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Research Paper

Production of Bacterial Keratinase Using Keratinous Waste Substrate

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Abstract

This study was conducted with the aim of isolation of efficient keratinase producing bacteria from the soils of chicken feather and human hair-dumping places, determining optimum keratinase production conditions and partially characterizing the stability of keratinase interms of keratinase activity assay with regards to some physicochemical parameters. Inoculation of soil sample suspensions on chicken feather and human hair modified meal agar plate resulted in the growth of three keratinolytic bacterial isolates ($Kf_1$and Kf_2 from chicken feather and Kh from hair cut dumping sites). These isolates were identified as Bacillus species on the basis of their biochemical characteristics. The optimum temperature of keratinase production for all isolates was recorded at 60°C with activities of 16.2 U/ml/min, 15.5U/ml/min and 12.3 U/ml/min for isolates Kf_1, Kf_2 and Kh, respectively. In all cases, pH 7 was optimum for keratinase production resulting in activities corresponding to 16.4 U/ml/min, 15.1U/ml/min and 12.4/ml/min for Kf_1, Kf_2 and Kh, respectively. Among the various carbon sources tested, potato gave 14.3 U/ml/min, 11.3 U/ml/min and 10.1U/ml/min for isolates Kf_1, Kf_2, and Kh, respectively. Regarding nitrogen sources, yeast extract gave 25.2 U/ml/min, 20.5 U/ml/min and 16.37 U/ml/min for Kf_1, Kf_2, and Kh, respectively. Studies on the effect of pH on the activity and stability of keratinase enzyme revealed that the crude enzyme had a maximum stability at pH 7.The maximum keratinase stability time for the three isolates was found to be 48 hr with keratinase activity of 8.5 U/ml/min, 6.5 U/ml/min and 5.9 U/ml/min for Kf_1, Kf_2, and Kh, respectively. These results generally indicate that the keratinase obtained in this study belongs to the class of hydrolases that are active and stable at pH 7. Thus, identification of the three Bacillus isolates at a molecular level and separation and identification of the types of keratinase produced by molecular techniques is recommended.

1. Introduction

Keratinase is an enzyme capable of degrading the insoluble structural protein called keratin. Keratin is a fibrous structural protein of hair, nails, horn, hoofs, wool, feathers, and of the epithelial cells in the outermost layers of the skin (Cai et al., 2008). Keratin present in feathers and human hair cannot be degraded by the usual proteolytic enzymes such as trypsin, pepsin and papain. This is because of the differences in composition and molecular conformation of the amino acids found in keratin (Janaranjani et al., 2015).

Keratinase enzymes are useful in the bio-conversion of keratin waste to feed and fertilizers. Other promising applications of keratinolytic enzymes include enzymatic deharing in leather and cosmetic industry, and detergent uses.

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Animal wastes can be used as a powerful nutrient source. But wastes like feathers and hair obtained as byproducts of meat and dairy processing cannot be degraded easily and hence they form a source of pollution. There are certain strains of microorganisms which produce extracellular enzymes which can serve as a substantial source of keratinase for the degradation of keratinous wastes (Adriano and Brandelli**,** 2007).

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In recent years, feather-treated with microbial keratinase is attracting wide attention with several applications. Keratinase–treated feather is considered as a viable source of dietary protein in food and feed supplements, as the enzyme-treated end product is thought to retain high nutritive value. Keratinases are projected to generate a potential worldwide market similar to other proteases having a price of 4.34 dollars (Manoj et al., 2016). Keratinolytic enzymes have several current and potential applications in agro industrial, pharmaceutical, and biomedical fields. Their use in biomass conversion into biofuels may address the increasing concern on energy conservation and recycling (Andriano et al**.,** 2007).

In Ethiopia, isolation of protease was conducted by Gessesse Mula et al. (2011). This report described the isolation of Bacillus species AR009 and *B. pseudofirmus* AL-89 from an alkaline soda lake in Ethiopia, Rift Valley Area. The use of 'nug' meal as a low cost substrate for the production of alkaline protease was also described with the intent of practical application (Genckkal, 2004). In recent years, limited efforts are also being made to improve the management of tannery waste in the local industries using protease. As concluded by Gessesse Mula et al. (2011), it is crucial to continue the efforts of searching for potent isolates in the environment because there are plenty of potential applications of keratinase in the food industry, animal feed processing and management of wastes of various industrial activities and municipal household garbage wastes.

