ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 9, pp. 13302-13306, September, 2016

International Journal of Recent Scientific Research

Research Article

OPTIMIZATION AND BIODEGRADATION OF FEATHER WASTE

Uma maheswari, N* and Balasundari, A

Department of Microbiology, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Thiruvarur District, Tamil Nadu, India

ARTICLE INFO

Article History:

Received 17th June, 2016
Received in revised form 12th July, 2016
Accepted 04th August, 2016
Published online 28th September, 2016

Key Words:

Bacillus, Turkey Feather, Keratin, Protease, Lipase.

ABSTRACT

Feathers are by-product waste of poultry processing plant and produced in large amount. Waste feathers from poultry were not a major concern for the environment or for the plants and poultry farms. But with the increasing production of poultry which might be accompanied with an increase in feather waste. In this study, feather-degrading bacteria and the production of keratinolytic and proteolytic enzymes by these organisms was investigated. Isolation of bacteria from poultry waste dump site and identified by the standard method, such as morphological and biochemical characteristics and they were tested for the ability to degrade feather. Six strains of *Bacillus* were identified from the sample; *Bacillus cereus, Bacillus subtilis, Bacillus coagulans, Bacillus megaterium, Bacillus licheniformis, Bacillus amyloliquefaciens.*Optimization was also done, includes pH, temperature, carbon, nitrogen sources. Compared to the other species, *Bacillus cereus* was showed maximum degradation of keratin at the pH 6 and temperature 40^0C, Protease (2.016 IU/ml) , ammonium phosphate as the best nitrogen sources. lipase (0.20 IU/ml), production Lipase production by *Bacillus cereus* were pH 8 and temperature 80^0C,yeast extract as nitrigen sources and sucrose as the main carbon sources for the protease, lipase optimization. Biodegradation of feather by *B .cereus* can be used for high level of productivity compared than other species. The keratinous waste can be biologically degraded by enzymes or the microbes itself to form useful products. These isolates are therefore promising organisms for biological means of waste management through biotechnological processes.

INTRODUCTION

Feather are produced as a waste of poultry processing plants in large quantities, millions of tons per year worldwide. Poultry feather constitutes the most abundant keratinous material in nature. The main component of the feather is keratin, a mechanically durable and chemically unreactive protein, which renders it difficult to digest by the most proteolytic enzyme. Recycling of feathers is a subject of interest because it is a potentially cheap and alternative protein supplement to be used in animal feed (Bertsch and Coello, 2005). Development of simple enzymatic treatment method will improve the nutritional value of keratin waste for use in animal feed (Gushterova and Haertle 2005). Thus, the bioconversion of poultry waste from a potent pollutant to a value-added product using microbial technology offers considerable opportunities (Suntornsuk and Suntornsuk 2003).

Keratin is highly stable insoluble protein present in hair, feather, nails, wool and horns (Onifade, 1998). A total 5-7% weight of mature chicken comprises of feathers which are composed of β-keratin. It is an insoluble protein of feathers and wool and is identified for their stability (Bradbury, 1973). Keratin contains several cross linking disulfide bonds, responsible for the stability of keratin and its resistance to enzymatic degradation. Feather is the waste obtained from chicken processing industry. Due to high protein content, keratin is used as a good source of protein and amino acids by general recycling. Feather meal is used as animal feed obtained by subjecting to high temperature and pressure which destroys certain amino acids and hence has low nutritional value.

Bacillus strains are ubiquitous microorganisms, which can grow on natural media without any special requirements. These properties can be exploited in the degrading feathers, which are produced in huge amounts throughout the world. Moreover, *Bacillus* strains are thermophile microorganisms and this property can be used in a controlled process for the efficient and fast degeneration of feathers. Other bacterial strains known by their keratinolytic activity includes *B.pumilis* (Burtt and Ichida, 1999a), *Streptomyces pactum* (Bokle *et al*., 1995) and *Streptomyces fradiae* (Kunert, 1989; Sinha *et al*., 1991), *Bacillus* species (Kao Ming - Muh and Hsing - Yao, 1995). The present study was aimed to foccus on isolation, identification of *Bacillus* from poultry dumped site, protease, lipase and

**Corresponding author: Uma maheswari, N*
Department of Microbiology, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Thiruvarur District, Tamil Nadu, India

keratinolytic degradation activity of the species, and optimization were also studied.

