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Microbes Mediated Keratin Extraction from Poultry Waste and Assessment of Its Efficacy in Growth Promotion of Zea Mays and Triticum aestivum

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Cover Page Footnote

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MICROBES MEDIATED KERATIN EXTRACTION FROM POULTRY WASTE AND ASSESSMENT OF ITS EFFICACY IN GROWTH PROMOTION OF *ZEA MAYS* AND *TRITICUM AESTIVUM*

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ABSTRACT

Feathers are a waste product of the poultry industry and are considered a major pollutant of the environment. Soil associated with poultry farms is a rich source of indigenous bacteria that are involved in biodegradation of keratin of the feathers of *Gallus gallus domesticus*. The present study was designed to understand the role of microbes in the degradation of a feather's keratin and its possible utilization as a bio-protein for plants. In our studies, Keratinolytic activity was high at 37-45 ℃ and at 7.5-9.5 pH. *Pseudomonas aeruginosa* was identified by 16S rRNA sequencing as the most active keratinolytic strain. At 37 ℃ and pH 9.0 the protein content was 5.67mg/ml, while at 45 ℃ and pH 7 the protein content from bacterial isolate IM6 was 6.52 mg/ml. However, all other ten bacterial isolates (IM1, IM2, IM3, IM4, IM5, IM7, IM8, IM9, IM10, IM11) also showed degradation potential. Fourier transform infrared (FTIR) was performed to confirm the presence of strong Amide-A, Amide-III, and disulfide bonds. In the plant microbial interaction experiments on the seed of *Triticum aestivum* and *Zea mays*, the isolates IM2 and IM6 were observed to increase the root, shoot and plant lengths as compared to all other bacterial isolates. The future implication of this study shows the importance of keratin degrading bacteria for the degradation and recycling of poultry feather waste, and introducing it as a value-added product for use in fertilizers, while also removing poultry waste from the environment and making the environment healthy for other living beings.

Keywords: Feathers, keratinolytic activity, FTIR, biodegradation, degradation potential.

INTRODUCTION

Poultry products are important sources of animal protein but also lead to a large amount of waste. This waste can be used as an important anaerobic fermentation material for biogas energy, fertilizers and animal feed. However, if not treated properly they cause water and soil pollution (Li et al., 2016). Keratin composed of amino acids can be converted into useful and biodegradable plastics by using various chemical and physical methods (Thyagarajan et al., 2013). Peptides and amino acids are also used for plant growth like IAA (indole acetic acid), hydrogen cyanide and ammonia (Bhange et al., 2016). Chicken feathers are made up of keratin protein (91 %), water (8 %) and lipids (1 %). Many microorganisms degrade the feathers and used it as nitrogen and carbon source.

Keratin is important component of poultry hairs, nails, horns, beaks and feathers (Wang et al., 2016). It is reported that keratin is the hardest biological protein with a low degradation rate (Bhari et al., 2020). It is usually hard to break keratin by ordinary enzymes like pepsin, however, some fungi and bacteria can degrade keratin and transform it in nutrients rich waste (Adelere and Lateef, 2019).

Femi-Ola et al., (2015) reported that a few microbes produce keratinase in the presence of keratin substrate and hence can degrade *Gallus domesticus* feathers, hairs, nails, wool, and scales at a wide range of pH and temperature levels. The degradation of keratin by microorganisms is an eco-friendly method that in turn produces biological products such as keratinolytic enzymes, peptides, and amino acid-rich keratin hydrolysates (Adelere and Lateef, 2019).

Keratinolytic bacteria such as Pseudomonas, Bacillus and others can be isolated from soils, hot spring, birds' nests, and keratin wastes (Gurav and Jadhav, 2013). Species of Bacillus are the most common keratinolytic bacteria with high efficiency in degrading keratins. The feathers that are broken down by degrading keratinolytic bacteria can not only reduce waste discharge and use of synthetic fertilizer, but can also release nitrogen fertilizers via conversing and recycling the nutrients (Tamreihao et al., 2017). Soil fertility is improved by the nitrogen that is released into the soil by the microbial decomposition of keratinous waste. Tamreihao et al., (2019) informed about improved in growth of rice plants that were cultivated in the soil administered with feather hydrolysate as compared to the control plant. The germination of rice and pea seeds was improved by the soil treated with nitrogen-rich organic manure (Kumar et al., 2017).