As the best knowledge of the author, there has been little work done on keratinase enzyme in Ethiopia. Therefore, this study was aimed to evaluate the *in-vitro* production of Keratinase from bacterial species grown on keratineous wastes (chicken feather and human hair).

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted in the Microbiology laboratory of School of Biological Sciences and Biotechnology at Haramaya University, which is located at a latitude of 9° 26' N, the longitude of 42° 3' E, and an altitude of about 2010 meters above sea level. The area receives an average annual rainfall of 741.6 mm (Ewonetu Kebede, 2017).

2.2. Research Design

The design of the research involved a laboratory based study of isolation, screening, biochemical characterization of bacterial isolates. The performance of bacterial isolates in terms of keratinase production was evaluated and stability of the activity of produced keratinase was done at various parameters. All experiments were conducted in triplicates and their values were recorded and used for subsequent analyses.

2.3. Soil Sample Collection

One hundred gram soil samples were collected from poultry feather and human hair cut dumping sites at Haramaya University. The samples were collected using sterile plastic bags and stored in the refrigerator until used for isolation of bacteria.

2.4. Preparation of Feather and Human Hair Cuts Meal

The feather and human hair cuts meal were prepared from native chicken feathers and human hair collected from poultry farm and barber's shop at HU, respectively, as described by Tork et al. (2008) with slight modifications. The feathers and human hair cuts were broken into smaller pieces of 2 cm long with scissors and washed several times with tap water. Defatting of feather and hair pieces was done by soaking them in a mixture of chloroform: methanol (1:1) for 2 days followed by treatment with chloroform: acetone: methanol (4:1:3) solution for 2 days. The solvent was replaced every day. The feathers and hairs were finally washed several times with tap water to eliminate the solvent, dried for 24 hr in sunlight, ground using an electrically operated blender and used as feather and human hair cuts meal.

2.5. Isolation, Screening and Identification of Keratinase Producing Bacteria

2.5.1. Isolation of Bacteria from Soil

Isolation of bacteria was performed by serial dilution and plating method on nutrient agar medium. One gram of soil sample was transferred to 10 ml of sterilized distilled water and mixed properly. Serial dilution was made up to 10^{-6} and 0.1 ml was taken from10^{-6} of the diluted sample was inoculated to nutrient agar media by spread plate method. Plates were incubated at 37ºC for 48 hr. The bacterial isolates were further sub cultured twice on nutrient agar media to obtain pure culture. Pure isolates were maintained in nutrient agar slants at 4ºC for further studies.

2.5.2. Screening of Keratinase Producing Bacteria

Suspension of nutrient agar slant was made and about 0.1 ml of the pure culture of three isolate was uniformly spread plated on the surface of an agar medium supplemented with the prepared feather and human hair cuts meal as a sole carbon and nitrogen source. Isolates obtained from poultry site soil was spread on agar supplemented with feather meal whereas isolates obtained from the soil samples collected from human hair cut dumping sites were spread on agar supplemented with human hair cuts meal. Then colonies that appeared on the media were picked and sub-cultured on nutrient agar (Harison and Sandeep, 2014).

The agar supplemeted with feather meal contained the following ingredients ($g.L^{-1}$): NaCl (0.5), KH_2PO_4 (0.7), K_2HPO_4 (1.4), $MgSO_4$ (0.1) and feather meal (10). The agar supplemeted with human hair cuts meal contained all the above-listed ingredients except that the feather meal was replaced with human hair cuts meal. The pH of the medium was adjusted to 7. The plates were incubated at 37°C till the colonies appeared. The presence of keratinase producing bacteria was indicated by growth and formation of hallo zone forming colonies on modified chicken feather meal agar or on human hair cuts meal agar media. Colonies on such media were sub-cultured and pure cultures were maintained on nutrient agar plate slants at 4°C (Shabaan et al., 2014).

2.5.3. Cultural, Microscopic and Biochemical Characterization of the Isolate

The isolates were identified using: a) their cultural characteristics such as colony morphology with respect to shape, texture of colony and pigmentation b) microscopic observation of the isolates under the high power magnifying lens of the compound light microscope after Gram staining. Motility tests were also performed to observe the morphology and motility of the cells and biochemical characteristics as described in Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986).