MATERIALS AND METHODS

Collection of soil sample

The soil sample,Turkey feather waste are collected from the poultry site in the place of Muthupet, Thiruvarur District, Tamil Nadu, India.The soil samples were collected at depths of about 3.0 to 4.0cm at the poultry site. It was taken in a sterile polythene bag and transferred to the laboratory and analyzed for the isolation of feather-degrading bacteria.

Isolation and identification of bacteria

Six- fold serial dilution of the samples was carried out and inoculated using the pour plate method; one millilitre (1ml) of 10^6 dilutions was inoculated on sterile Petri dishes, after which the sterilized media was poured aseptically on the inoculated plates. The plates were incubated at 37^0C for 24 hours. After incubation, morphologically different colonies observed on the plates were subcultured on nutrient agar plates to obtain a pure culture of the organisms and subsequently transferred into nutrient agar slants. The slants were kept in the refrigerator at 4^0C as a stock culture.

Pure cultures of the isolates were identified based on their cultural, morphological and physiological characteristics in accordance with the taxonomic schemes, preliminary tests are performed include Gram stain, spore stain, motility test, catalase test, oxidase, coagulase, urease, indole production, hydrogen sulphide production, nitrate reduction, methyl red, Voges- Proskauer, oxidative/fermentative test and utilization of carbon sources.

Screening test

Six strains were used to be screened for the degradation of feather wastes. Growth was evaluated by the OD method (optical density). The strains were grown an a minimal medium containing 0.1g/L Mg So_4 , 2g/L KH_2 PO_4 , and 2g/L glucose.8 g of clean feather cut in slices of 2 cm was distributed into 250 ml Erlenmeyer flasks containing 100 ml of distilled water. The flasks were sterilized at 121˚C for 25 min and inoculated. After 16 days of incubation, keratinase, lipase, protease activity was determined.

Keratin degradation (Khanam et al, 2004)

Collection of sample

Feather waste is collected in clean plastic bags, transferred immediately to the laboratory and stored in a refrigerator until examined. Turkey feather was washed with tap water and detergent. After making them fat-free, the solvent was evaporated, washed three to four times with distilled water and air-dried in a ventilated oven at 40^0C for 72 hrs. Prepared the feather meal, feathers were milled in a ball mill and passed through a small - mesh grid to remove coarse particles.

Procedure

Isolated and identified *Bacillus* species in soils such as *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus amyloliquefaciens* were tested so keratin degradation capability using feather and keratin substrates. Substrates were cleaned, washed with dextran, air dried, cut into small fragments, further washed five times distilled water and air dried .These shorts fragments were further dried at 80˚C till constant weight. To support initial growth of bacteria feather meal agar was used.

Composition of feather meal agar plate

Feather meal powder preparation

Poultry feathers were washed extensively, boiled at 30 - 40 psi for 2 -3 hrs. Dried in hot air oven for 4 hrs at 50^0C. The dried feathers were pulverized and the powder was used as feather meal.

Initial growth of bacteria was supported by a feather meal agar plate was employed, which contained (g/l) NH_4 cl-0.5, Nacl - 0.5, K_2 HPO_4 -0.3, KH_2PO_4 -0.4, Cl_26H_2 - 0.1, yeast extract - 0.1 and feather meal powder - 10, agar powder and the pH was adjusted to 7. 100 ml Erlenmeyer flask 75 ml of feather meal agar was taken, 1 g keratin substrate (Turkey feather) was added and sterilized at 1.1 kg pressure $(cm^{-2})^{-1}$ for 15 min. After cooling each flask was incubated with 6 days old bacterial colonies and incubated at 28 ± 2^0C for 20 days. A set of control flask without inoculums was also kept.

