InnoVationInfo Scholar Journal of Applied Sciences and Research

Sch J Appl Sci Res 2018
Volume 1: 6

Isolation, Characterization and Partial Purification of Keratinase from Keratinolytic Bacteria

Prakash Bhuyar[1*]
Sourabh Zagade[2]
Rajesh Revankar[2]
Mashitah M Yusoff[1]
Mohd Hasbi Ab Rahim[1]
Natanamurugaraj Govindan[1]

[1]*Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Malaysia*
[2]*Faculty of Life Science, Garden city College, Bangalore, India*

Abstract

Background: Enzymes are proteins that catalyze the chemical reactions, such as biosynthetic and derivative, that occurring in living cells. In enzymatic reactions, the molecules at the beginning of process are called substrates and they are converted in to different molecules, called the product. Almost all processes in a biological cell need enzyme for catalytic reactions. The use of purified enzymes for generating a useful product or service constitutes enzyme technology. Enzymes have a wide variety of applications in industry, medicine research etc. Some out of this one industrially important enzyme is keratinase. It is an extracellular and proteolytic enzyme.

Methods: Keratinolytic bacteria were isolated from poultry contaminated soil identification done by using morphological methods and biochemical test. Enzyme production was done followed by partial purification methods like salting out technique, dialysis and Ion exchange chromatography.

Results: The bacterial strains were isolated from the poultry waste and evaluated for their Keratinolytic activity on skim milk agar media. The keratin degrading bacteria or keratinolytic bacterial culture was isolated from soil sample and Colony characteristics, Gram nature and motility of the keratinolytic culture were studied. It is Gram negative short rods, non-motile in nature and colony characteristics and morphology was studied. The biochemical testes of keratinolytic culture were also studied and their results were found. The enzyme production was observed between temperature 25-50°C and pH 5.5-9.5 The highest enzyme activity was observed at 35°C, decrease in enzyme activity is observed with increase in temperature above 40 ^{0}C. The highest enzyme activity was obtained at pH 7. However, further increase in pH was not favorable on enzyme production. It was reported that proteases secreted by Genus *Pseudomonas* presented activity at a wide range of pH (7.0 to 9.0) and temperature (30°C to 40°C).

Keywords: Azokeratin, Keratin, Keratinolytic bacteria.

Introduction

Background

Keratin is a family of fibrous structural protein of hair, nails, hoofs, wool, and feather and of the epithelial cells in the outer most layer of the skin. Keratin is the polypeptide chain of the amino acid. The length of keratin fibers depends on their water containing complex hydration increases their length by 10-12%. Two types of keratin can

Article Information

Article Type: Research
Article Number: SJASR-166
Received Date: 06 March, 2018
Accepted Date: 11 September, 2018
Published Date: 17 September, 2018

***Corresponding author:** Dr. Prakash Bhuyar, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Malaysia. Tel: +60168369703, Email: prasadmbhuyar(at)gmail.com; pkt17002(at)ump.edu.my

Citation: Bhuyar P, Zagade S, Revankar R, Yusoff MM, Ab-Rahim MH, et al. (2018) Isolation, Characterization and Partial Purification of Keratinase from Keratinolytic Bacteria. Sch J Appl Sci Res. Vol: 1, Issu: 6 (40-45).

be distinguished on the basis of physical character, histology and chemical composition. Soft keratin is outer layer of epidermis, itself is the type example of soft keratin. Such material is most conveniently isolated from the thickened skin of cattle noses and lips. Hard keratin- Horn, nails, claw and hoof are typical example of hard keratin. Hard keratin is characteristically higher is sulfur, having up to 5% preponderantly in the form of combined cystine. Keratinase is an extracellular enzyme. It is proteolytic enzyme in nature. It was classified a Proteinase of unknown mechanism as recommended by the nomenclature, the committee on the international union of biochemistry with EC number 3.4.99 [1]. recently some worker defined keratinase as serine protease due to its 97% sequence homology with alkaline protease and it is also inhibited by same inhibitor that inhibit serine protease.