The isolates were biochemically characterized using catalase, carbohydrate fermentation, oxidase, starch hydrolysis, indole production, methyl red, Vogas-Proskauer, citrate utilization, triple sugar iron, nitrate reduction and amino acid utilization tests for the purpose of preliminary identification of the isolates.

2.6. Production of Crude Keratinase using the Bacterial Isolates

Keratinase was produced by employing submerged fermentation using two types of sterile media. The first one was prepared from chicken feather meal (1%), yeast extract (0.01%), NaCl (0.05%), KH_2PO4 (0.03%), K_2HPO4 (0.04%) and $MgCl_2$ (0.01%), at a pH of 7. The second media composed of human hair cuts meal (1%), yeast extract (0.01%), NaCl (0.05%), KH_2PO4 (0.03%), K_2HPO4 (0.04%) and $MgCl_2$ (0.01%), at a pH of 7. These media were inoculated by 2-3 inocula taken from pure cultures of isolates and incubated for 3 days at 37ºC. After incubation, portions of the broth cultures were centrifuged at 10,000 rpm for 10 min and the supernatants were used as source of crude enzymes (Lin and Yin, 2010). The amount of keratinase was estimated using keratinolytic (keratinase) assay method described in section 2.7.

2.7. Assay for Keratinolytic Activity

The assay of keratinolytic activity was conducted by using soluble keratin as a substrate. Soluble keratin was made by mixing separately 5 gm of chicken feather and 5 gm of the human hair cut with 250 ml of dimethylsulfoxide (DMSO) and heating the obtained mixture in a reflux condenser at 100°C for 2 h. Soluble Keratin of each was precipitated by addition of chilled acetone at −20°C for 2 hr, followed by cooling and centrifugation at 10000 rpm for 10 min. The resulting precipitate was washed twice with distilled water and dried at 40ºC in a hot air oven. The keratin solution was prepared by dissolving 1gm of soluble keratin in 20 ml of 0.05 M NaOH, adjusting the pH to 7 with 0.1 M HCl

and diluting the resulting solution to 200 ml with 0.05 M phosphate buffer pH 7 (Vigneshwaran et al., 2010).

The keratinolytic activity was assayed by the modified method of Cheng et al. (1995) by incubating 1.0 ml of crude enzyme which was diluted in 0.05 M Tris-HCl buffer (pH 7.0) with 1.0 ml of 1% soluble keratin solution at 50ºC in a water bath for 20 min. The reaction was stopped by adding 2.0 ml of 0.4 M trichloroacetic acid (TCA). The precipitate formed by the addition of TCA was removed by centrifugation at 10,000 rpm for 10 min. After centrifugation, 0.2 ml of the supernatant was taken and diluted with 1.0 ml of distilled water. To the resulting dilution, 5.0 ml of alkaline copper reagent was added, shaken well, and incubated for 10 min. After incubation, 0.5 ml of Folin Ciocalteau (FC) reagent was added and incubated again in the dark for 30 min to allow blue color development. The control was prepared by incubating enzyme solution with 2 ml of TCA without the addition of keratin solution. The absorbance was measured at 660 nm using a UV-spectrophotometer. One unit of alkaline keratinase was defined as the amount of enzyme required to liberate 1 μg of tyrosine per min per ml under the standard assay conditions (Vigneshwaran et al., 2010).

2.8. Effect of Physico-chemical Parameters on Keratinase Production

2.8.1. Effect of pH, Temperature, and Concentration of Substrate on Keratinase Production

The effect of three different physicochemical parameters, i.e. pH, temperature, and concentration on keratinase production was assessed for the isolate. The effect of pH and temperature on keratinase production were individually tested by adjusting the production media to different pH values (pH 5, 6, 7, 8, 9 and 10) and incubating to varying temperatures (50°C, 60°C, 70°C,and 80°C). Similarly, the effect of substrate concentration on the production of keratinase was assessed by growing the isolates in media containing different concentrations of feather and human hair meal as a substrate (2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml) were used. These fermentation media were assayed every day for keratinase production till a decline is observed in the enzyme activity (Saibabu et al., 2013).

2.8.2. Effect of Carbon and Nitrogen Source on the Production of Keratinase

Separately, 5% of Wheat flour, corn flour, sorghum flour, and potato were used as carbon source in place of glucose to find out their effect on keratinase production. The cultures were incubated at 37°C and pH 7 for 48 hr and the sources of carbon used in the production medium were varied by replacing glucose with the above-mentioned substrates (Akcan, 2012)

The effect of different nitrogen sources were tested by adding five gm of nitrogen-containing substrates such as tryptone, peptone, and yeast extract in the production medium separately. The fermentation media having the three isolates in different flask were incubated at 37^{O}C and pH 7 for 48 hr and tested for keratinase production (Venkata et al., 2013).

