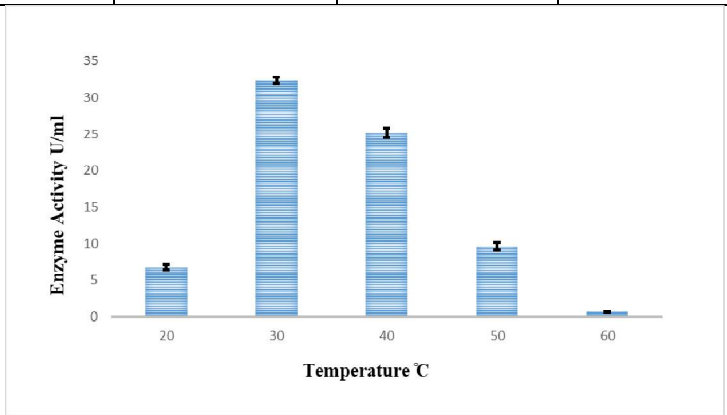


Figure 3:- Effect of different temperature on Keratinase production by *Bacillus tequilensis* S-5.

Effect of incubation temperature on keratinase production was determined in the range of 20ºC to 60ºC.Result showed that the *Bacillus tequilensis* S-5 produced Keratinase over the temperature range of 20ºC to 60ºC with maximum keratinase activity of 32 U/ml was observed at after 48h of incubation at 30ºC. Optimal production at 30ºC has been reported for *Arthrobacter* sp. KFS-1 keratinase [14]and *Bacillus* sp. Okoh-K1 keratinase [13]. Similar low temperature (27ºC-28ºC)havebeen reported for *Streptomyces* sp[15] and *Alcaligenes* sp. ACS05-001[11]. Whereas, Thermostable keratinase from *Bacillus pumilus* KS12 showed optimal production at 37ºC by [16]. On the other hand showed that a temperature range of 40ºC-45ºC was optimal for keratinase production from *Bacillus licheniformis*. More higher optimal temperatureof 50ºC- 70ºC have been reported as optimal for keratinase production from *Streptomyces thermonitrificans* [17, 18] and *Streptomyces* sp. S.K1-02[19]respectively.

Table 3:Effect of initial medium pH on keratinase production by *Bacillus tequilensis* S-5.

pH	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
5.0	3.9	±0.3	7.6
6.0	8.7	±0.3	3.4
7.0	32.1	±0.2	0.8
8.0	26.0	±0.5	1.9
9.0	16.6	±0.2	1.2
10.0	11.2	±0.4	3.5

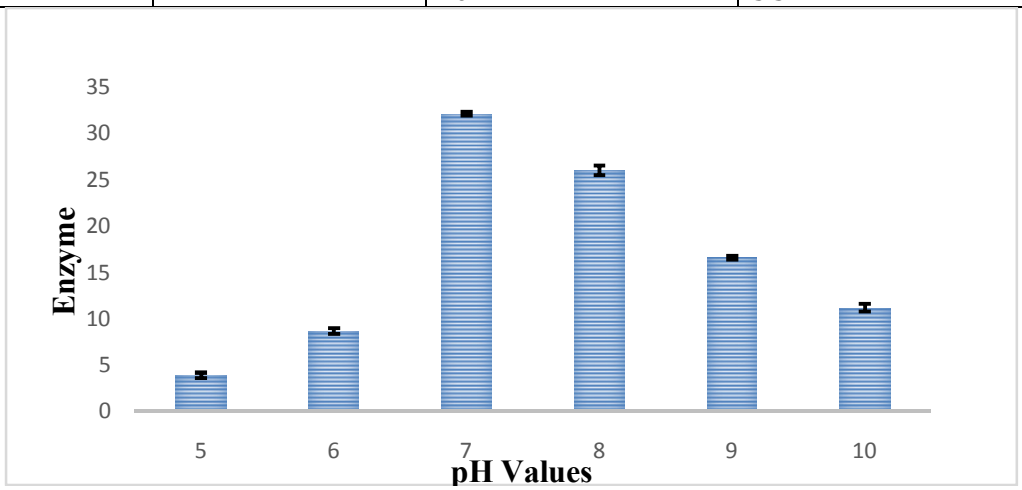


Figure 4:-Effect of initial medium pH on keratinase production by *Bacillus tequilensis* S-5.

