Ameliorating Effect of Plant Growth Promoting Bacterium *Staphylococcus sciuri* PSD 11 Isolated from Feather Waste Dumping Site

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Abstract

Feather degrading bacterium was isolated from poultry waste dumping site. Total thirteen bacterial isolates were stipulated on the basis of their growth pattern on nutrient agar and screened for proteolytic activity. Among these, eight isolates exhibited casein hydrolysis and hence, exposed to feather degradation for keratinolytic ability. Studied strain having significant keratinolytic potential was explored for biochemical characterization as well as for molecular sequencing using 16S rDNA. Sequenced bacterium having maximal keratinolytic activity was characterized as *Staphylococcus sciuri*. *S.sciuri* exhibited 99.05 U/ml of enzyme activity on optimized media and it also susceptible to commonly used antibiotics (eg: Azithromycin). This bacterium was effective to customize feather waste into nutrient loaded hydrolysate and exploits as plant growth enhancer for tomato and black mustard plant. This potent microbe exhibited keratinolytic efficacy towards biotechnological and agricultural applications. Thus, isolated keratinolytic bacterium could be a potential agent for total degradation of feather waste and showed eco-friendly association with environment as it helps to reduce environmental pollution.

Keywords: - *Staphylococcus sciuri*, feather waste, proteolytic activity, keratinolytic efficacy, 16S rDNA, plant growth enhancer.

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1. Introduction

Animal wastes can be developed as potential nutrient source for crop development. From last few decades, research has been carried out comprehensively to upsurge the agronomic importance of animal wastes, including poultry wastes. The improved production facilities and massive expansion of poultry industry generates huge amount of waste which constituent's plentiful source of protein [1]. Keratin is an insoluble, fibrous polypeptide and is vital structural constituent of the epidermis and its extremities such as hair, nails, feathers, wool and horns. The secondary structure classified into α-keratin or β- keratin. On the basis of its sulphur content, is divided into hard (feather, hair, hoof and nail) and soft (skin and callus) keratin [2]. The recalcitrance nature of keratin causes its effective hydrolysis using keratinase enzyme produced by bacteria and fungi which are isolated from leather and poultry wastes [3]. Keratinolytic microbes and their enzymes may have significant applications in poultry and leather processing industries with generation of non-polluting products. Some species of *Bacillus*, Actinomycetes and Fungi are active producers of these enzymes [4]. The various commercial industries like detergents and medicines has vast applications of keratinase due to its wider range of substrate specificity and therefore it was more beneficial than any other common protease. In detergent industry, keratinase can deteriorate fibrous proteins and thus it is highly productive enzyme employed for the removal of strains of oil splits, fruit squash and other tough dirt strains. In leather industries, keratinase utilized for softening and tenderization of leather. It is also exploited to break down the cheap quality and defective wool. Excess amount of keratin waste from slaughter houses, leather and fur industry which impacts negatively on soil and air quality [5]. The chicken feather waste was employed for the development of biofertilizers by research community. Feather meal is a low and simply obtainable source of nitrogen (15% N) and may acts as prospective biofertilizer. The plant growth stimulating ability of protein hydrolysate could be effective strategy in agricultural processes. Therefore, microbial break down of feather acts as substituent for development of slow-release nitrogen fertilizers. The degenerated by-product of chicken feathers causes release of sufficient quantity of tryptophan which is essential amino acid and play key imp role in IAA synthesis which is main precursor for root development of plat. The composting method is an effective method of creating a balanced organic matter that can be utilized as a source of nutrient and soil conditioner [6].

In current study, the keratinolytic bacteria were isolated and characterized from poultry waste dumping site. The isolated keratin producing bacterium has ability to decompose or degrade feather waste. Feather degraded hydrolysate was utilized as plant growth promoter and can be used to increase soil fertility.

2. Materials and method

2.1. Sample collection

The soil samples were collected from poultry waste dumping site near poultry farm at Ichalkaranji region, Kolhapur, Maharashtra, India. The samples were collected in sterile autoclavable plastic bags and stored at R.T. for further studies (Figure no. 1).

Figure 1:(a) Poultry site; (b) Soil sample

2.2. Bacterial isolation

Approx. 1 gm of soil sample were added into minimal media which composed with (gm/l): KH_2PO_4, 3; Na_2HPO_4, 6; NaCl, 5; NH_4Cl, 2; $MgSO_4$, 0.1; glucose, 8 and kept for enrichment at 30ºC for 48 hr. The enrichment was repeated for 3 times. On the last day, enriched sample was subjected to primary screening for isolation of micro-organisms.

2.2.1. Primary screening

The primary screening of enriched soil sample was carried out on nutrient agar. Serially diluted enriched sample were spread on media and incubated at 30ºC for 24 hr. Then, distinct isolates were selected and spread on casein agar media having composition 125 ml/l skimmed milk, 875 ml/l nutrient broth, 15 gm/l agar and pH was adjusted at 7.2 then after, plates were incubated at 30ºC for 24 hr and observed for zone of hydrolysis. Protease positive isolates were selected and used for secondary screening.

2.2.2. Secondary screening

To check potency of keratinolytic activity, feather meal broth was used, which composed with (gm/l): NaCl, 0.5; $MgCl_2.6H_2O$, 0.24; K_2HPO_4, 0.3; NH_4Cl, 0.5; KH_2PO_4, 0.4; yeast extract,0.1; feather waste,10; pH 7.5 [7]. 1 ml suspension of selected strains were inoculated into media and kept for 30ºC under constant stirring in shaking incubator. The cultures were monitored for feather degradation which continues for 7 days. Degradation efficiency of micro-organism was calculated by following formula;

$$\frac{\text{Weight of degraded feather}}{\text{Weight of feather}} \times 100$$

2.2.3. Enzyme activity assay

Keratinase assay was performed according to the method of Bhange et. al.[8]. 20 mg of minced feathers were suspended in 3.8 ml of 100 mM Tris–HCl buffer (pH 8.0); further 200 µl of culture supernatant was added to it. The prepared sample was incubated at 32°C and 150 rpm for 1 hr and activity was ceased by cooling the tubes in ice cold water. Further, sample was centrifuged at 5000 rpm for 5 min; absorbance of resultant supernatant was evaluated at 280 nm and to check the difference enzyme control was used. Unit of enzyme activity was calculated as the amount of enzyme required to liberate 1 µmol/min of tyrosine under same standard conditions mentioned above.

2.2.4. Effect for carbon and nitrogen sources on enzyme activity

To examine effect of various carbon and nitrogen sources on enzyme activity methodology of Pustake et.al.[9] was slightly modified. Carbon and nitrogen sources were screened to check their efficiency on the

keratinase activity of potent isolate. 1% w/v of several carbon sources (dextrose, fructose, lactose, CM cellulose, starch) as well as inorganic nitrogen sources (ammonium chloride, ammonium sulphate, urea, glycine, sodium nitrate) and organic nitrogen sources (soya peptone, yeast extract, beef extract, peptone) were used to study their influence on keratinase activity.