2.8.3. Effect of pH, Temperature and Incubation Time on Keratinase Activity

The optimal pH for keratinase activity was determined over a pH range of 5, 6, 7, 8, 9, and 10. To determine the pH range over which keratinase remain stable, the crude enzyme was pre-incubated in buffers over a pH range of 5, 6, 7, 8, 9 and 10 at 50°C for 60 min. Citric acid/sodium phosphate buffer was used for pH between 5 and 7; barbital/HCl for pH between 8 and 9 and sodium carbonate buffer for pH between 10. To determine the thermo-stability, the enzymes were pre-incubated in barbital buffer (pH 7) at temperatures over the range of 50, 60, 70 and 80°C (Minghai, 2012).

The time required to attain the maximum enzymes activity was optimized for each isolate. To do this, each isolate was inoculated into chicken feather and human hair cuts meal media and incubated at 37°C. The keratinase activity was measured at regular intervals for 5 days (24 hr, 48 hr, 72 hr, 96 hr, and 120 hr). From the graph, the time required to attain the highest enzyme production was determined (Anitha and Eswari, 2012).

2.9. Data Analysis

All data gathered in this study were subjected to statistical analysis using SPSS version 20. The degradability and enzyme activities were analyzed by taking the mean values of triplicates. Microsoft office excels work sheet 2007 was used for the analysis of generated data and the preparation of graphs.

3. Results

3.1. Isolation. Screening and Cultural Characterization of Keratinase Producing Bacteria

Among the three bacterial isolates obtained, Kf_1 and Kf_2 were the isolates from the poultry farm site and Kh was the isolate from human hair-dumping site. The isolates were able to grow on the modified human hair cut and feather meal agar plates. Two isolates (Kf_1 and Kf_2) have circular, white and smooth texture. But for Kh isolate the morphology of the colony was white darkish, oval shaped and its texture was smooth like that of the other isolates. All the three isolates (Kf_1, Kf_2 and Kh) are Gram positive and motile.

3.2. Biochemical Characterization

The results of the biochemical tests of the three isolates i.e., Kf_1, Kf_2, and Kh are summarized in Table 1.

3.3. Identification of Keratinase Producing Bacterial Isolate

Based on the cultural, microscopic and biochemical characteristics of the isolates, isolate kf_1, Kf_2 and Kh were identified as bacillus species.

3.4. Production of Keratinase Using Bacterial Isolates

The results of this study indicated that the activity of released keratinase from Kf_1, Kf_2, and Kh isolates was 14.2±0. 45 U/ml/min, 11.9±0.35 U/ml/min, and 10. 9±0.34 U/ml/min, respectively (Table 2). The highest and lowest keratinolytic activities were consistently recorded for isolates Kf_1 and Kh, respectively.

3.5. Effect of Physico-chemical Parameters on Keratinase Production

3.5.1. Effect of pH on keratinase production

The highest keratinase production was recorded for Kf_1 at pH 7 (showing a keratinase activity of 16.4 U/ml/min). The optimum keratinase production by Kf_2 and Kh, at pH 7 was 15.1 U/ml/min and 12.3 U/ml/min, respectively. The lowest keratinase production of 4.5 U/ml/min, 4 U/ml/min, and 2.7 U/ml/min was recorded at pH 10 for Kf_1, Kf_2 and Kh isolates, respectively (Figure1).

Table 1: Biochemical characterization of the bacterial isolates

Biochemical Characteristics	Bacterial Isolates		
	Kf_1	Kf_2	Kh
Catalase test	+	+	+
Indole test	-	-	-
Citrate utilization test	+	+	+
Starch hydrolysis test	+	+	+
Oxidase test	-	-	-
Methyl red test	-	-	-
H_2S formation test(TSI) test	+	+	-
Slant color	Yellow (acid)	Yellow (acid)	-
Bottom color	Red (alkali)	Red (alkali)	Red (alkali)
Lactose utilization test	-	-	+
Carbohydrate Fermentation Test			
Glucose	+	+	+
Sucrose	+	+	+
Lactose	-	-	+