Feather-degrading activity (Dalev, 1994)

Residual in the culture broth of lipase and protease production media was harvested by filtration through whatmann no.1 filter paper washed twice with buffer and dried at 65^0C to constant weight. The feather degradation percentage was calculated from the difference in residual feather dry weight between control and treated sample.

Determination of keratinase activity

For 2 ml of azokeratin (1% w/v), 0.5 ml of diluted enzyme was added, incubated for 30 min at 45˚C. The enzymatic reaction was stopped by adding 2.5 ml of 10% TCA (Trichloro acetic acid) and the allowed to settle for 30 min and then filtered. To 1 ml of the filtrate add 5 ml of 0.5 ml sodium bicarbonate solution and 0.5 ml of diluted folin phenol cioculate reagent and incubated for 30 min. This was inoculated with 1ml of inoculum containing approximately 3.0× 10^6 CFU/ml. The absorbance was measured at 660 nm using spectrophotometer by using blank.

Optimization

Physical optimization was done by using pH levels (6,7,8), temperature (40^0C,50^0C,80^0C) and chemical optimization was done by using Carbon sources (Starch, Maltose, Sucrose),Nitrogen sources (Ammonium chloride, Ammonium phosphate, Sodium nitrate, Urea, Yeast extract, Peptone).

Assay of protease

Protease activity was determined using the method prescribed improved Anson assay (Lanoe and Dunnigan, 2004).The reaction mixture consists of 1ml of 1.5% casein solution and 1ml of the enzyme sample. The reaction mixture was incubated for 10 minutes at 37^0C prior to the addition of 2ml of 0.4M TCA. The free amino acids released from casein hydrolysis were estimated by Lowry *et al.* (1951) method. One unit of protease activity was defined as 1µmol^{-1} of tyrosine released per minute by 1ml of the enzyme.

Assay of lipase

The assay of lipase was carried out using two different media.

Medium A (g/l) (Nityananda *et al*., 2004) Starch 20, peptone 20 ; NH_4Cl 3.8 ; Mg SO_4 1 ; K_2HPO_4 5 ; Olive oil 1% at pH 8.

Medium B (g/l) (Kanwar *et al* ., 2004) Glucose 10, Yeast extract 5; Tap water 1 liter ;Olive oil 1% at pH 7.

Estimation of total protein was done by the standard method of Lowry *et al*., 1951.

RESULTS AND DISCUSSION

Isolation and identification of bacteria

Keratin degrading bacteria were isolated from the collected soil sample and allowed to serial dilution. The colonies were observed on nutrient agar plates after incubation of 24 hrs. Uninoculated plate with medium was maintained as a control. Isolates are identified as *Bacillus cereus, Bacillus subtilis, Bacillus coagulans, Bacillus megaterium, Bacillus licheniformis, Bacillus amyloliquefaciens.*

Six of the isolates were found to be Gram positive spore forming and motile rod-shaped bacteria. Based on their cultural, morphological and biochemical characteristics, the bacterial isolates recovered were identified as Bacillus species (Table - 1).

Table 3 Assay of protease, lipase production

Organisms	Protease productivity (IU/ml)	Lipase productivity (IU/ml)
B.cereus	2.4 ± 0.42	2.1 ± 0.03
B.subtilis	1.9 ± 0.17	1.7 ± 0.10
B.coagulans	1.4 ±0.11	1.4 ± 0.02
B.megaterium	1.4 ± 0.10	1.3 ± 0.03
B.licheniformis	1.2 ± 0.61	1.3 ±0.01
B.amyloliquefaciens	0.7 ± 0.17	1.1 ± 0.13

Optimization Study

Optimization parameters such as pH, temperature, carbon, nitrogen sources were performed only for *Bacillus cereus* due to the high degradation efficiency.