2.3. Biochemical characterization of potent strain

The strain having potent keratinolytic activity was selected and explored for biochemical characterization such as Gram staining, IMVIC test (Indole, Methyl Red, Voges Proskauer and Citrate), starch hydrolysis, gelatin liquefaction, urease activity, effect of NaCl concentration, pH and temperature.

2.3.1. Gram staining

Gram staining was performed as per method of Abiola and Oyetayo [10] with slight modifications. A thin smear of 24 hr old bacterial culture was prepared on clean grease free slide and fixed it by passing through gentle flame. Initially, smear was stained by crystal violet for 60 second and rinsed with water. Then, smear was flooded with Gram's iodine for 60 second and again rinsed with water. It was decolorized by using ethanol for 30 second and rinsed with water. At the last, safranin was used as counter stain for 60 second and again rinsed with water. The smear was allowed to air dry and observed under oil immersion lens of microscope using cidar wood oil. Gram positive bacteria appeared as violet color while Gram negative showed pink color.

2.3.2. IMVIC analysis

IMVIC test includes indole (I), methyl red (MR), vogues proskauer (VP) and citrate utilization (C). The tests were performed according to method of Hussain et.al. [11] with minor changes. In indole test bacteria were incubated in tryptophan broth at 30ºC for 24 hr after that Kovac's reagent (0.5 ml) were added to it and observed for pink red ring formation. For MR test, micro-organism were incubated in MRVP broth at 30ºC for 24 hr and after incubation 5 drops of methyl red were added to it and observed for formation of red colored ring. VP test was performed by using MRVP broth and bacteria were incubated in it at 30ºC for 24-48 hr. After incubation, 0.6 ml of alpha-naphthol solution (VP-A reagent) and 0.2 ml of potassium hydroxide (VP-B reagent) were added to it and observed for pink red color. For citrate utilization, micro-organism were streaked on citrate agar and observed for color change of media from intense blue to deep green within 24-72 hr at 30ºC.

2.3.3. Catalase

Catalase activity of isolate was performed according to method of Syahri et.al. [12] using 3% hydrogen peroxide (H_2O_2).Bacterial suspension was taken on glass slide and 100 µl of 3% H_2O_2 were added onto it. Slide was observed for formation of bubbles which indicates positive catalase activity of bacteria.

2.3.4. Urease

Urease test was used to determine the ability of micro-organism to split urea through the production of urease enzyme. For this purpose, (gm/l): urea, 20; NaCl, 5; KH_2PO_4, 2; peptone 1; dextrose, 1; phenol red, 0.012; agar 15 gm; and pH was adjusted to 6.7. Bacterial culture were spread on plate and incubated at 35-37ºC for 48 hr and observed for color change. The formation of pink color indicates positive urease activity [13].

2.3.5. Starch hydrolysis

Starch hydrolysis test of micro-organisms was performed to check their capability to degrade substrates with carbon composites and able to grow on it. It was performed as per method of Abiola and Oyetayo [10] with slight modifications. 2 gm of starch was solubilized in nutrient agar and plates were prepared. Bacterial suspension was spread on it and incubated at 30ºC for 24 hr. After growth, plates were flooded with Gram's

iodine. Clear zone around colony indicates hydrolysis of starch due to alpha amylase activity of bacteria while unhydrolyzed starch forms blue color in presence of iodine.

2.3.6. Gelatin liquefaction

Gelatin hydrolysis was performed on nutrient gelatin media. For this, 23 gm/l nutrient agar and 8 gm/lit gelatin were used. Nutrient gelatin plates were inoculated with bacteria and incubated at 30ºC for 24 hr. After adequate microbial growth, plates were flooded with saturated ammonium sulphate to precipitate unhydrolyzed gelatin and observed for clear zone around bacterial colony within 5-10 min [13].

2.3.7. Phenylalanine deaminase

This test was performed to determine ability of bacteria to oxidatively deaminate phenylalanine to phenylpyruvic acid. The media containing yeast extract 3 gm; NaCl 5 gm; Phenylalanine 2 gm; Na_2HPO_4 1 gm; agar 15 gm and D/W 1000 ml were used. The plates were spread with bacterial suspension and incubate at 30ºC for 24 hr. After incubation, plates were flooded with 10% ferric chloride and observed for light to deep green color [14].

2.3.8. Effect of NaCl concentration, pH and temperature

The influence of NaCl concentration, pH and temperature on growth of micro-organism was studied by using method of Hussain et.al. [11] with slight modification. For this purpose, salt concentration from 1% to 10%, pH ranging from 2 to 10 and temperature varying from 10 to 50ºC were studied.

2.3.9. 16 S rDNA sequencing

Phylogenetic relationship of micro-organism helps to evaluate relationship of isolated strain with already known micro-organisms. The 16S rDNA gene sequencing was carried out and it was compared with other sequences deposited in NCBI GenBank database by using BLAST. Depending upon maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7.

2.4. Estimation of liberated end compounds

Feather waste degradation by micro-organism causes liberation of some useful an end products like proteins as well as amino acid. Estimation of these products was carried out till complete degradation was achieved. Protein estimation was carried out by Lowery method using BSA as standard [15]. Amino acid content was calculated by Ninhydrin method using Leucine as standard [16].

2.5. Antibiotic susceptibility test of micro-organism

After 16 S rDNA sequencing, identified isolate was tested for antibiotic susceptibility on nutrient agar according to method of Begum et. al. [17] with some modification. Disc diffusion method was performed and commercially available seven antibiotic discs of penicillin (P, 10 mcg), chloramphenicol (C, 10 mcg), streptomycin (S, 10 mcg), gentamycin (GEN, 10 mcg), azithromycin (AZM, 15 mcg), amoxicillin (AMC, 30 mcg) and clarithromycin (CLR, 15 mcg) was employed for this investigation. Above pre-treated antibiotic plates were incubated at 37ºC for 24 hr. Sensitivity of micro-organism against each antibiotic was determined by measuring zone of inhibition (mm) on next day.

2.6. Plant growth promotion by feather hydrolysate

After degradation of feather waste by micro-organisms, the remaining hydrolysate was tested for their ability of plant growth stimulation. For this study tomato (*Solanum lycopersicum*) and black mustard seeds (*Brassica nigra*) were used.

2.6.1. Plantation and dose application

To evaluate plant growth promotion, feather waste hydrolysate was used. The study was carried out during month of January to April using pot method. The both seeds were surface sterilized by 70% ethanol and further washed properly with distilled water and sowed at the middle of (16^{th} Jan) January. 5ml of feather hydrolysate was provided to both seedlings plants; after every 5 days and was continued for one month until germination was attained. The water was supplied to both the plants test and control, in which control was devoted of hydrolysate.

2.6.2. Evaluation of morphological parameters of plants

Both control and hydrolysate treated plant were monitored for above stipulated time and at the end of April, plants were fully developed into mature plant and attained maximum growth. After maximum growth both plants were analysed for its biochemical and morphological investigation of parameters including shoot and root length, root hairs, number of leaves, flowers, chlorophyll, protein, phenolic and flavonoid content was analysed.