(+) Indicates positive result and (-) Indicates negative result

Table 2: Keratinolytic activity of keratinase produced by the isolate

No.	Isolates	Activity U/ml/min
1	Kf_1	14.2±0.45
2	Kf_2	11.9±0.35
3	Kh	10.9±0.34
4	Cf	1.2±0.45
5	Ch	1±0.26

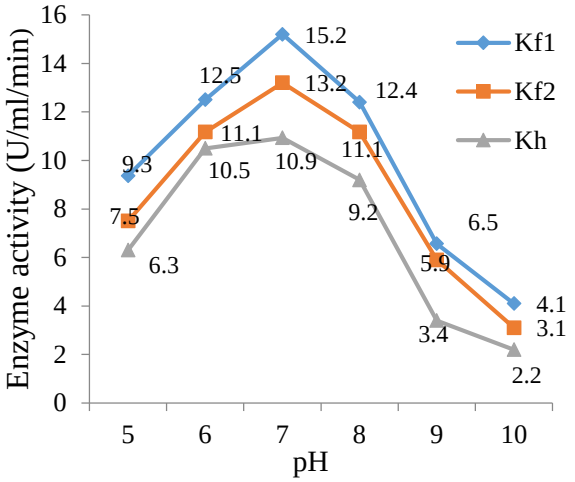

Figure 1: Effect of pH on enzyme production

3.5.2. Effect of Temperature on Keratinase Production

The highest keratinase production was recorded at 60°C for Kf_1, Kf_2 and Kh with an activity of 16.2 U/ml/min, 15.5 U/ml/min and 12.3 U/ml/min respectively. The lowest keratinase production was recorded for Kf_1, Kf_2 and Kh at 80°C was 14.1, 13.2 and 8.7, respectively (Figure 2).

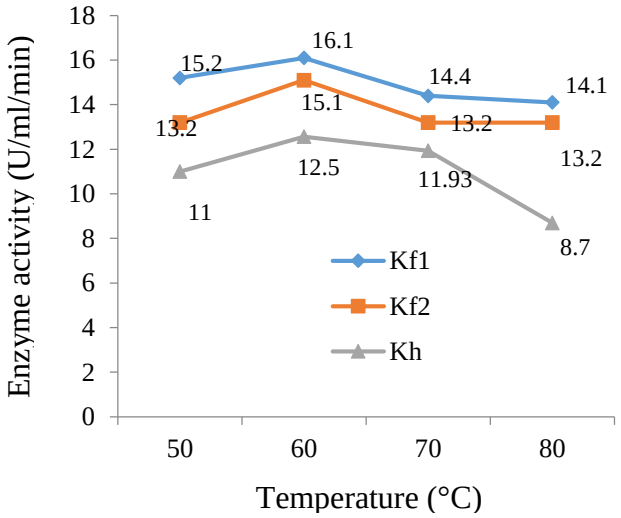

Figure 1: Effect of temperature on enzyme production

3.5.3. Effect of concentration of substrates on keratinase production

The highest keratinase production was recorded at 10 mg /ml of feather meal and human hair cut concentration (specific activities of 10.5, 8.5 and 5.5 U/ml/min for Kf_1, Kf_2 and Kh, respectively). The lowest keratinase production was recorded at 2 mg/ml of feather meal and human hair concentration for all bacterial isolates (Figure 3).

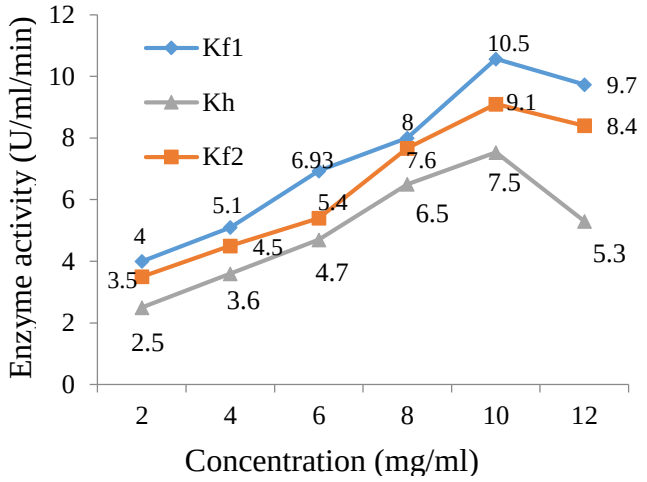

Figure 3: Effect of feather and human hair cut concentration on keratinase production

3.5.4. Effect of the Type of Carbon and Nitrogen Source on the Production of Keratinase

The highest keratinase production was recorded with the addition of yeast extract for all isolates (with the activities of 25.2 U/ml/min, 20.5 U/ml/min and 16.4U/ml/min for Kf_1, Kf_2, and Kh, respectively). The lowest keratinase production was recorded on maize (with specific activities of 7.9 U/ml/min for Kf_1 and Kh; and 8.5U/ml/min for Kf_2) (Figure 4).