Table 4 Optimization of pH and Temperature for protease, lipase production

Name of the organism	pH	Protease	Lipase
B.cereus	6	2.30	2.56
	7	1.80	1.00
	8	1.54	2.08
Name of the organism	Temperature	Protease	Lipase
B.cereus	40^0C	90.5	20
	50^0C	38.8	40
	80^0C	58.7	44

Table 1 Bio chemical characterization of isolated organisms

Name of the Test	*B.cereus*	*B.subtilis*	*B.coagulans*	*B.megaterium*	*B.licheniformis*	*B.amyloliquefaciens*
Gram staining	+	+	+	+	+	+
Motility	Motile	Motile	Motile	Motile	Motile	Motile
Methyl Red	+	-	+	-	+	-
Voges-Proskauer	+	+	+	+	+	+
Oxidase	-	-	-	-	+	+
Catalase	+	+	+	+	+	+
Indole	-	-	-	-	-	-
Urease	-	-	-	-	-	-
Citrate	+	+	-	+	-	-
Nitrate utilization	_	-	-	+	-	+

Keratin degradation

Maximum keratin degradation was noted in *B. cereus* 63.5%, followed by *B.subtilis* 61.0%, *B. coagulans* 57.5%, *B.megaterium* 50.5%, *B. licheniformis* 45.5%, *B.amyloliquefaciens* 17.0% .(Table - 2).

Table 2 Keratin degradation

Organisms	Keratin degradation (%)
B.cereus	63.5
B.subtilis	61.0
B.coagulans	57.5
B.megaterium	50.5
B.licheniformis	45.5
B.amyloliquefaciens	17

Assay of protease

The protease productivity was estimated in all the isolated six bacterial strains such as *B. cereus, B. subtilis, B.coagulans, B. megaterium*, *B. licheniformis*, *B. Amyloliquefaciens.*The maximum assay was noticed in *B. cereus* (2.4 ± 0.42 IU/ml), followed by *B. subtilis* (1.9 ± 0.17 IU/ml), *B. coagulans* (1.4 ± 0.11 IU/ml), *B. megaterium* (1.4 ± 0.10 IU/ml), *B. licheniformis* (1.2 ± 0.61 IU/ml), *B. amyloliquefaciens* (0.7± 0.17 IU/ml). (Table -3).

The pH value (8.0) was showed maximum activity for protease production of *B . cereus*. (Table - 4).

Temperature

The temperatures optimum level 60^0 C was suitable for proteases production of *Bacillus cereus* using turkey feather as substrate. (Table - 4)

Carbon and Nitrogen sources of protease

The protease activity was optimized using different carbon supplementation medium such as starch, maltose, and sucrose. The sucrose content was present in a high amount of protease (70.6 IU/ml) compared than starch and maltose (56.5&51.6 IU/ml).(Table - 5)

The protease productivity was optimized using different nitrogen supplementation medium such as Ammonium chloride, Ammonium phosphate, and Sodium nitrate. The Ammonium phosphate content was present in a high amount of protease (73.3 IU/ml) Lowest production was determined as Ammonium chloride (70.3 IU/ml) and Sodium nitrate (60.3 IU/m).The results were presented in the table - 5.

Table -5 Corbon and Nitrogen sources of protease optimization

Carbon sources	Protease (IU/ml)
Starch	56.60 ± 0.14
Maltose	51.0 ± 0.14
Sucrose	70.6 ± 0.07
Nitrogen sources	Protease (IU/ml)
Ammonium chloride	70.3 ± 0.07
Ammonium phosphate	73.3 ± 0.07
Sodium nitrate	60.3 ± 0.07

Assay of lipase

The six strain of bacteria produced lipase in one or other media. The lipase productivity were estimated in six bacterial strains such as *B. cereus* (2.1±0.03 IU/ml) *B.subtilis,* (1.7±0.10 IU/ml) *B. coagulans,*(1.4±0.02 IU/ml) *B. megaterium,* (1.3±0.03 IU/ml) *B.licheniformis,*(1.3±0.01 IU/ml) *B. amyloliquefaciens* (1.1±0.13 IU/ml).(Table - 3).