2.6.3. Biochemical analysis of plants

2.6.3.1. Chlorophyll estimation

Chlorophyll content was estimated as per protocol of Arnon [18] with few modifications. Thoroughly washed 1g fresh leaves were crushed in (80 % v/v) acetone to prepare slurry and attain the volume 20ml. The extract was refrigerated overnight followed by centrifugation and absorbance of supernatant was measured spectrophotometrically at 645 nm and 663 nm (Shimadzu UV-1800, Japan).

Chlorophyll a (μg/L) = $(12.7 \times A663) - (2.69 \times A645)$

Chlorophyll b (μg/L) = $(22.9 \times A645) - (4.68 \times A663)$

Total Chlorophyll (μg/L) = $(20.2 \times A645) + (8.02 \times A663)$

2.6.3.2. Estimation of free proteins and amino acids

The procedure of Gurav and Jadhav [19] was followed to determine the free proteins and amino acids. For sample preparation, 0.5g of vegetative part of it homogenised in 80% ethanol and 100 mM phosphate buffer (pH 7.0) respectively. Extracts were centrifuged and estimated for protein and amino acids by using Lowry [20] and Ninhydrin method respectively [21].

2.6.3.3. Determination of total phenolics and flavonoid

The total phenolics content was calculated by folin-ciocalteu's reagent [22]. Reaction mixture were prepared and incubated for 90 min in dark. After incubation, the absorbance was measured at 765 nm and total phenolics were calculated by using gallic acid calibration curve. The total flavonoid was determined by measuring absorbance at 415 nm (Shimadzu UV-1800, Japan) by using standard quercetin curve [23].

2.6.4. Chemical analysis of soil

Chemical analysis of sample soil was carried out to study the effect of feather hydrolysate on plant growth. The Kjeldahl's method and TOC analyser was used to determine total nitrogen and carbon content respectively. Similarly, samples were prepared to check its P, K, Ca, Mg and Zn content by the method of Hseu [24]. Diluted samples were analysed for the presence of P, K (flame photometry), Zn, Mn, Ca and Mg (atomic absorption spectrophotometer).

2.7. Statistical analysis

All the experimental sets were performed in triplicates (n=3). The statistical analysis was carried out

on the basis of mean and standard derivation (SD).

3. Result and discussion

3.1. Isolation of potent bacterial strain from soil

In present work, it was observed that the enriched minimal media containing micro-organisms exhibiting keratinolytic potential. Total 13 micro-organisms were potent and are selected on the basis of their growth on nutrient agar and named as PSD 1 to PSD 13.

3.1.1. Proteolytic activity

Isolates selected from serially diluted sample were listed in table no.1 and checked for their proteolytic activity on casein agar. Out of which, eight isolates showed significant and prominent hydrolytic zone (Figure no.2). Isolate PSD 11 showed remarkable casinolytic activity and screened for its keratinolytic activity as well.

Figure 2: Plates showing zone of casein hydrolysis by eight isolates during screening

Table 1: Strains isolated using synthetic casein medium

No.	Strains	Growth on nutrient agar	Caseinase activity	Zone of hydrolysis (mm)	Keratinolytic activity
01	PSD 1	+	+	11	+
02	PSD 2	+	+	12	+
03	PSD 3	+	+	8	-
04	PSD 4	+	-	-	-
05	PSD 5	+	+	7	-
06	PSD 6	+	-	-	-
07	PSD 7	+	-	-	-
08	PSD 8	+	+	5	-
09	PSD 9	+	+	2	-

10	PSD 10	+	+	10	+
11	PSD 11	+	+	15	+
12	PSD 12	+	-	-	-
13	PSD 13	+	-	-	-

3.2. Keratinolytic activity

Isolates with potent keratinolytic activity was recognized by cross examining their ability to hydrolyse feathers. This study was carried out by using feather meal broth, a minimal media in which feathers were utilized as whole carbon and nitrogen source. Entire feathers were accompanied in media to study feather degradation by visual identification (Figure no.3). The maximum degradation of feathers showed by PSD 11 than PSD 1, PSD 2 and PSD 10, this noticeable change was observed in 7 days while rest of three strains were delay in this biochemical process. The obtained results were closely related to Nagarajan et.al. [6] and Bhange et.al. [8].The results were further assisted by their considerable increase in turbidity of media which indicates that feather was utilized for microbial growth [25] and therefore, PSD 11 was explored for further characterization. Feather degrading efficiency of bacterium under suitable conditions was found to be 70% (weight of initial feather: - 0.5 gm/50 ml and weight of degraded feather :- 0.35 gm).

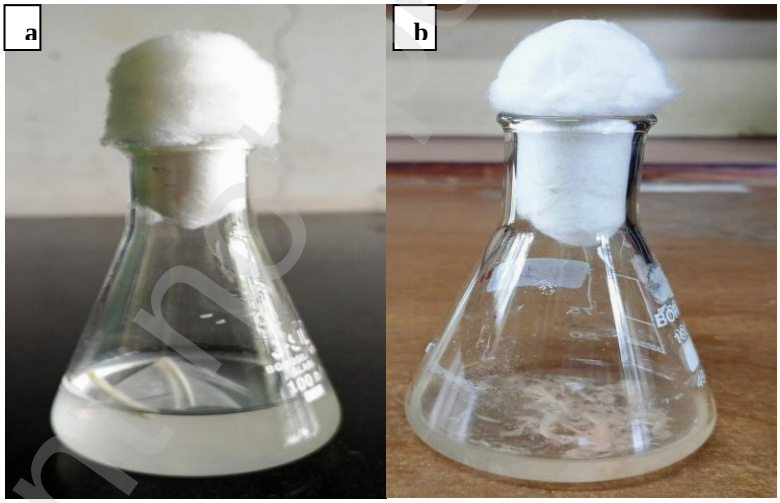

Figure 3: (a) Control without micro-organism; (b) Test sample with micro-organism

3.3. Enzyme activity

Keratinolytic ability of PSD 11 was continuously evaluated during microbial growth. It was observed that, the activity was enhanced day by day [26] and the change was significant. Activity was minimum on zero day (1.15 U/ml) while at the end of seventh day maximum activity was attained. The figure no.4 indicated the increase in enzyme activity within seven days and after that it was ceases.

In earlier reports, keratinolytic activity was observed after 7th days were found to be 74.66 U/ml, 50 U/ml and 119 U/ml [27,28,29]. In current study, it was observed that activity was increased up to 99.05 U/ml in same period which was more significant in comparison of earlier studies.