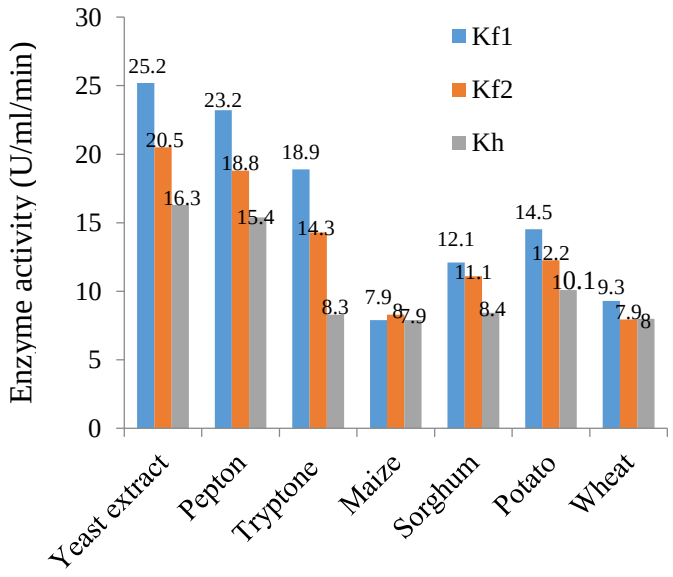

Figure 4: Effect and types of carbon and nitrogen source on keratinas production (Nutrient)

4. Discussion

4.1. Isolation, Screening and Identification of Keratinase Producing Bacteria

The study showed that Kf_1 and Kf_2 grew and formed a hallo zone on the feather meal agar plates and Kh on the modified human hair cut. This happened because the isolates might be released extracellular keratinase which breaks down keratin and increases the amino acid (tyrosine) content of the medium.

The cultural, microscopic and biochemical data for those keratinolytic bacteria that were isolated from poultry feather and human hair cut dumping sites indicated that the three isolates (Kf_1, Kf_2 and Kh) belonged to the Genus *Bacillus* according to the descriptions provided in Berge's Manual of Systematic Bacteriology (Brenner et al., 2004).

4.2. Effect of pH, Temperature, and Concentration of Substrate on Keratinase Production

In the present study keratin degradation in vitro was found to be associated with the release of a large amount of extracellular keratinase into the production medium by the grown isolates. The highest keratinase production was recorded for Kf_1 at pH 7. Srivastava et al. (2011) reported pH 8.5 as optimum for keratinase production in *Bacillus spp*. Cheng et al. (1995) also reported the highest keratinase production by *B. licheniform* was obtained at initial pH of 8.5. Similarly, *B. megatherium* was identified as a better alkalophilic keratinase producer by Srivastava et al. (2011). The variation in keratinase production of the previous study may be due to bacterial species difference or other environmental factors.

The highest keratinase production was recorded at 60°C for Kf_1, Kf_2 and Kh. Similarly, Sivakumar et al. (2012) reported that keratinase enzyme activity for *Bacillus* over a broad range of temperature (30-80°C) production is found to be optimal at 50°C. Further increase in the temperature to 80°C reduces keratinase enzyme activity. Laxman et al. (2004) also observed that the enzyme showed broad temperature specificity with a maximum activity at 60°C. The relative activity of enzyme was found to be higher in the temperature range of 50–70°C than at the normal assay temperature (40°C), while the enzyme retained nearly 50% of its activity at 80°C.