Keratinase has brought revolution to many commercial industries like detergents, medicines, leather industries etc. Keratinase is preferred than any other common protease considering its broad spectrum of substrate specificity. Following are the application of keratinase. Detergent application of keratinase has been also suggested by Gupta and Ramnami [2]. They can degrade a fibrous protein that means keratinase is most efficient enzyme to be implemented in detergents as they can remove strains of oil splits, fruit squash and other strong dirt strains. Keratinase in leather industries is used for softening and tenderization of leather. Keratinase is also used to degrade low quality and damaged wool. Large keratin waste from slaughterhouses, leather and a fur plant contaminates the soil and air. Traditional method to degrade keratin containing wastes are energy inefficient; But degradation by keratinase is eco-friendly and energy efficient method. Prions proteins are causative agents for transmissible neurodegenerative disease including Scarapi, Bovine spongiform encephalopathy (mad cow disease). Keratinase can degrade these proteins [3].

Keratinase isolate from certain bacterial strains also used for formulating drugs on vein blockage. In pharmaceutical industry keratinase is used for making of ointments, medicines etc.

Keratinase are produced only in the keratin containing substrate, it mainly attacks on the disulfide (-s-s-) bond of the keratin substrate [4]. The keratinase production by various microorganisms were reported by many researchers. It was found that keratinase was produced by fungi, Streptomyces & bacteria were produced in alkaline pH & almost thermophilic temperature. These enzymes have wide range of substrate specificity such as it can degrade the fibrous protein like fibrin, elastin, collagen & other non-fibrous protein like casein, BSA, gelatin etc. Keratin is an insoluble macromolecule requiring the secretions of extracellular enzymes for biodegradation to occur. Keratin comprises long polypeptide chains which are resistance to the activity of non-substrate specific proteases. Adjacent chains are linked by disulphide bonds which are responsible for the stability and resistance to degradation of keratin. The degradation of keratinous material is important medically and agriculturally [5,6]. Secretion of keratinolytic enzyme is associated with dermatophytic fungi, for which keratin is the major substrate [6]. The keratinase productions by various organisms were reported by a number of workers. It was found that keratinase produce by fungi, *Streptomyces* and bacteria were produced in nearly at alkaline pH and almost thermophilic temperatures. These enzymes have a wide range of substrate specificity for fibrous protein fibrin, elastin, collagen and other non fibrous protein like casein, bovine serum albumin, gelatin etc.

The solubilization of feather keratin was studied. The samples of chicken body feather were treated with a solution containing mercaptoethanol and a protein denaturant such as urea, or with a solution containing mercaptoethanol and an anionic surface active agent such as sodium do decyl benzene sulphonate (DBS) or sodium lauryl sulphate [7]. Current research was centered on the potential use of keratinase of bacterial origin for the industrial treatment of keratin-containing compounds. Keratin, as well other insoluble proteins, was generally not recognized as a substrate for common proteases [8]. Its hydrolysis was, however, affected by specific proteases (Keratinases) which have been found in some species of *Bacillus* [9]. Feather degrading bacteria were isolated from poultry waste. Among those isolates, three strains identified as *Bacillus subtilis, Bacillus pumilus & Bacillus Cereus*- degraded feather effectively & produced 142, 96 & 109 units of keratinolytic activities respectively. The optimal conditions for the enzyme production by *Bacillus subtilis* were 40 ºC and pH 5.0-9.0, for *Bacillus pumilis* 40ºC and pH 5.0-6.0 & for *Bacillus cereus*_30°C. The maximum keratinolytic activities of *Bacillus subtilis, Bacillus pumilis* & *Bacillus cereus*_161, 149 & 117 units/ml after 84, 72 & 60 hrs of cultivation. The production of soluble protein showed the same tendency as that of keratinolytic protease [10]. Keratin hydrolysates were prepared from goat and sheep hairs. The hairs were hydrolyzed under controlled alkaline conditions and the materials were neutralized to pH 7.0 to 8.0 and then filtered. Then the filtrate was concentrated to solid contents, which was further dried and made into powdered form [11]. Keratinase from Streptomyces spp. was extracted and found to degrade keratin from human epidermis. They purified the enzyme by DEAE-cellulose column. *Pseudomonas aeruginosa* strain isolated from poultry waste was tested for its abilities to hydrolyze the feather. The effect of different production parameters such as pH, temperature, carbon source, nitrogen source, incubation time, inoculums size was studied by isolated stain [12]. Determine the keratinolytic ability of a range of bacteria and subsequently, to characterize the keratinase showing the greatest biotechnological potential [8]. The keratinolytic ability of the purified Proteinase was examined by incubation with the insoluble substrate, keratin azure, feather meal and native and chicken feather downs [4]. The main objectives of Isolation, characterization and partial purification of extra cellular enzyme keratinase are Isolation of keratinase producing bacteria from poultry waste, identification of bacteria, up to genus level. isolation of keratinase enzyme, determination of crude, partially purified enzyme activity and characterization of enzyme.