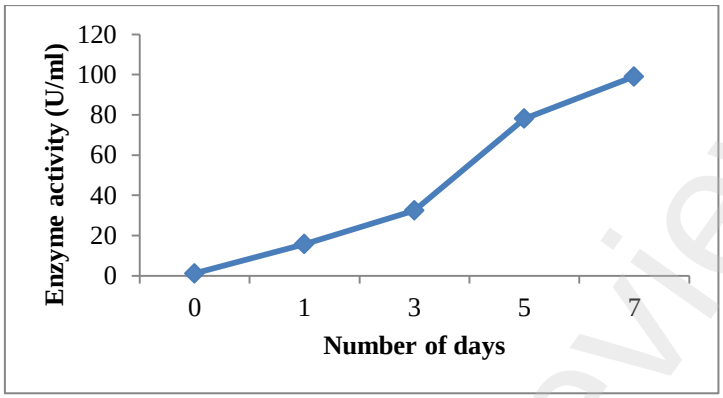

Figure 4: Enzyme activity with respect to number of days

3.4. Enzyme activity in presence of carbon and nitrogen sources

Carbon and nitrogen source plays important role in any media formulation. Simple and easily available sources are usually preferred. Enzyme activity may be increased or decreased in presence of these sources and it depends on nature of sources used. Enzyme activity were analysed with respect to various simple to complex carbon and nitrogen sources (organic as well as inorganic). Among these different carbon sources used, enzyme activity was maximal in presence of dextrose (74.01 ± 0.66) while least in starch (44.29 ± 0.51). In comparison with organic and inorganic nitrogen sources utilized in current study yeast extract (organic source) and ammonium chloride (inorganic source) exhibited 62.27±0.56 U/ml and 44.61±0.45 U/ml enzyme activity respectively. Thus, dextrose, ammonium chloride and yeast extract was employed as carbon and nitrogen source for efficient feather degradation and enzyme activity with respect to these components were illustrated in figure no.5 and 6.

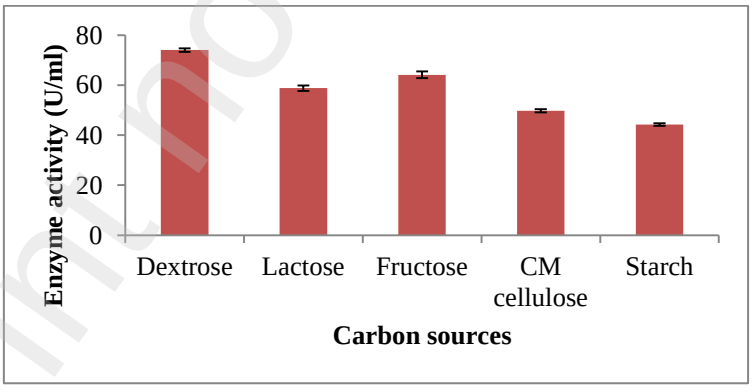

Figure 5: Effect of carbon sources on enzyme activity

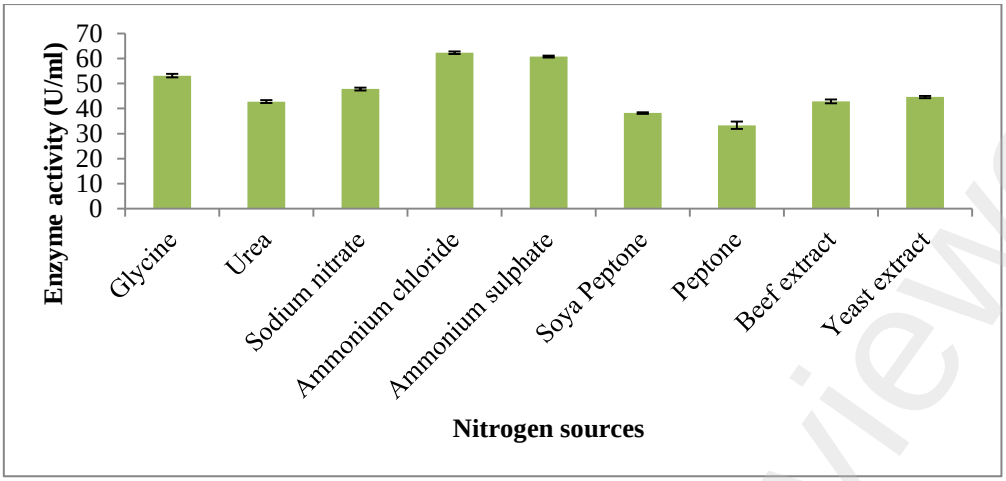

Figure 6: Effect of nitrogen sources on enzyme activity

3.5. Microscopic and biochemical assay

The strain PSD 11 was characterized by biochemical and microscopic studies. The colony characterization and biochemical assays were listed in table no.2 and 3 respectively. Figure no.7 showed growth of PSD 11 on nutrient agar which was further identified by 16 S rDNA sequencing.

Table 2: Colony characteristics

Size	Shape	Margin	Elevation	Color	Opacity	Consistency
3 mm	Circular	Regular	Convex	White	Opaque	Mucoid

Table 3:- Biochemical characterization of PSD 11 (+: positive; -: negative; W: weak)

No.	**Test**	**Result**
01	Gram nature	Gram positive
02	Morphology	Clustered Cocci
03	Motility	Motile
04	Indole	+
05	Methyl red	+
06	Voges-Proskuer	-
07	Citrate utilization	-
08	Catalase	+
09	Urease	+
10	Starch hydrolysis	+
11	Casein hydrolysis	+
12	Gelatin liquefaction	+
13	Phenylalanine deaminase	-
14	NaCl (%)	
	1.0	+
	2.0	+
	4.0	W
	6.0	W

	8.0	-
	10.0	-
15	Growth pH	
	2	-
	4	-
	6	-
	8	+
	10	W
16	Growth temperature (ºC)	
	10	+
	20	+
	30	+
	40	W
	50	-

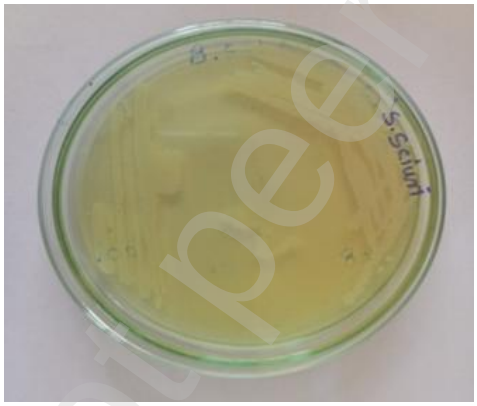

Figure 7: Growth of *S. sciuri* on nutrient agar

3.6. Identification by 16S ribosomal RNA sequencing

The 16S ribosomal RNA sequencing of PSD 11 was carried out. From sequencing, it was revealed that isolate PSD11 showed high similarity with *Staphylococcus sciuri* strain DSM 20345 based on nucleotide homology and phylogenetic studies. The analysis was carried out by using MEGA7 software and results were given in figure no.8. BLAST similarity alignment of micro-organism with respect to NCBI GenBank was given in figure no.9.The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [30].