The highest keratinase production was recorded at 10 mg/ml of feather and human hair cut meal. Anitha and Eswari (2012) reported that significant improvement in yield of keratinase was observed on the medium with 2% raw feather. Keratinase production at the level of 0.5% feather meal was ten times less than that of 3% feather meal. This was because the amount of substrate supplied for growth and enzyme production was sufficient for the two isolates. Kf_1 and Kf_2 showed a higher keratinase production this is because of Feather protein is an excellent source of metabolizable protein (Klemersrud et al., 1998), and microbial keratinases enhance the digestibility of feather keratin (Lee et al., 1991; Odetellah et al., 2003; Korniłłowicz-Kowalska and Bohacz, 2011).

4.3. Effect of the Type of Carbon and Nitrogen Source on the Production of Keratinase

The three isolates showed the highest keratinase production by using yeast extract substrate as a nutrient. In previous study, supplementation of yeast extract resulted in maximal keratinase production by *Stenotrophomonas sp* (Ramnani and Gupta, 2004). Some cost effective substrate such as soybean meal have been successfully used for keratinase production (Vidyasagar et al., 2007). Different bacteria have different preference for either organic or inorganic nitrogen for growth and enzyme production although complex nitrogen sources are usually used for alkaline protease production (Panday et al., 2000). The choice of carbon and nitrogen sources has a major influence on the maximum yield of enzymes. Highest keratin hydrolyzing activity was achieved at higher yeast extract concentrations in a shorter period than in the presence of lower yeast extract concentrations (Prakasham et al., 2006). Sivakumar et al. (2012) also reported that the activity of crude keratinase assessed by incubating keratinase with different carbon and nitrogen sources. Extra nitrogen (yeast extract) have positive effects on keratinase production while other carbon and nitrogen sources have negative effects.

5. Conclusion

Keratinase is one of the most important groups of industrial enzymes with considerable application in the animal feed processing leather industry, medical activity, beverage industry and others sectors. In this

study, Kf_1, Kf_2 and Kh keratinolytic bacterial isolates that belong to *Bacillus* genus were identified by cultural, microscopic and biochemical characterization. The isolates produced maximum keratinase at pH 7, 60°C and 48 hours fermentation conditions. The study showed that maximum keratinase production was obtained from the use of potato as carbon source and yeast extract as nitrogen source.

Generally the three bacterial isolates Kf_1 and Kf_2 isolated from soil of poultry site and Kh from human hair cut damp sites are efficient in the production of the keratinase enzyme. Identification of the three Bacillus isolates at a molecular level, and purification and identification of the types of keratinase is recommended.

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Reference

Adriano, B. (2007). Useful enzymes for bioprocessing agro-industrial wastes and beyond. *International Biodeterioration and Biodegradation*, 60(5): 32-45.

Akcan, N. (2012). Production of extracellular protase in submerged fermentation by Bacillus licheniformis ATCC12759. *African Journal of Biotechnology*, 11(7):1729-1735.

Andriano, B., Riffel, A., Bellato, C. M., Souza, H. M., Eberlin, M. N. & Tavares, C. A. (2007). Purification and characterization of a keratinolytic metalloprotease from Chryseobacterium sp. kR6. *Journal of Biotechnology*, 128(5): 693–703.

Anitha, A. & Eswari, R. (2012). Impact of newly isolated bacillus megaterium (a1) on degradation of feather waste. *International Journal of Pharmaceutical and Bio Sciences*, 3(1):212-221

Brandelli, A. & Riffel, A. (2005). Production of an extracellular keratinase from *Chryseobacterium* sp. growing on raw feathers, *Journal Biotechnology*, 8(1): 35-42

Brenner, D. J., Krieg, N.R, & Staley, J.T. (2004). Bergey's manual of systematic bacteriology (2nd edition), Part B, Volume 2, Springer, New York

Cai, C., Lou B. & Zheng, X. (2009). Keratinase Production and Keratin degradation by Mutant Strain of *Bacillus subtilis*. *Journal of Zhejiang University science*, 9(2):60-67.

Daniel Yimer, Ameha Kebede. & Melaku, Alemu. (2014). Production and characterization of bacterial protease from isolates of soil and agro-industrial wastes MSc. thesis, Haramaya University, Haramaya, Ethiopia.

Ewonetu, Kebede. 2017. Growth Performance and Rearing Costs of Fayoumi and White Leghorn Chicken Breeds. *East African Journal of Sciences*, 11(1): 37-42.

Gessesse Mula, Lyantagaye, A.F., Nyine-Wamwize, S.L., Mattisson, L. & Pandy. (2011). Industrial Enzyme for sustainable bio economy: large scale production of application in environment and agriculture in eastern Africa, Nairobi, *LRI*.