Bacillus tequilensis S-5 was able to grow in the range ofpH 5.0-10, but maximum keratinase production occurred at pH 7.0. Microbial keratinase production has been reported at acidic, neutral and alkaline pH_s. Similar to our finding alkaline optimal pH of 8.5 has been reported for *Aspergillus* sp. DHE7[20]. On the other optimal pH of 7.0 has been reported for *Bacillus cereus*[12]. Whereas a number of *Bacillus* sp. have been reported to exhibit optimal pH_s in the acidic range (pH 50.-6.0) such as *Bacillus cereus* Wu2[21], *Bacillus pumillus*[22], *Bacillus cereus* MCM B-326[23]and *Bacillus hirikoshii*[24].

Table 4:-Effect of shaking rate on keratinase production by *Bacillus tequilensis* S-5.

RPM (Round per Minute)	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
100	**14.8**	**±0.4**	**3.2**
150	**31.9**	**±0.3**	**1.2**
200	**43.8**	**±0.5**	**1.1**
250	**26.2**	**±0.5**	**1.9**

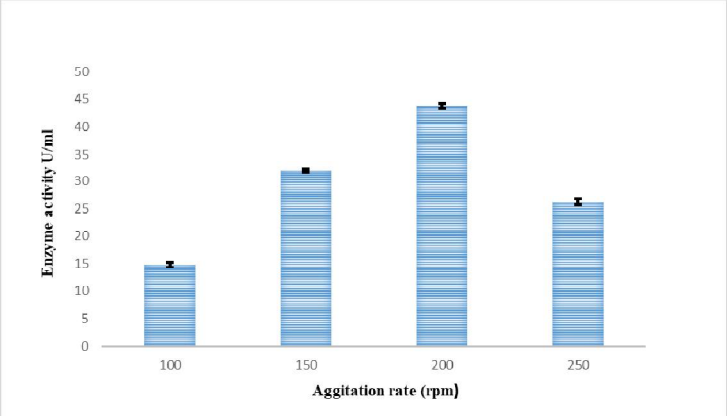


Figure 5:-Effect of shaking rate on keratinase production by *Bacillus tequilensis* S-5.

Aeration (shaking) plays an important role in the fermentative production of various metabolites. Optimal keratinase production by *Arthrobacter* sp. KFS-1 was also observed at 200rpm [14], whereas optimal agitation rate of 150 rpm has been reports for *Bacillus* sp. OKoh-K1 [13].So in this study, the effect of shaking rate was investigated on keratinase production and the result showed that shaking rate enhance extracellular keratinase activity of *Bacillus tequilensis* S-5. The optimum keratinase activity of *Bacillus tequilensis* S-5 of 43.8U/ml was observed at 200 rpm.

Table 5:-Effect of different carbon sourcein raw chicken feather medium in48hrs.

Carbon Source (0.2%)	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
Fructose	**18.7**	**±0.17**	**0.9**
Lactose	**18.3**	**±0.42**	**2.2**
Sucrose	**17.2**	**±0.5**	**2.9**
Maltose	**20.8**	**±0.61**	**2.9**
Starch	**24.9**	**±0.38**	**1.5**
Glycerol	**20.8**	**±0.28**	**1.3**
Glucose	**19.2**	**±0.48**	**2.5**
Control	**35.9**	**±0.51**	**1.4**

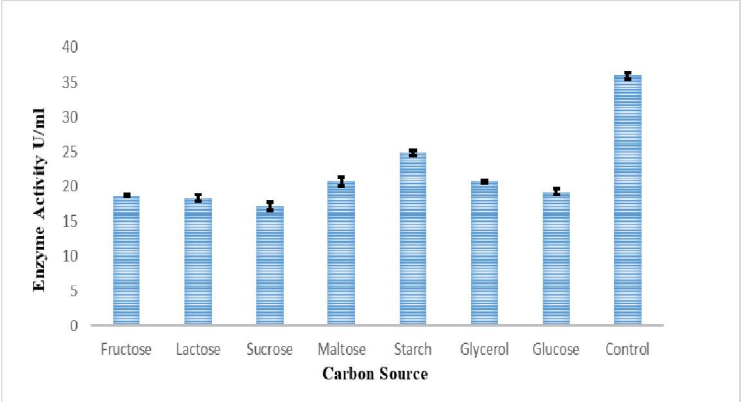


Figure 6:-Effect of different carbon source on keratinase production by *Bacillus tequilensis* S-5.