Methods

Isolation of keratinase producing bacteria by primary screening – caseinolytic activity

Soil sample containing keratinolytic bacteria was collected from poultry area. About 1 gm of poultry waste was suspended in 9 ml of saline solution (0.9 % NaCl). A volume of 0.1 ml of suspension is serially diluted and then spread on the surface of milk agar plate incubation of this plate is carried out at 37°C for 24 hrs. Isolation of keratinolytic bacteria from soil The bacterial strains were isolated from the poultry waste and evaluated for their Keratinolytic activity on skim milk agar media. Study of culture, morphology and biochemical characteristics of 'Keratinase' isolates Gram nature and Gram nature of 'Keratinase' was studied by Hucker and Cohn method. Biochemical are performed and motility was observed by Hanging drop method.

Biochemical tests for identification

Hydrogen Sulphide production an inoculum from a pure culture is transferred aseptically to a sterile triple sugar iron agar slant. The inoculated tube is incubated as 35-37°C for 24 hrs. and the results are determined. Present in TSIA is an iron compound. The iron ions (Fe^{2+}) have a high affinity for sulfide ions. The result shows that H_2S combines with the iron to make FeS, as blank compound. In tubes of TSIA containing bacteria producing hydrogen sulfide, the agar turns black from the FeS. Indole Test: To detect the ability of an organism to breakdown tryptophan to indole. Method: The culture was inoculated in sterile peptone water and incubated for 24 hours. After incubation the breakdown of tryptophan was analyzed by adding Kovac's reagent. Methyl Red Test: To test the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Method: To MR - VP broth a loopful of culture was inoculated containing MR indicator and incubated for 24 hrs. Voges Prosakeur- To detect the production of acetylmethylcarbinol, a natural product formed from pyruvic acid in the course of glucose fermentation. Method: To MR - VP broth a loopful of culture was inoculated containing Barritt's reagent and incubated for 24 hrs. Citrate utilization test- To determine if an organism is capable of utilizing citrate as sole carbon source for metabolism with resulting alkalinity. Method: Streak a Simmons's Citrate agar plate with the organism and incubate at 37°C for 48 hrs. Gelatin liquifaction Test: For this gelatin agar media was produced and bacterial suspension of isolated strain was spot inoculated in the centre of medium plate and incubated at 37°C for 24 hrs. for the growth development. After incubation, $HgCl_2$ is added and results in clear zone which indicated that this test is positive.

Enzyme Production

Inoculum preparation the selected bacterial colony after its identification and characterization inoculated into the feather basal medium. An incubated on shaker incubator at 35°C for 7 days. Production of enzyme- After 7 days of incubation, 10 ml of culture medium was transferred to 500 ml medium. All incubations were done at 35°C with continuous shaking.

Filtration: The culture medium was filtered through Whatmann No.1 filter paper to remove undegraded residues.

Centrifugation: After incubation the broth was collected and centrifuged at 10,000 rpm at 4ºC for 10 mins. Supernatant containing the extra cellular secretions and pellet was discarded.

Synthesis of substrate (Azo-Keratin)