Harison, M. and Sandeep, S. (2014). Degradation of keratinous waste products by keratinolytic bacteria isolated from soil. *International journal of engineering and computer science*, 3(8):7588-7595

Janaranjani, K., Praveenkumar, G. & Suneetha, V. (2015). Screening of keratinase producing bacteria by using feathers of owl, crow, hen, sparrow, peacock and cock. *Research Journal of pharmaceutical, biological and chemical sciences*. 6 (11):1802-1810.

Johnvesly, B., B.R. Manjunath & Naik, G.R. (2002). Pigeon pea wastes as a noval, inexpensive substrate for production of a thermostable alkaline protease from thermoalkalophilic *Bacillus* sp. *Bioresource Technology*, 82(7): 61-64.

Kim, J.M., Lim, W.J. & Suh, H.J. (2001). Feather degrading Bacillus species from poultry waste. *Process Biochemistry*, 37(4): 287-291.

Korniłłowicz-Kowalska, T. (1997). Studies on the decomposition of keratin wastes by saprotrophic microfungi. P.I. Criteria for evaluating keratinolytic activity. *Acta Mycology*, 32(5):51–79.

Laxman, R.S., S.V. More, M.V., Rele, B.S. Rao V.V. Jogdand, M.B. Rao, R.B. Naidu, P. Manikandam D.A. Kumar, J. Kanagraj, R. Samvarama and R. Puvanakrishnan (2004). Process for the Preparat of Alkaline Protease. *US Patent*, 4(6): 777129.

Lin, H.H. & Yin, L.J. (2010). Feather meal and rice husk enhanced keratinase production by *Bacillus licheniformis*YJ4 and characterization of produced keratinases. *Journal of marine science and Technology*, 18(3):458-465

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biology Chemistry*, 193(1):265-75.

Manoj, K., Anshumali, M., and Praveen, K.M. (2016). Microbial enzymes: industrial progress in 21st century. *Journal of Biotechnology*, 6(2): 174.

Minghai, Han., Wei, Luo1., Qiuya, Gu. & Xiaobin, Yu. (2012). Isolation and characterization of a keratinolytic protease from a feather-degrading bacterium *Pseudomonas aeruginosa* C11. *African Journal of Microbiology Research*, 6(9): 2211-2221.

Panday, A., C.R. Scocoll, P., Nigam, V.T., Soccoll, L.P.S., Vendenberghe. & R. Mohan. (2000). Biotechnological potential of agroindustrial residues II. Cassava bagasse. *Bioresource Technol*, 74(3): 81-87.

Prakasham, R.S., S.C.H. Rao & P.N. Sarma, (2006). Green gram husks an inexpensive substrate for alkaline protease production by *Bacillus* sp. In solid state fermentation, *Bioresource Technol*., 97(1): 1449-1454.

Saibabu, V. & Niyongabo, N. (2013). Isolation Partial purification and characterization of keratinase from *Bacillus megaterium.International. Research Journal of Biological Sciences*, 2(2): 13-20.

Shabaan, M. T., Attia, M., Sabhaa M. El-Sabagh & Amany, A. M. (2014). Isolation, Screening and Selection of Efficient Feather Degrading Bacteria. *Current Science International*, 3(4): 488-498.

Sivakumar, T., Shankar, P. and Ramasubramanian, V. (2012). Optimization for Keratinase Enzyme Production Using *Bacillus thuringiensis* TS2. *Academic Journal of Plant Sciences*, 5(3): 102-109.

Sneath, P.H.A., Mair, N.S., Sharpe, M.E. & Holt J.G. (1986). .Bergey's Manual of Systematic Bacteriology, Vol. 2 Baltimore., Williams and Wilkins.

Srivastava A., Sharma, A. & Suneetha, V. (2011). Feather waste biodegradation as a source of amino acid. *European Journal of Experimental Biology*, 1(2): 56-63

Venkata, S., Francois, N.., Niyonzima. & Sunil, S. (2013). Isolation, Partial purification and Characterization of Keratinase from *Bacillus* megaterium. *International Research Journal of Biological Sciences*, 2(2):13-20.

Vigneshwaran, C; Shanmugam, S., & Kumar,T. S. (2010). Screening and characterization of keratinase from *Bacilluslicheniformis*isolated from Namakkal poultry farm. *Researcher*, 2(4): 89-96.