Addition of different carbon sources to the medium showed that there was decrease in keratinase production. Similar to our result, addition of sugar to production medium suppressed growth and keratinase production from *Bacillus*sp. FK46[25]. Suppression of enzyme production by sugar is also common in fungi and other microorganisms [21]. Protease activity of *Streptomyces* sp. was observed to be decreased by glucose [17].

Table 6:- Effect of different organic and inorganic nitrogen source on keratinase production from *Bacillus tequilensis* S-5

Nitrogen Source (Organic and Inorganic)	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
Casein Hydrolysate	20.5	±0.8	3.9
Peptone	17.9	±0.6	3.3
Beef Extract	21.9	±0.4	1.8
Yeast Extract	35.7	±0.3	0.9
Casein	17.5	±0.4	2.2
Tryptone	16.7	±0.7	4.1
Ammonium Chloride	14.2	±0.4	2.8
Ammonium Nitrate	20.7	±0.1	0.4
Ammonium Dihydrogen orthophosphate	15.7	±0.3	1.9
Ammonium Sulphate	38.2	±0.6	1.67
Control	35.1	±0.5	1.5

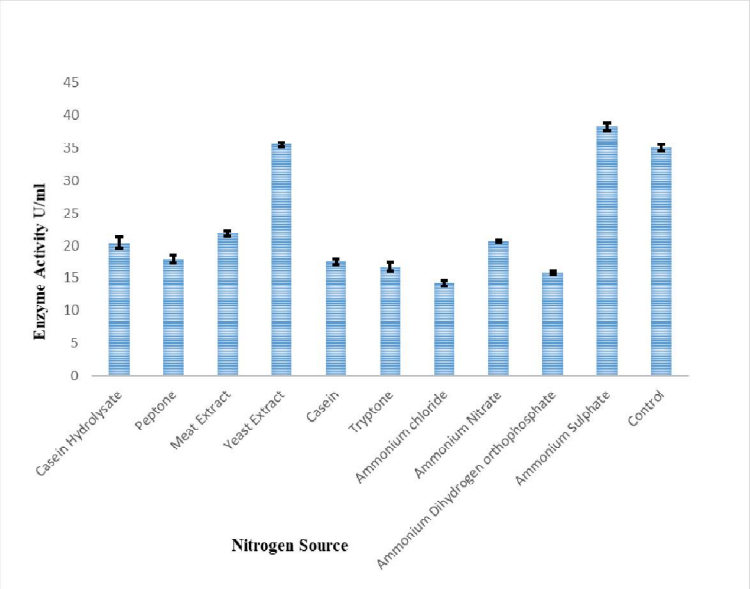


Figure 7:-Effect of different organic and inorganic nitrogen source on Keratinase production by *Bacillus tequilensis* S-5.

To investigate the effect of nitrogen source on Keratinase production, media containing different nitrogen sources (0.2% concentration) to cultivate *Bacillus tequilensis* S-5. The maximum keratinase activity (35.7 and 38.2 U/ml) were obtained with yeast extract and ammonium sulphate. So_4, respectively however further increasing the concentration of yeast extract and ammonium sulphate resulted in decrease in keratinase production (data not shown) similar to our results addition of NH_4Cl at 0.2% concentration enhanced keratinase production from *Bacillus cereus* Wu2 [21]. Similarly, nitrogen supplement improved keratinase production from *Alcaligenes* sp. AQ05-001 [11]and *Bacillus weihenstephanensis*PKD-5 [26].

Table 7:-Effect of inoculum level on Keratinase production by *Bacillus tequilensis* S-5.