Azo-keratin was prepared by a similar method similar to a known procedure for azo-albumin. The use of azo-albumin as a substrate in the colorimetric determination of peptic and tryptic activity. Ball-milled feather powder was prepared [5]. 1 g portion of the feather powder (the keratin source) was placed in a 100 ml round- bottomed reaction flask with 20 ml of deionized water. The suspension was mixed with a magnetic stirrer. Two ml of 10% NaHCO (w/v) were mixed into the feather suspension. In a separate 10 ml tube, 174 mg of Sulfanilic acid were dissolved in 5 ml of 0.2N NaOH. Sixty-nine mg of $NaNO_3$ were then added to the tube and dissolved. The solution was acidified with 0.4 ml of 5N HCl, mixed for 2 min and neutralized by adding in 0.4 ml of 5N NaOH This solution was added to the feather keratin suspension and mixed for 10 min. The reaction mixture was filtered and the insoluble azo-keratin was rinsed thoroughly with deionized water. The azo-keratin was suspended in water and shaken at 50°C for 2 hrs and filtered again. This wash cycle was repeated until the pH of the filtrate reached 6.0-7.0 and the spectrophotometric absorbance of the washing at 450 nm was less than 0.01 [13]. Finally, the wash cycles were repeated at least twice using 50 mM Potassium phosphate buffer, pH 7.5. The azo-keratin was washed once again with water and dried in vacuum overnight at 50°C. The resulting product is a chromogenic substrate that can be incubated with enzyme solution to produce and release soluble peptide derivatives that cause an increase in light absorbance of the solution [13].

Partial purification

Ammonium sulphate precipitation: 70 ml of supernatant was used for the precipitation with 80% ammonium sulphate. All subsequent steps are carried out at 4°C. The resulting precipitate was obtained by centrifugation at 10000 rpm for 10 min. The precipitate was dissolved in 50 mM phosphate buffer.

Dialysis principle: Dialysis is a separation technique that facilitates the removal of small, unwanted compounds from macromolecules in solution by selective and passive diffusion through a semi-permeable membrane. A sample and buffer solution is placed on opposite sides of the membrane. Sample molecules that are then the membrane-pores are retained on the sample of the membrane, but small molecules and buffer salt pass freely through the membrane, reducing the concentration of those molecules in the sample. Changing the dialysis buffer removes the small molecules that are no longer in the sample and allows more contaminants to diffuse into the dialysate. In this way, the concentration of small contaminants within the sample can be decreased to acceptable or negligible levels.

Determination of total protein concentration by lowery method

Phenolic groups like tyrosine and tryptophan react with Folin's reagent and produce blue color complex with maximum absorption at 660 nm. The protein concentration can be detected by comparing with BSA as a substrate. Different solution of BSA (1mg/ml) ranging from 0.1-7 mg/ml were taken alkaline copper sulphate was added in it and incubated for 30 min. The protein concentration of enzyme was determined by plotting graph of OD vs concentration in µg.

Enzyme optimization

Purified enzyme was used for enzyme Optimization. Optimization of enzyme was done to determine the effect of pH and temperature on activity of enzyme. Effect of Temperature The effect of temperature on keratinase activity was determined by the addition of 20 µl keratinase (3 mg/ml) to 1.5 ml phosphate buffer (100 mmol 1, pH 7.5) containing 15 mg azo keratin, and incubating at a range of temperatures (25, 30, 35, 40, 45 and 50°C) for 10 h. Peptide release was determined spectrophotometrically (280 nm; 1unit of activity (U) the amount of enzyme causing an increase of 1.0A 280 units within 1 minute). Effect of pH The effect of temperature on keratinase activity was determined by the addition of 20 µl keratinase (3mg/ml) to 1.5 ml phosphate buffer (100 mmol) with different pH (5.5, 6.0, 6.5, 7.0, 705, 8.0, 8.5, and 9.0) containing 15 mg azo keratin, and incubating at 35°C for 10 hrs. Peptide release was determined spectrophotometrically (280nm; 1unit of activity (U) the amount of enzyme causing an increase of 1.0A 280 unit within 1 minute).

Chromatography

Ion exchange chromatography: Procedure-10 ml column using the glass column was prepared, the pump and adaptor attached and allowed a 1-2 ml/min flow rate. Regenerated with a 25 ml wash High Salt Buffer (50 mM buffer at pH 7.5 plus 0.1M NaCl). Column equilibrated with the 50-100 ml of Equilibration Buffer (50 mM buffer at pH 7.5) Checked to the sample has more than 50 mM NaCl or KCl. If it does, it must be dialyzed overnight before using. If starting from lysates, no further preparation of the sample is necessary 36 hrs. Save a sample of the lysates for later analysis. Freeze in a microfuge tube. Sample is loaded and 1-2 ml/min flow rate is maintained. Washed with 3 column volumes of Equilibration Buffer. Elute protein using either an isocratic (40 ml) or gradient elution (100 ml total). 8 ml fractions throughout this step was collected. Follow up with a High Salt buffer wash to remove any tightly bound proteins and regenerate your resin for the next use. 8 ml fractions throughout this step were collected. Each tube of or the protein for total protein concentration was analyzed.