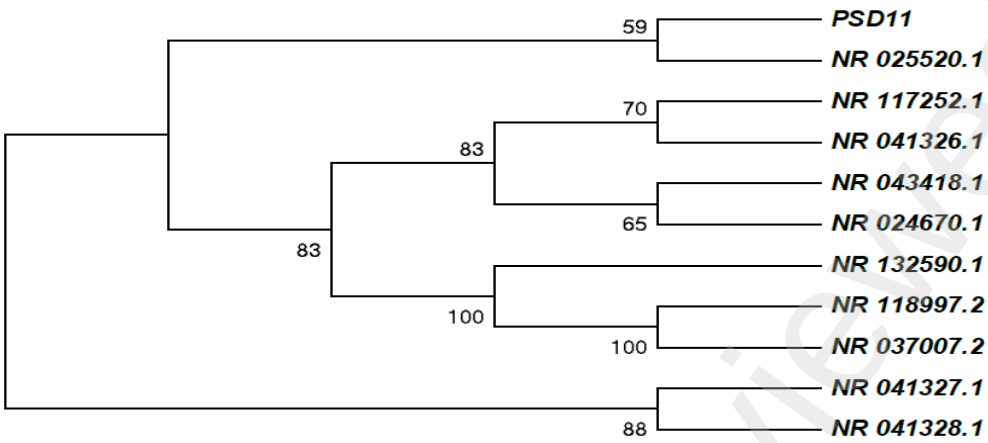

Figure 8: Molecular Phylogenetic Analysis of PSD 11(*S. sciuri*) by Maximum Likelihood Method

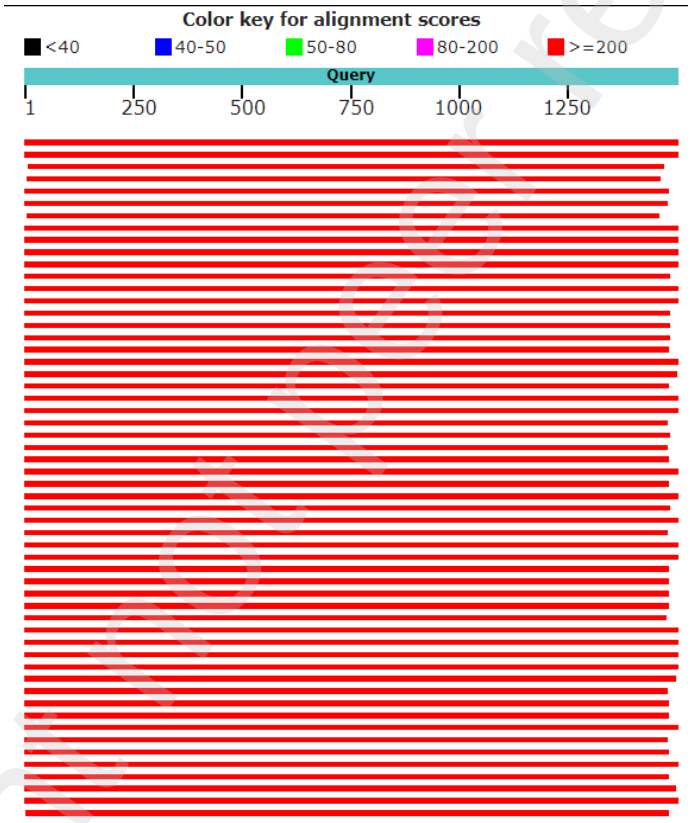

Figure 9: Alignment view of *S.sciuri* using combination of NCBI GenBank

3.7. Analysis of liberated end products

It was found that *S.sciuri* isolated from poultry waste dumping site has good capacity to degrade feather waste. It utilizes feather as whole carbon and nitrogen source therefore, results in generation of free proteins and amino acids. Figure no. 10 depicted, rise in amount of free amino acids and proteins during feather degradation by *S.sciuri* which indicated that feather waste was utilized by micro-organism for growth.

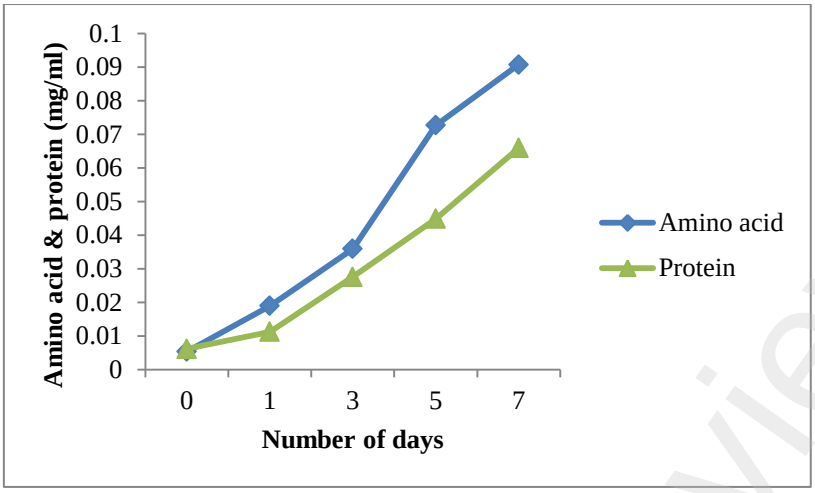

Figure 10: Analysis of degraded end products during feather degradation by *S.sciuri*

3.8. Antibiotic susceptibility analysis

Antibiotics are the vital secondary metabolites synthesized by bacteria during stationary phase of growth. More than 80% antibiotics were extracted from soil micro-organisms. *Staphylococcus sciuri* isolated from poultry waste dumping site were tested for antibiotics sensitivity against commercially available antibiotics. It was observed that, isolate was susceptible to penicillin, streptomycin, gentamycin, chloramphenicol, azithromycin, amoxicillin and clarithromycin. *S.sciuri* was more sensitive to amoxicillin and less sensitive to streptomycin with zone of inhibition about 31 mm and 19 mm respectively [17] while marginal changes was observed for other antibiotics. Antibiotics susceptibility of *S.sciuri* was depicted in figure no.11 and 12. Due to susceptibility against various antibiotics it can be concluded that, isolated micro-organism is non-pathogenic and can be explored for different applications in agricultural field and as a probiotics in poultry as well as aquaculture feed.