Inoculum Level %	Enzyme activity U/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
0.5	18.5	±0.57	3.0
1.0	30.7	±0.3	0.9
2.0	43.8	±0.2	0.6
3.0	22.1	±0.2	0.9
4.0	10.5	±0.26	2.4
5.0	9.4	±0.4	4.2
6.0	7.1	±0.28	3.9

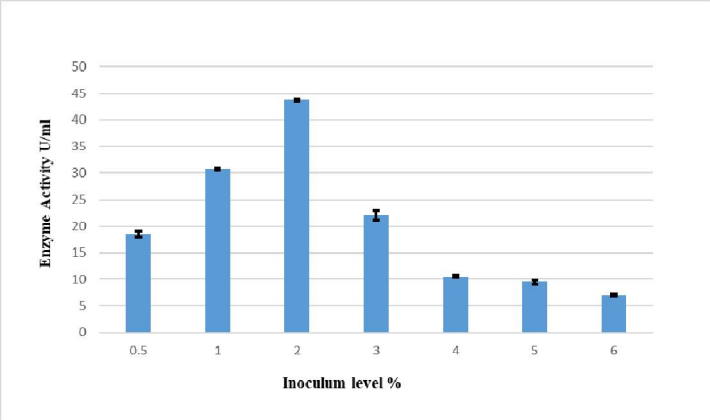


Figure 8:-Effect of inoculum level on Keratinase production by *Bacillus tequilensis*S-5.

In the present investigation effect of different inoculum levelswas investigated on keratinase production by *Bacillus tequilensis* S-5. Different inoculum levels ranging from 0.5 to 6% were tested. The maximum keratinase activity 43.8 U/ml was obtained in 2% inoculum which was the control. Similar inoculum level (1-2% v/v) have been reported as optimal for keratinase production by different researchers [10, 27-29].

Table 8:-Effect on incubation period on keratinase production by *Bacillus tequilensis* S-5.

Incubation Time	Enzyme activityU/ml	Standard Deviation (S.D.)	Coefficient of Variation (C.V.) %
24 h	22.8	±0.2	0.8
48 h	43.5	±0.4	0.9
72 h	11.3	±0.2	1.7

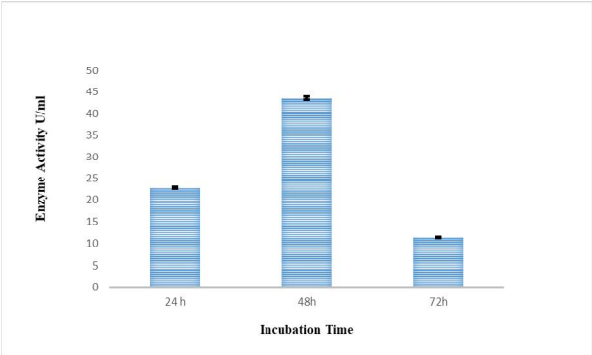
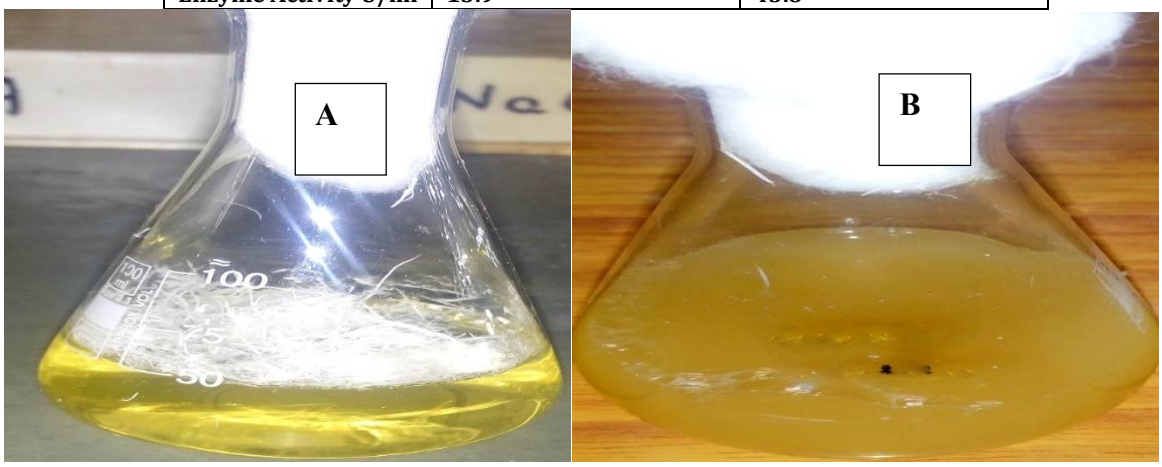


Figure 9:-Effect on incubation period on Keratinase production by *Bacillus tequilensis* S-5.