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Similarly, introducing feather hydrolysate can cause early germination of *Cicer arietinum* seeds (Verma et al., 2016). So, soil fertility can be increased through the biodegradation of keratin waste. It directly increases soil health and indirectly human health and hence is beneficial for the ecosystem (Adelere and Lateef, 2016). Keeping in consideration the previous studies, the present research work was planned to check the efficacy of indigenous poultry soil bacteria with the capacity to degrade keratin and their role in plant growth promotions in soil.

MATERIAL AND METHODS

Dry soil from a poultry shed dumpsite and the feathers of *Gallus gallus domesticus* were collected. Feathers were washed, autoclaved, dried and ground to form a powder.

i. Isolation, Characterization, Screening and 16S rRNA Sequencing of Bacterial isolates from Gallus gallus domesticus Feather

Isolation of bacteria from poultry waste was performed following the modified method of Martinus, 1901 described by Kumar et al. (2016). Isolates were further sub-cultured using skim milk agar to analyze proteolytic activity. Eleven isolates formed clear zones on plates, whose growth was also checked on minimal agar. Selected bacterial isolates

were characterized morphologically and biochemically following Cappuccino and Sherman (2002) techniques. The genetic identification of isolate IM6 was done using 16S ribosomal gene sequence analysis using commercial services from IBM.

ii. Effect of varying Temperature and pH on Degradation and Protein Estimation of Feathers

To study the effect of different temperatures on keratin degradation, the isolates were individually incubated in L-Broth at 37° C for 24 hours. 66 test tubes, contained 10 ml fresh keratin media and feathers in each, were divided into 6 batches of 11 test tubes. Three batches of 11 test tubes were kept at 3 different temperatures of 25º C, 37º C, and 45º C, at pH 7, while for checking growth rates at different pH, the remaining three batches were incubated at pH 5, pH 7, and pH 9 at 37º C temperature. To study the Protein contents of degraded feathers by bacterial isolates, the Lowry method was used as mentioned by Assinkv et al. (2023).

iii. Biofilm Assay and Fourier Transform Infrared (FTIR) Spectroscopy Analysis of Keratin Hydrolysate

Biofilm formation for keratin degrading bacterial isolates was done according to the method of Qurashi and Sabri (2012). The FTIR spectrum of the extracted keratin hydrolysate was analyzed for the detection of Amide groups and functional groups. Keratin hydrolysate was sent to a private laboratory in Lahore, Pakistan.

iv. Plant Microbe Interaction (PMI)

To study plant microbe interaction, the seeds of *Zea mays* and *Triticum aestivum* were sterilized with 0.1 % $HgCl_2$ and then soaked in pellet suspended normal saline for 20 minutes. These seeds were placed in petri dishes, which have wet filter paper and were sterilized. These dishes were kept in the dark for 3 days. The parameters like dry weight, fresh weight, root, shoot, and plant length were observed and measured for the following 15 days.

v. Keratin as Fertilizer in Control Conditions and in the Field

The seed of *Triticum aestivum* and *Zea mays* were sterilized with 0.1 % $HgCl_2$. Keratin hydrolysate culture, which was not centrifuged, was filtered using a sieve of 0.2 mm to remove any undigested feather. The seeds were sown in a plastic glass in controlled conditions and in pots in the field. Different dilutions of hydrolysate i.e., 10X, 20X, 30X, 50X, 70X were used in the glasses. Distilled water was used in control instead of hydrolysate. In the field, 15 seeds per pot for *Triticum aestivum* and 10 seeds per pot were used for the *Zea mays*. The seeds of *Triticum aestivum* were given 50x (0.09 mg/ml) and 70x (0.07 mg/ml) keratin hydrolysate dilutions. The seeds of *Zea mays* were given 20x (0.24 mg/ml) and 70x (0.07 mg/ml) keratin hydrolysate dilutions in the field. The result was checked after 3 days in controlled conditions while after 30 days in the field. The number of germinated seeds, root lengths, and shoot lengths was recorded and compared with controls.

vi. Proline and Soluble Protein Estimation from Field Plants

To extract the L-proline from the plant, the method of Tonon et al., (2004) was adapted. While, soluble protein was extracted by following the protein analysis method of Lowery et al., (1951).