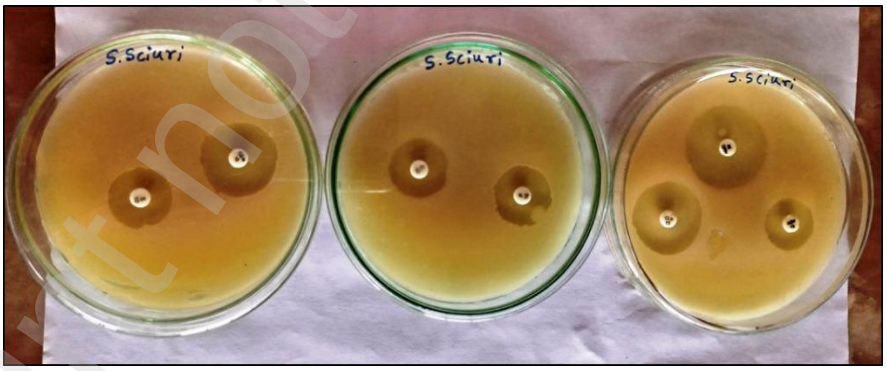

Figure 11: Zone of inhibition by *S.sciuri* against antibiotics

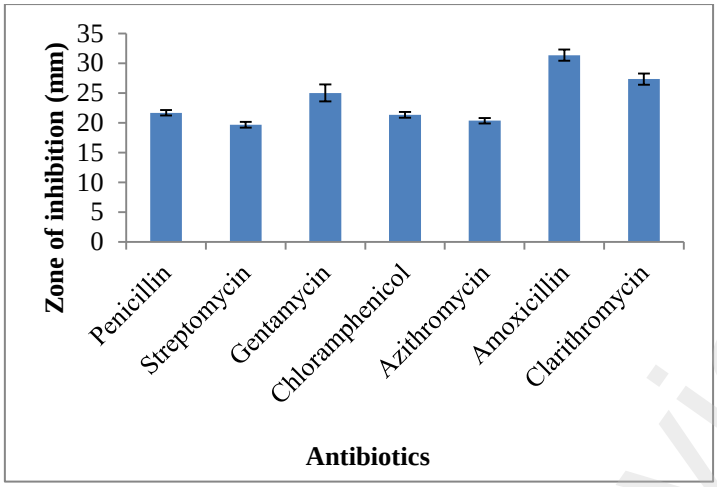

Figure 12: Antibiotics susceptibility of *S.sciuri*

3.9. Effect of degraded feather waste hydrolysate on plant growth

The following morphological and biochemical analysis of plants and soil supplemented with feather waste hydrolysate were carried out.

3.9.1. Plant growth promotion study

It was observed that, there is significant enhancement in phytochemicals in case of test while marginal change was observed in control plant. This confirms, feather waste hydrolysate was rich in nutritional factors that act as effective plant growth stimulator so it has positive effect on test plants. Besides this, time duration study revealed that, tomato plants nourished with feather hydrolysate showed germination after 5 days and healthy growth of all vegetative parts were observed after one week but in control plant delayed in germination as well as all phytochemical parameters. Similar observation was obtained in case of black mustard plant. In which, germination was observed within 3 days in test plant and healthy growth of all vegetative parts of plant was noted after 1 week as compared to control plant. There was no significant change was observed in other phytochemical parameters.

3.9.2. Effect of hydrolysate on morphological parameters of plants

The organic components of feather hydrolysate were utilized by tomato and black mustard plant. Figure no.13 and 14 depicted the results of feather hydrolysate. The hydrolysate treated plants exhibited better morphological characters than control one (table no. 4 and 5). In case of tomato plant, treated one showed shoot and root length of about 49.5±0.03 cm and 26±0.11 cm respectively. More number of leaves were observed in treated plant than control one and also exhibited increase in leaf length as well as width of about 7±0.03 cm and 3.6±0.05 cm. In case of black mustard plant, shoot and root length of test plant was higher than control one. It showed 54±0.079 cm and 22±0.011 cm shoot and root length respectively. Significant number of leaves and all morphological parameters was observed in test plant as compared to control. This significant change was observed due to microbial degraded feather hydrolysate. Similar results were observed after application of feather [31,8] and fish waste hydrolysate [32] in *V. radiata* and *C. arietinum* L.

Table 4: Morphological characters of control and treated tomato plant ((n=3) ± SD

Parameters	Shoot length cm	Root length cm	No.of leaves	Leaf length cm	Leaf width cm
Control	38±0.02	21±0.13	37±1.02	4.1±0.04	2.2±0.07
Test	49.5±0.03	26±0.11	52±1.05	7±0.03	3.6±0.05

Table 5: Morphological characters of control and treated black mustard plant ((n=3) ± SD)

Parameters	Shoot length cm	Root length cm	No.of leaves	Leaf length cm	Leaf width cm
Control	29±0.083	16±0.05	18±1.13	4.5±0.16	2.8±0.05
Test	54±0.079	22±0.011	26±1.17	7.9±0.19	4.7±0.07

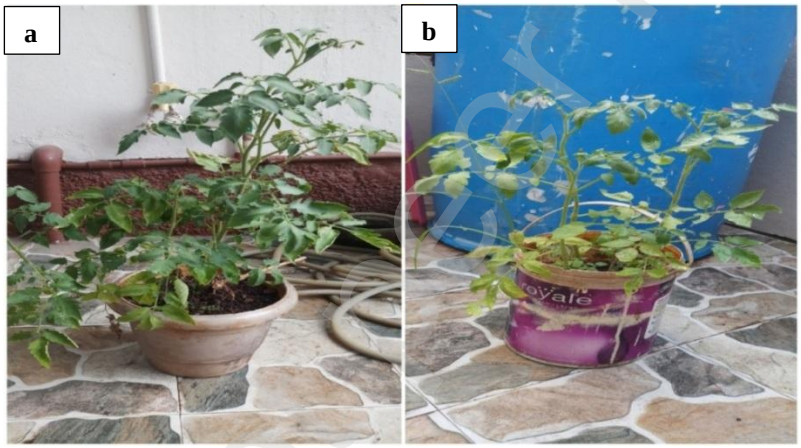

Figure 13: Tomato plant (a) Test; (b) Control

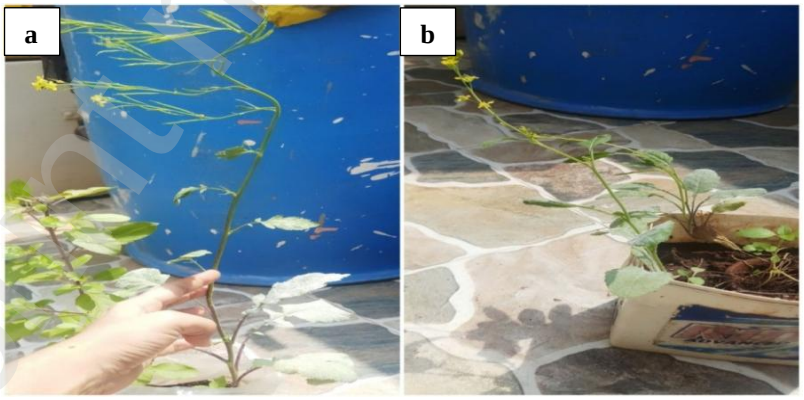

Figure 14: Black mustard plant (a) Test; (b) Control

3.9.3. Biochemical parameters of plants

The hydrolysate of feather degradation by microbes contains important nutrients which act as plant growth stimulant so it has potential applications in agriculture particularly horticulture field which has great commercial value. It is potent source of small peptides and amino acids that involved in protein synthesis, secondary metabolism and signalling mechanism of organism. Some amino acids in feather hydrolysate act as

precursor of plant growth hormones thus; it may helpful to develop tolerance in various stress conditions [33]. Hence, plants treated with feather waste organic hydrolysate exhibited effective increase in biochemical characters of plants like concentration of photosynthetic pigments, proteins, amino acids, flavonoid and phenolic. Some amino acids in feather hydrolysate may acts as precursor for plant growth hormone thus helpful to develop tolerance in various stress conditions.