Incubation period is an important determinant of the cost of production of any fermentative product. In this regards maximum keratinase production from the *Bacillus tequilensis* S-5 strain was observed after 48 h of incubation as compared to 24h & 72h. This is a reasonably short incubation period, which is shorter than the optimal incubation period reported by other researchers: 3days [12]. 4 days [30,31], 5 days [32] and 7 days [33].

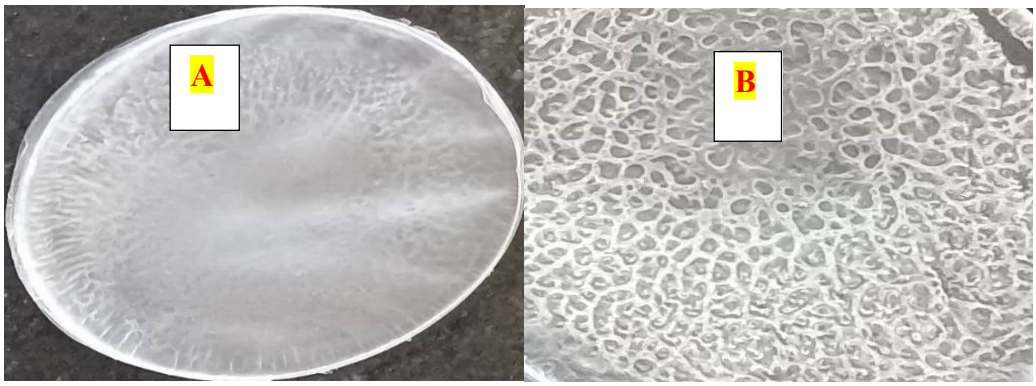
Table 9:-Comparison of fermentation condition of unoptimized and optimized Medium for Keratinase production by *Bacillus tequilensis* S-5.

Medium Comoponent	Unoptimized Medium	Optimized Medium
Feather %	2%	1.5%
Temperature	40ºC	30ºC
pH	7.0	7.0
Agitation Rate	150 rpm	200 rpm
Carbon source	Nil (other carbon source)	Nil (other carbon source)
Nitrogen Source	Yeast Extract	Yeast Extract
Inoculum Level %	2%	2%
Incubation Time (h)	48	48
Enzyme Activity U/ml	13.9	43.8



(A) Before inoculation of *Bacillus tequilensis* S-5 keratinase.
(B) After inoculation of *Bacillus tequilensis* S-5 keratinase.

Figure 10 :- Flask contain 100 ml of fermented broth having 43.8 U/ml of keratinase and 5 gm of feathers. (A) Picture taken at 0h. (B) Picture taken after 12h of incubation. It is visible from figure10 that the *Bacillus tequilensis* S-5 keratinase successfully hydrolyzed chicken feathers in 12h the O.D. 660nm of hydrolyzed feather solution was determined after appropriate dilution, which showed as [34]. There was no there increase in the O.D. 660nm when the flask was incubated for 24h and more. This hydrolysate passed through muslin cloth with no visible feather fibers observed on the cloth except for the central shaft of the feather.



(A) Bioplastic film Before degradation **(B) Bioplastic After degradation**

Figure 11:-Bioplastic filmproduced from keratin powder & polyvinyl alcohal

CONCLUSION

Optimization study for keratinase production from *Bacillus tequilensis* S-5 strain result in a more than7-fold increase in enzyme titre. This is a significant enhancement in enzyme production. The keratinase successfully hydrolyzed feathers to soluble protein lysate which can be used as animal/bird meal and biofertilizer. The keratin produced through enzymatically hydrolyzed chicken feather waste was successfully used for manufacturing bioplastic which was biodegradable as well. So, it can be concluded that the keratinase from *Bacillus tequilensis* S-5 has immense industrial potential.