The protein concentration was determined by following formula:

Protein concentration=OD × Sample dilution/ Extention coefficient × Pathlength

OD=Optical density at 280 nm

Extention Coefficient =extinction coefficient of 1, so 1 OD = 1 mg/ml protein.

Pathlength =When using a 1 cm cuvette, the pathlength is 1

Sample dilution =Dilution of sample done by 1 ml

Calculated Extinction Coefficients for proteins measured in a 1 cm cuvette [14-16].

Molecule	Calculated Extinction Coefficient (mg/ml) cm^{-1}
BSA	0.66

Results and Discussion

The bacterial strains were isolated from the poultry waste and evaluated for their Keratinolytic activity on skim milk agar media. The keratin degrading bacteria or keratinolytic bacterial culture was isolated from soil sample and Colony characteristics, Gram nature and motility of the keratinolytic culture were studied. It is Gram negative short rods, non-motile in nature and colony characteristics and morphology was shown (Figure 1-8). The biochemical testes of keratinolytic culture were also studied and the results were shown (Figure 1-8). The enzyme production was observed between temperature 25-50°C and pH 5.5-9.5 The highest enzyme activity was observed at 35°C, decrease in enzyme activity is observed with increase in temperature above 40°C. The highest enzyme activity was obtained at pH 7. However, further increase in pH was not favorable on enzyme production. It was reported that proteases secreted by Genus *Pseudomonas* presented activity at a wide range of pH (7.0 to 9.0) and temperature (30°C to 40°C).

Morphological characterization and Biochemical tests of keratinolytic bacteria

As shown in Figure 1-7 the colony characteristics of well isolated colony, isolated on nutrient agar containing keratinolytic bacteria incubated at room temperature for 24 hrs. Carbohydrate utilization tests were carried out. Biochemical tests were also carried out. According to Burgeys Mannual, testes were carried out for identification of bacteria up to genus level. It was found that isolated strain will may be belongs to Genus *Pseudomonas.*

As per standard curve of BSA protein unknown sample that is dialyzed sample concentration was obtained it was about 36.48 mg/ml.

Totally around 60 fractions were taken from Ion Exchange Chromatography. The fraction number 35 to 55 Showed maximum value of OD. Therefore, they were pooled and stored for further study.

Th graph shows the enzyme activity of selected fractions from Ion Exchange chromatography and the enzyme activity of the selected fraction as per formulae (Figure 4-8).

This experiment was performed at pH optima of enzyme determined earlier. According to observation table, the maximum activity of enzyme was observed at 35°C. Therefore, 35°C is the optimum temperature for keratinase.

As we purify the enzyme, the enzyme activity goes on increasing from crude enzyme sample to ion-exchange

chromatography sample.

A unit of keratinase activity was defined as a 0.01-unit increase in the absorbance at 280 nm as compared to the control after 15 min reaction [17].

Conclusion

Keratinolytic bacteria isolated from poultry waste and identified as *Pseudomonas species.* They showed the production of alkaline keratinase enzyme. The cultivation condition was investigation to optimize alkaline keratinase production.

The goal of this study was to enhance the production which has achieved to optimization of cultivation condition like pH, temperature etc.

The maximum enzyme activity of isolated enzyme was shown at pH 7.0 and temperature 35°C. After partial purification up to ion-exchange chromatography the activity is increased.

Declarations

List of abbreviations

$C_6H_{12}O_9$: Citric Acid Dihydrate, $CaCl_2.2H_2O$: Calcium Chloride Dihydrate, HCl: Hydrochloric Acid, K_2HPO_4: Dipotassium phosphate, LM: Light Microscope, $MgSO_4.7H_2O$: Magnesium Sulfate Heptahydrate, Na_2MG EDTA: EDTA Magnesium Disodium, $NaNO_3$: Sodium Nitrate, NaOH: Sodium Hydroxide, $(NH_4)_5[Fe(C_6H_4O_7)_2]$: Ferric Ammonium Citrate, OD: Optical Density, PAST: Paleontological Statistics Software Package, sp.: Species, TLC: Thin Layer Chromatography, UV/VIS: Ultraviolet/Visible.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

Not applicable

Funding

This is bachelor degree self-funded project by corresponding author.