Effect of P^H on the activity of lipase

The P^H optimum (6.0) was suitable for lipase production of *Bacillus cereus* using turkey feather as substrate. (Table - 4).

Effect of temperature on the activity of lipase

The temperature optimum (80^0C) was suitable for lipase production of *Bacillus cereus* using turkey feather as substrate. (Table - 4).

Carbon and Nitrogen sources of lipase

The lipase activity was optimized using different carbon supplementation medium such as starch, maltose, and sucrose. Maximum production was determined in the level of sucrose (61.3 IU/ml).Lowest production determined as starch (48.6 IU/ml) and maltose (42.3 IU/ml). The estimated results were presented.(Table - 6).

The lipase productivity was optimized using different nitrogen supplementation medium such as Urea, Yeast extract, peptone. The yeast extract content was present in a high amount of lipase (68.6 IU/ml).Lowest production determined as urea (57.3IU/ml) and peptone (49.3 IU/ml). The results were presented. (Table - 6).

Table 6 Carbon and Nitrogen sources of lipase

Carbon sources	Lipase (IU/ml)
Starch	48.6 ± 0.14
Maltose	42.3 ± 0.07
Sucrose	61.3 ± 0.07
Nitrogen sources	Lipase (IU/ml)
Urea	57.3 ± 0.07
Yeast extract	68.6 ± 0.14
Peptone	49.3 ± 0.07

Estimation of protein

Feather waste was first characterized for their chemical composition. Major compounds including protein were determined. The protein content was high amount noted in 1200mg/ml of serum albumin (2.888) with an average value of 81% respectively. The chemical composition of turkey feather wastes indicates a very balanced medium, which may not need other nutrients for culturing the degrading microorganism. The protein levels of the crude and purified enzyme were estimated by the standard method (Table-7).

Table 7 Protein estimation by Lowry's method

Concentration of bovine serum albumin (mg/ml)	Optical density (660)
200	0.405
400	1.22
600	1.917
800	2.018
1000	2.28
1200	2.888

Overall findings were highlighted that, *B. cereus* was the best one for feather degradation. In optimization study for the assay of the protease was better in the pH 6 and temperature 40^0C respectively. In the sucrose content (70.6 ± 0.07 IU/ml) was given the high amount of protease. Nitrogen source of ammonium phosphate (73.3 ± 0.07 IU/ml) was present in a high amount of protease. The level of protein (2.888) was estimated.

Lipase enzyme has optimum pH 8 and temperature 80^0C respectively. In the carbon sources of sucrose content was present in a high amount of lipase (61.3 ± 0.07).Yeast extract was showed (68.6 ± 0.14 IU/ml) high level of lipase production as nitrogen sources.

Our study reports correlated to that Kannan *et al.*, degradation of a chicken feather by *B.subtilis* at 42.1^0C on 20^{th} day at decreased up to 40^{th} day.

Our study reports revealed that Lakshmi *et al.,* for efficient degradation of a feather by keratinase producing *Bacillus* species at pH 8.5, temperature 45-55^0C and keratinase yield 518-520 KU/ml.

CONCLUSION

In conclusion, the protease and keratinase activities of the *Bacillus* species have revealed that they have the potential biotechnological use in processes involving keratinase hydrolysis. Feather studies on the kinetic properties of the keratinases and proteases will provide necessary information for optimal activities of the enzymes, particularly for *Bacillus* species.

Animal feed typically includes a carbohydrate source and a protein source. Common protein sources used in animal feed include soy meal; fish meal; blood meal; meat or poultry by - products and meat and poultry meal. These protein sources are generally expensive feather waste too is high in protein and very inexpensive, but cannot be used directly in animal feed, as it is difficult for animals to digest. Typical treatments to form feather meal are expensive. These treatments also tend to destroy some amino acids, which are heat - sensitive amino acids. It would also solve the waste disposal problem of poultry waste and with limited resources recycling of keratinacious waste would be beneficial, financially and environmentally.

Acknowledgements

The authors are thanks to the Dr.V.Dhivaharan, Dean, Department of Life Science, Sengamala Thayaar Educational Trust Women's College Mannargudi, for his encouragement for extending the facilities to carried out the research work.