REFERENCES

1. Baid, S., Vashishtha, A., Ahmed, A., Verma S., &Meghwanshi, G.K. (2016). Bacterial Keratinase catalysed bioremediation of keratin rich wastes for potential agricultural and other applications, *J. Phytol. Res*. 29(1 & 2): 17-30.
2. Lakshmi, P.J., Chitturi, C.H., Lakshmi, M.K. (2013). Efficient degradation of feather by keratinase producing *Bacillus* sp., Int. J. Microbiol., Article Id 608321, Https://Doi.Org/10.1155/2013 /6083 21,2013.
3 Sharma I.& Kango N. (2021). Production and characterization of keratinase by *Ochrobactrum intermedium* for feather keratin utilization, *Int. J. Biol. Macromol.* 166: 1046-1056.
4 Cavello, I.A.,Hours R.A.& Cavalitto S.F. (2012). Bioprocessing of "hair waste" by *Paecilomyces lilacinus* as a source of a bleach-stable, alkaline, and thermostable keratinase with potential application as a laundry detergent additive: characterization and wash performance analysis, *Biotechnol. Res Int.* 1–12.
5 Bressollier, P., Letoumeau, F., Urdaci, M.&Vemcuil, B. (1999). Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*, *J.Appl. Environ. Microbiol.* 65: 2570-2576.
6 Prasad, V.H., Kumar, G.,Kartik, K.L.&Rao, K.V.B. (2010). Screening of extracellular Keratinase producing bacteria from feather processing area, *J. sci. res*. 2(3): 559-565, 2010.
7 Qiu, J., Wilkens, C., Barrett K.,& Meyer A.S. (2020). Microbial Enzymes Catalyzing Keratin Degradation, Classification, Structure, Function. *Biotechnol Adv*., 44: 107607. Doi:10.1016/*J. Biotechnol*.
8 Dabi, N.K., Vashistha, A., Ahmed A., &Meghwanshi, G.K. (2021). Screening of native bacterial isolates for Keratinase production and its application in detergent formulation and wash performance, *J. phytol Res*. 34(2): 159-168.
9 Gradisar, H., Kern, S., Friedrich, J. (2000). Keratinase of *Doratomyce microspores*. *Appl. Microbiol. Biotechnol*. 53:196-200.
10 Thanaa, H.A., Nadia, H.A., & Latifa, A.M. (2011). Production, Purification and Some Properties of Extracellular Keratinase from Feathers-Degradation by *Aspergillus oryzae* NRRL-447. *J. of Appl. Sci. Environ. Sanit.* 6(2): 123-136.
11 Yusuf, I., Ahmad, S.A., Phang, L.Y., Syed, M.A., Shamaan, N.A., Khalil, K.A., Dahalan, F.A.,&Shukor, M.Y. (2016). Keratinase production and biodegradation of polluted secondary chicken feather wastes by a newly isolated multi heavy metal tolerant bacterium-*Alcaligenes* sp. AQ05-001, *Journal of Environmental Management*, 1-14.
12 Shalaby, M.M., Samir, R., Goma, F.A.M., and Rammadan, M.A. (2021). Enhanced fusidic acid transdermal delivery achheived by newly isolated and optimized *Bacillus cereus* Keratinase, *Biotechnology reports*, 30.
13 Nnolim, N.E., Okoh, A.I.,& Nwodo, U.U. (2021). Elucidation of coding gene and characterization of alkaline metallo-keratinase produced by acidophilic *Bacillus* sp. Okoh-K1 grown on chicken feather, *Environmental Technology & Innovation*, 21, 101285.
14 Nnolim, N.E., Ntozonke, N., Okah, A.I., Nwodo, U.U.(2020). Exoproduction and characterization of a detergent-stable alkaline keratinase from *Arthrobacter* sp. KFS-1, *Biochimie*, 177, 53-62.
15 Demir, T., Hames, E.E., Oncel, S.S.,&Sukan, F.V. (2015). An optimization approach to scale up keratinase production by *Streptomyces* sp. 2M21 by utilizing chicken feather, *International Biodeterioration & Biodegradation*, 103, 134-140.
16 RajpuT, R.,& Gupta, R. (2013). Thermostable keratinase from *Bacillus pumilus* KS12: Production, chitin crosslinking and degradation of Sup35NM aggregates, *Bioresource Technology*, 133, 118-126.
17 Lin, X.,Inglis, G.D., Yanke, L.J.,and Cheng, K.J. (1999).Selection and characterization offeather degrading bacteria from conola meal compost, *J. Ind. Microbiol. Biotechnol*., 23, 149-153.
18 Mohamedin, A.H. (1999). Isolation, identification and some cultural conditions ofa protease producing thermophilic *Streptomyces* strain grown on chickenfeather as a substrate, *Int. Biodeterior. Biodegrad*., 43, 13-21.
19 Letourneau, F.,Soussote, V., Bressollier,P., Branland, P., andVerneuil, B. (1998). Keratinolytic activity of *Streptomyces* sp. S. KI-02: a new isolated strain, *Lett. Appl. Microbiol*., 26, 77-80.
20 Ghonemy, D.H.E.,&Ali, T.H. (2021). Effective bioconversion of feather-waste Keratin by Thermo-Surfactant Stable Alkaline Keratinase produced from *Aspergillus* sp. DHE7 with promising biotechnological application in detergent formulations, *Biocatalysis and Agricultural Biotechnology*, 35, 102052.
21 Lo, W.H., Too, J.R.,& Wu, J.Y. (2012). Production of keratinolytic enzyme by an indigenous featherdegrading strain *Bacillus cereus* Wu2, *Journal of Bioscience and Bioengineering*, 114(6), 640-647.
22 Kim, J.M., Lim, W.J., andSuh, H.J. (2001). Feather-degrading *Bacillus* species from poultry waste, *Process Biochem*., 37, 287-291.
23 Nilegaonkar, S.S., Zambare, V.P., Kanekar, P.P., Dhakephalkar, P.K., and Sarnail, S,S. (2007). Production and partial characterization of dehairing protease from *Bacillus cereus* MCM B-326, *Bioresour. Technol*., 98, 1238-1245.
24 Joo, H.S., Kumar, C.G., Park, G.C., Kim, K.T., Paik, S.R., and Chang, C.S. (2002). Optimization of the production of an extracellular alkaline protease from *Bacillus hirikoshii*, Process *Biochem*., 38, 155-159.
25 Suntornsuk, W.,& Suntornsuk, L. (2003). Feather degradation by *Bacillus* sp. FK 46 in Submerged cultivation, *Bioresour. Technol*., 86, 239-243.
26 Sahoo, D.K., Das, A., Thatoi, H., Mondal, K.C.,&Mohapatra, P.D.K. (2012). Keratinase production and Biodegradation of whole chicken feather keratin by a newly isolated bacterium under submerged fermentation, *Appl.Biochem.Biotechnol*., 167, 1040-1105.
27 Gajju, H., Bhalla, T., Agarwal, H. (1996). Thermostable alkaline protease from thermophilic *Bacillus coagulans* PB-77, *Indian J. Microbiol*. 36, 153–156.