Author's contributions

Authors are highly contributed towards throughout completing of research project.

Appendix

A. Composition of nutrient agar medium –

Peptone – 1 gm

Beef extract – 0.3 gm

NaCl – 0.5 gm

Agar-agar – 2.5 gm

D/W – 100 ml

pH – 7.2

B. Composition of milk agar medium –

Peptone – 1.0 gm

Meat extract – 0.3 gm

NaCl – 0.5 gm

Agar – 2.5 gm

D/W – 95 ml

pH – 7.2

Milk – 5 ml

C. Composition of peptone water broth –

Peptone – 1 gm

NaCl – 0.5 gm

D/W – 100 ml

pH – 7.5

1% Bromothymol blue – 1.2 ml

D. Reagent preparation –

a) TCA – 110 mM

Commercial 0.9 gm TCA dissolved in 500 ml D/W.

b) Folin's reagent – 0.5 N

2.5 ml 2.0 N Folin's reagent dissolved in 10 ml D/W

c) Std. BSA solution – 1gm/1ml

100 mg BSA Dissolved in 100 ml D/W

References

1. Owen GC, Roberts GW, Ferrier IN, Lee Y, Crow TJ, et al. (1983) Peptides, the limbic lobe and schizophrenia. Brain Res 288: 199-211.
2. Gupta R and Ramnami P (2006) Microbial keratinases and their prospective applications: Applied Microbiology and Biotechnology 70: 21-33.
3. Caughey B (2001) Interactions between prion protein isoforms: The kiss of death? TIBS 26: 235-242.
4. Bockle B, Galunsky B, Muller R (1995) Characterization of a Keratinolytic Serine Proteinase from Streptomyces pactum DSM 40530. Applied and Environmental Microbiology 3705-3710.
5. Shin RW and Strohm LA (1993) Policy Regimes for the International Waste Trade. Review of Policy Research 12: 226-243.
6. Matsumata T (1996) In: Principle and practiceof clinical mycology. Wiley & Sons Ltd, USA pp: 103–129.
7. Kikkawa M, Chonan Y, Toyoda H (1974) Solubilization of keratin 6: Solubilization of feather keratin by oxidation with performic acid. Hikaku Kagaku 20:151-162.
8. Allpress JD, Mountain G, Gowland PC (2002) Production, purification and characterization of an extracellular keratinase from Lysobacter NCIMB 9497. Letter in Appl Microbiol 34: 337-342.
9. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535.
10. Kim JM, Lim WJ, Suh HJ (2001) Feather-degrading Bacillus species from poultry waste. Process Biochem. 37: 287–291.
11. Sastry PA, Pearlstone JR, Smillie LB, Paranchych W (1985) Studies on the primary structure and antigenic determinants of pilin isolated from Pseudomonas aeruginosa K. Can J Biochem Cell Biol 63: 284–291.

12. Raju EVN and Divakar G (2013) Production of keratinase by using pseudomonas aeruginosa isolated from poultry waste. Int J Pharm Chem Biol Sci 3: 79-86.

13. Burtt EH and Ichida JM (1999) Occurrence of feather-degrading bacilli in the plumage of birds. The Auk 116: 364-372.

14. Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY.

15. Fasman G (1992) Practical handbook of biochemistry and molecular biology. CRC press, Boston.

16. Gill SC and von Hippel PH (1989) Calculation of protein extinction coefficients from amino acidsequence data. Anal Biochem 182: 319-326.

17. Tom Sinoy ES, Bhausaheb CP, Rajendra PP (2011) Isolation and identification of feather degradable Microorganisms. Letter in Bioinformatics and Biotechnology 2: 128-136.

Citation: Bhuyar P, Zagade S, Revankar R, Yusoff MM, Rahim M H Ab, et al. (2018) Isolation, Characterization and Partial Purification of Keratinase from Keratinolytic Bacteria. Sch J Appl Sci Res. Vol: 1, Issu: 6 (40-45).