3.9.3.1. Chlorophyll content

There was increase in chlorophyll a concentration of treated plant than control while decrease in overall chlorophyll pigment and chlorophyll b concentration was observed. Table no.6 illustrated the change in chlorophyll concentration. This significant change was observed because along with soil macro and micronutrients, hydrolysate provides additional nutrients which enhance the growth, development and improve in chlorophyll content as well [34].The rise in photosynthetic pigments may cause rapid synthesis of carbohydrates thus improve yield in plants [35].Chlorophyll a content in treated plant was found to be 3.650±0.45 µg/ml for tomato while 3.603±0.49 µg/ml for black mustard. Tomato contains 11.230±0.32 µg/ml and black mustard contains 11.006±0.41 µg/ml chlorophyll b. The overall chlorophyll content was found to be 14.874±0.44 µg/ml in tomato while 14.604±0.44 µg/ml in black mustard. The results were analogous to *T. aestivum* and *V. radiata* treated with feather and fish waste hydrolysate respectively [36, 32].

Table 6: Concentration of chlorophyll

Variants	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total Chlorophyll (µg/ml)
Control	1.906±0.28	15.422±0.41	17.321±0.48
Test (Tomato)	3.650±0.45	11.230±0.32	14.874±0.44
Control	1.877±0.40	15.870±0.49	17.741±0.41
Test (Black mustard)	3.603±0.49	11.006±0.41	14.604±0.44

3.9.3.2. Free protein and amino acids

The absorption of macro and micronutrients in hydrolysate causes enhancement in amino acid and protein synthesis in treated plants than control [33] (table no.7) which may help to the plant for easy absorption, results in healthy growth of plants. The concentration of free proteins and amino acids in test plant was significantly higher than control one. Hence, feather waste hydrolysate promotes rapid growth and development of treated plants.

Table 7: Biochemical analysis of vegetative tissue in control and treated plants (n=3) ±SD

Variants	Protein (mg/g fresh weight)	Amino acid (mg/g fresh weight)	Total phenolics (mg GAE /g fresh weight)	Total flavonoids (mg Que /g fresh weight)
Control	1.88 ± 0.34	0.724 ± 0.03	3.11 ± 0.83	1.65 ± 0.57
Test (Tomato)	3.48 ± 0.13	1.25 ± 0.38	3.34 ± 1.0	1.85 ± 0.32

Control	3.68 ± 0.45	1.516 ± 0.42	2.83 ± 0.04	2.89 ± 0.32
Test(Black mustard)	6.77 ± 0.27	3.022 ± 0.08	4.78 ± 0.35	3.68 ± 0.17

(GAE- gallic acid equivalent; Que- quercetin equivalent; n=3±SD)

3.9.3.3. Total phenolics and flavonoid

The natural antioxidants, polyphenols are present in all plants and have vital function in defence mechanism of it. Besides this, polyphenols have effective health benefits in human beings also [16]. The vegetative parts of plants contains high amount of phenolic and flavonoid. The treatment of feather waste hydrolysate to tomato and black mustard plants showed little bit increase in total phenolics and flavonoid content (table no.7) as compared to control plant.

3.9.4. Chemical analysis of soil

Soil composition and its chemical parameters are crucial factors for growth and development of any crop. The pre-treatment of hydrolysate on soil and observed its effect on plants and results showed that tremendous positive change was occurred in all constituents of soil tabulated in table no.8. The increased concentration of macro and micronutrients were observed in test soil. The current study showed enrichment of C and N percentage which indicates raise in soil fertility. With the supplement of hydrolysate, it was found that, other macro and micro components of soil drastically increased viz; Mg, P, Zn, K, Mn, Ca, Fe, N, K, Cu and proteins. Hydrolysate treated soil contains increasing amount of total nitrogen, phosphorus, potassium of about 250.8±0.22%, 20± 0.021 mg/l and 65± 0.02 mg/l respectively. Thus, this micro-organism degraded feather waste can be considered as a best source of nutrients which improve soil in terms of soil fertility or quality and crop productivity. The high amount of carbon and nitrogen causes increase in plant biomass. Therefore, feather waste hydrolysate efficiently employed as soil conditioner which elevates the water holding and buffering ability of soil [37, 38].

Table 8: Chemical analysis of control and soil treated with feather hydrolysate

Sample	**P (mg/l)**	**K (mg/l)**	**Ca (mg/l)**	**Mg (mg/l)**	**Mn (mg/l)**	**Zn (mg/l)**	**Total C (%)**	**Total N (%)**
Control	9.52±0.02	52.5±0.02	197.5± 0.016	11.05± 0.01	BDL	4.892± 0.05	1.18±2.07	235.2±0.30
Test	20± 0.021	65± 0.02	205.7± 0.020	11.11± 0.02	BDL	5.077±0.014	2.88±0.08	250.8±0.22

(Test: - 5ml hydrolysate; n=3±SD)

4. Conclusion

Feather degradation ability of Gram positive *Staphylococcus sciuri* could be efficiently utilized in feather waste management as this bacterium utilizes whole feather as sole carbon and nitrogen source. This micro-organism showed keratinolytic activity 99.05 U/ml so it may acts as potential source for keratinase. The feather degradation product that is hydrolysate made by *S. sciuri* can be employed as plant growth stimulator also helps to improve soil and crop productivity. Thus, this bacterium helps to maintain the ecological balance and protect our mother earth from environmental pollution at some extent also improves the importance of feather waste in agricultural sector.

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6. Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7. References