References

Bertsch, A. and Coello N, A. 2005. Biotechnological process for treatment and recycling poultry feathers as a feed ingredient, *Bioresour Techno*l, 96, 1703-1708.

Bockle, B. Galunsky. B, Muller, R. 1995. Characterization of a keratinolytic serine proteinase from *streptomyces pactum* DSM 40530. *Appl. Environ. Microbiol*.61, 3705-3710.

Burtt EH, lchida JM 1999. U.S. PATENT No 5,877,000. Keratinase produced by *Bacillus licheniformis.*

Bradbury, J.H. 1973. The structure and chemistry of keratin fibers. *Advances in proteinchemistry*, 27, 111-211.

Burtt. EH, lchida JM 1999. Occurrence of feather-degrading bacilli in the plumage of birds. *App. Biotech*. 116(2), 364-372

Cheng-gang cai.Bing-gan lou.Xiao-dong zheng.2008. Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis.J. of Zhejianguniversity science* .9(11), 60-67.

Dalev,P. 1994. Utilisation of wate feather from poultry Slaughter for production of a protein concentrate. *Bioresour. Technol.*, 48 (3), 265- 267

Gushterova A, Braikova, D, Goshev, l, Christov, P, Tishinov, K, Vasileva-Tonkova, E, Haertle, T, Nedkov, P, 2005. Degradation of keratin and collagen containing wasted by newly isolated thermoactinomycetes by alkaline hytrolysis, Letters*, Appl. Microbiol*, 40,335-340.

Ichida, J.M.Krizova, L.Lofevre, C.A.Keener, H.Elwell, D.L.Burtt, E.B.2001. Bacterial inoculums enhances keratine degradation and biofilm formation in poultry compost. *J. Microbial methods* 47, 99-208.

Kunert J 1989 Biochemical mechanism of keratin degradation by the *Actinomycetes streptomyces* fradiae and the fungus *Microsporum gypseum*-a comparaison. J .*Basic Microbiol*. 29:597-604.

Khanam, S.J.B. Agrawal, S.C.Jain, P.C. 2004. Keratin degrading and enzyme production ability of fungi from soil.*Indian J. of microbiology*. 44(4):261-264.

Kanwar., S.S.Srivastava, M.Cnimni, S.S. Ghazi,I. A. and Joshi, G.K. 2004. Action of iodine on stained keratin. *Acta. Microbial immunol hungarica*.51:57-53.

Lin X, Lee CG, casale ES, Shih JCH.1992. Purification and characterization of a keratinate from a feather – degrading *Bacillus licheniformis* strain. *Appliedand Environmental microbiology*. 58 (10), 3271-3275.

Lowry, OH, Rosebrough NJ, Farr AL, Randall RJ .1951. Protein measurement with the folin phenol reagent. *J.Biol chem.,* 193, 265-275

Ming-Muh K, Hsing-Yao L .1995. The Study of the selection of feather degrading microorganisms. *J. Chin. Inst. Environ. Eng*.5, 37-43.

Nityananda Roy. Lalitagauri Ray. and parimal chattopadhyas. 2004. Production of lipase in a fermenter using a mutant strain of *corynebacterium* species: Its partial purification and immobilization. Indian J. *Exp. Biol.*42: 202-207.

Onifade, A.A., A1- Sane *et al*., 1998. *Biosource Technology* 66, 1-11.

Suntornsuk W and Suntornsuk L, 2003. Feather degradation by *Bacillus sp*. FK 46 in submerged cultivation, *Bioresour Technol*, 86 239-243.

Sinha U, Wolz SA, Lad PJ 1991. Tow new extracellular serine proteases from *streptomyces fradiae*. *Lnt J. Biochem*. 23:979-984.

****

How to cite this article:

Uma maheswari, N and Balasundari, A.2016, Optimization and Biodegradation of Feather Waste. *Int J Recent Sci Res.* 7(9), pp. 13302-13306.