28 Kanekar, P., et al. (2002). Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India, *Bioresour. Technol*. 85 (1), 87–93.
29 Jani, S.A., et al. (2017). Production of alkaline keratinolytic protease by *Bacillus* sp. B13 from feather waste, *Int. J. Curr. Microbiol. App. Sci* 6 (5), 1538–1552.
30 Abdel-Fattah A.M., et al. (2018). Biodegradation of feather waste by keratinase produced from newly isolated *Bacillus licheniformis* ALW1, *J. Genet. Eng. Biotechnol*.
31 Poopathi, S., et al. (2014). Purification and characterization of keratinase from feather degrading bacterium useful for mosquito control–A new report, *Trop. Biomed*. 31 (1), 97–109.
32 Yadav, A., et al. (2011). Optimization of keratin degrading enzyme from Thermophillic strain of *Streptomyces sclerotialus*, *Res. J. Microbiol*. 6 (9), 693.
33 Brandelli, A., Sala, L., &Kalil, S.J. (2015). Microbial enzymes for bioconversion of poultry waste into added-value products, *Food Res. Int.* 73, 3–12.
34 Okoroma, E.A., et al. (2012). Identification and characterization of a *Bacillus licheniformis* strain with profound keratinase activity for degradation of melanised ferather, *Int. Biodeterior. Biodegradation*, 74,54-60.

CITATION OF THIS ARTICLE

N K Dabi, A Ahmed, G K Meghwanshi. Optimization, Application and Some Properties of Extracellular Keratinase from Chicken Feathers-Degrading Bacillus Tequilensis S-5. Bull. Env.Pharmacol. Life Sci., Spl Issue [1] 2022 : 1188-1197