1) J.M. Kim, W.J.Lim, H.J. Suh, Feather-degrading *Bacillus* species from poultry waste, Process Biochemistry, (2001) 287–291.
2) Y.Ghasemi, M.Shahbazi, S.R.Amini, M.Kargar, A.Safari, A.Kazemi, N.M. Najafabady, Identification and characterization of feather-degrading bacteria from keratin-rich wastes, (2012) Ann. Microbiol., 737–744.
3) R.Ouertani, H.Chouchane, M.Mahjoubi, H.Khdhira, A.S.Masmoudi, A.Cherif, M.Neifar, Feather degradation efficiency and hide dehairing ability of a new keratinolytic *Bacillus halotolerans* strain, isolated from a tannery wastewater (2020), Applied Bionics and Biomechanics,102-110.
4) A.Riffel, A.Brandelli, Isolation and characterization of a feather-degrading bacterium from the poultry processing industry (2002), Journal of Industrial Microbiology & Biotechnology,255-258.
5) P.Bhuyar, S.Zagade, R.Revankar, M.M.Yusoff, M.H.Ab Rahim, N.Govindan, Isolation, characterization and partial purification of keratinase from keratinolytic bacteria (2018), Scholar Journal of Applied Sciences and Research,40-45.
6) S.Nagarajan, P.Eswaran, R.P.Masilamani, H. Natarajan, Chicken feather compost to promote the plant growth activity by using keratinolytic bacteria (2017), Waste Biomass Valor,531–538.
7) V.Sekar, M.Kannan, R.Ganesan, B.Dheeba, N.Sivakumar, K.Kannan, Isolation and screening of keratinolytic bacteria from feather dumping soil in and around Cuddalore and Villupuram, Tamil Nadu (2015), Proc.Natl.Acad.Sci., 567-575.
8) K.Bhange, V.Chaturvedi and R. Bhatt, Ameliorating effects of chicken feathers in plant growth promotion activity by a keratinolytic strain of *Bacillus subtilis* PF1 (2016), Bioresources and Bioprocessing, 1-10.
9) S.O.Pustake, P.K.Bhagwat, P.B. Dandge,Statistical media optimization for the production of clinical uricase from *Bacillus subtilis* strain SP 6 (2019),Heliyon,1-7.
10) C. Abiola and V.O. Oyetayo, Isolation and biochemical characterization of microorganisms associated with the fermentation of kersting's groundnut (*Macrotyloma geocarpum*) (2016), Research Journal of Microbiology,1-10.
11) T.Hussain, A.Roohi, S.Munir, I. Ahmed, J.Khan, V.E. Hermann, K.Y.Kim, M.Anees,Biochemical characterization and identification of bacterial strains isolated from drinking water sources of Kohat, Pakistan (2013), African Journal of Microbiology Research,1579-1590.
12) Y.F.Syahri, Baharuddin, Fachruddin, A.Yani, Biochemical tests and identification of potential indigenous bacteria from nickel post-mining land in Pomalaa (2019), IOP Conf. Series: Earth and Environmental Science,1-8.

13) H.Isenberg, Clinical microbiology procedures handbook second edition(1992), American Society for Microbiology.
14) P.M. Tille and B.A. Forbes,Bailey and Scott's diagnostic microbiology (13th edition) (2014), St.Louis,Missouri:Elsevier.
15) J.H. Waterborg, The Lowry method for protein quantitation (1996),Researchgate,7-9.
16) H.B.Mahesha,Estimation of amino acid by ninhydrin method (2012),Researchgate,1-2.
17) K.Begum, S.J.Mannan, R.Rezwan, M.Rahman, S.Rahman, A.N.Kamal, Isolation and characterization of bacteria with biochemical and pharmacological importance from soil samples of Dhaka City (2017), Dhaka Univ. J. Pharm. Sci.,129-136.
18) D.I.Arnon, Copper enzymes in isolated chloroplasts polyphenoloxidase in Beta vulgaris (1949), Plant Physiology 24(1).
19) R.G. Gurav & J.P. Jadhav, A novel source of biofertilizer from feather biomass for banana cultivation (2013), Environmental Science & Pollution Research 20(7),4532-4539.
20) O.H.Lowry, N.J.Rosebrough, A.L.Farr, & R.J. Randall, Protein measurement with the folin phenol reagent (1951), Journal of Biological Chemistry 193(1), 265-275.
21) S. Moore & W.H.Stein, A modified ninhydrin reagent for the photometric determination of amino acids & related compounds (1954), Journal of Biological Chemistry 211(2),907-913.
22) V.L. Singleton & J.A. Rossi, Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents (1965), American Journal of Enology & Viticulture 16(3),144-158.
23) C.C.Chang, M.H.Yang, H.M. Wen & J.C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods (2002), Journal of Food & Drug Analysis 10(3).
24) Z.Y. Hseu, Evaluating heavy metal contents in nine composts using four digestion methods (2004), Bioresource Technology 95(1),53-59.
25) A.Reyes, I. D.Ambita, J. L.Batalon, B. L.Aba, A.Cortes, C. G. Macabecha and A. Montecillo, Isolation and characterization of keratinolytic bacteria from soil samples of poultry waste dumping sites (2018), International Journal of Agricultural Technology Vol. 14(7),1787-1800.
26) G. Govinden and D. Puchooa, Isolation and characterization of feather degrading bacteria from Mauritian soil (2012), African Journal of Biotechnology Vol. 11(71),13591-13600.
27) M.Manirujjaman, R.Amin, A. A. Nahid and M. S. Alam, Isolation and characterization of feather degrading bacteria from poultry waste (2016), African Journal of Bacteriology Research Vol.8 (3),14-21.
28) S.G.Joshi, M.M.Tejashwini, N.Revati, R.Sridevi and D.Roma, Isolation, identification and characterization of a feather degrading bacterium (2007), International Journal of Poultry Science 6 (9), 689-693.
29) M.Akhter, L.W.Marzan, Y. Akter and S. K. Kazuyuki, Microbial bioremediation of feather waste for keratinase production: an outstanding solution for leather dehairing in tanneries (2020), Microbiology Insights Volume 13,1–12.
30) M. Kimura, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences (1980), Journal of Molecular Evolution 16,111-120.

31) T.Paul, S.K.Halder, A.Das, S.Bera, C.Maity, A.Mandal, P.S.Das, P.K.Mohapatra, B.R.Pati & K.C.Mondal, Exploitation of chicken feather waste as a plant growth promoting agent using keratinase producing novel isolate *Paenibacillus woosongensis* TKB2 (2013), Biocatalysis & Agricultural Biotechnology 2(1),50-57.

32) P.K.Bhagwat, K.K.Bhise, M.V.Bhuimbar and P.B. Dandge, Use of statistical experimental methods for optimization of collagenolytic protease production by *Bacillus cereus* strain SUK grown on fish scales (2018), Environmental Science & Pollution Research 25(28), 28226-28236.

33) G.Colla, S.Nardi, M.Cardarelli, A.Ertani, L.Lucini, R.Canaguier & Y.Rouphael, Protein hydrolysates as biostimulants in horticulture (2015),Scientia Horticulturae 196,28-38.

34) T.F. Neales, Components of the total magnesium content within the leaves of white clover & perennial ryegrass (1956), Nature 177,388–389.

35) N.Paradikovic, T.Vinkovic, I.V.Vrcek, I.Zuntar, M.Bojic & M. Medic- Saric, Effect of natural biostimulants on yield & nutritional quality: an example of sweet yellow pepper (*Capsicum annuum* L.) plants (2011), Journal of the Science of Food & Agriculture 91(12),2146-2152.

36) K.K.Bhise, P.K.Bhagwat & P.B.Dandge, Synergistic effect of *Chryseobacterium gleum* sp. SUK with ACC deaminase activity in alleviation of salt stress & plant growth promotion in *Triticum aestivum* L. (2017), 3 Biotech 7(2).

37) N.J. Barrow, A comparison of the mineralization of nitrogen & of sulphur from decomposing organic materials (1960), Crop Pasture Science 11(6),960–969.

38) R.J. Haynes & R. Naidu, Influence of lime, fertilizer & manure applications on soil organic matter content & soil physical conditions: a review (1998), Nutrient Cycling in Agroecosystem 51(2),123–137.