Statistical Analysis

SPSS software version 25.00 was used for the analysis of statistical data. Analysis of variance (ANOVA) was performed to check pair-wise comparison of mean and calculating difference in each experiment for least significant difference (LSD) method at $p < 0.05$ level of significance.

RESULTS

i. Bacterial isolation and Screening for Determination of Keratinolytic activity using Skim milk assay and Minimal agar media

From 30 different bacterial colonies, 11 isolates (IM1, IM2, IM3, IM4, IM5, IM6, IM7, IM8, IM9, IM10, and IM11) were selected on the basis of positive results which appeared in the form of clear zones on skim milk agar and minimal agar media.

i. Morphological and Biochemical Characterization of Bacterial isolates

Most of the isolates were gram negative and also showed negative results on spore staining and capsule staining. On studying biochemical characterization, they showed DNAs test negative, catalase test positive, motility test positive, indole test negative, citrate positive, urease test positive, methyl red test positive, and Voges-Proskauer test negative.

ii. Effect of varying Temperature and pH on Bacterial growth

The effect of varying temperature conditions on bacterial growth showed that there was a general trend of significant ($p > 0.05$) increase in cell densities at temperature 45 ℃ as compared to temperature 25 ℃ and 37 ℃ for all bacterial isolates (Figure 1).

The effect of varying pH conditions on bacterial growth was noticed and a general trend of significant ($p > 0.05$) increase in cell densities at pH 9.0 were found as compared to pH 5.0 and pH 7.0 (Figure 2).

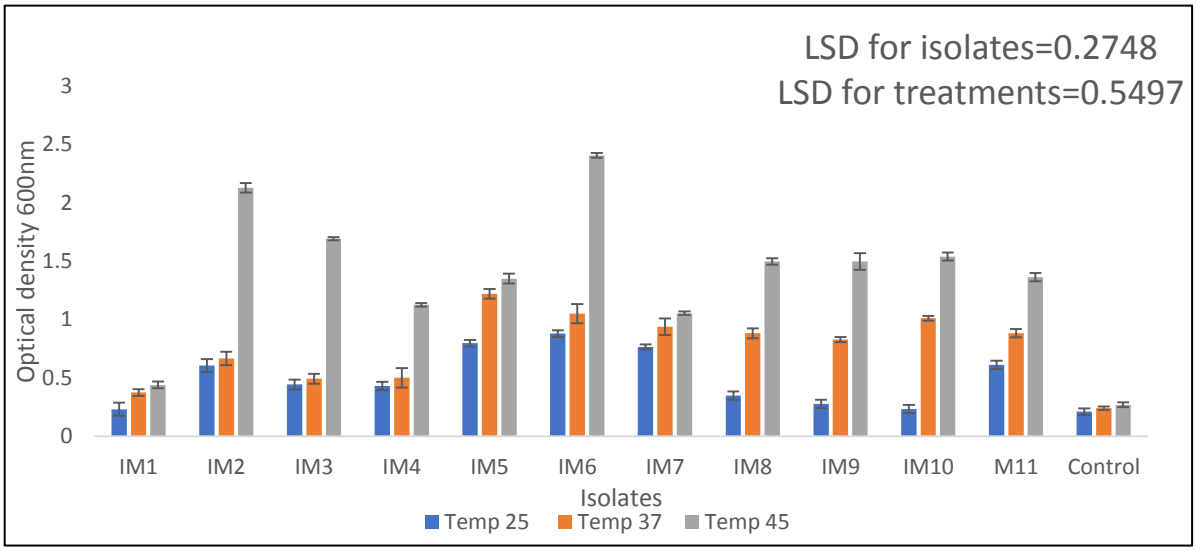

Figure 1: Effect of different temperatures (25, 37 and 45 °C) on bacterial growth.

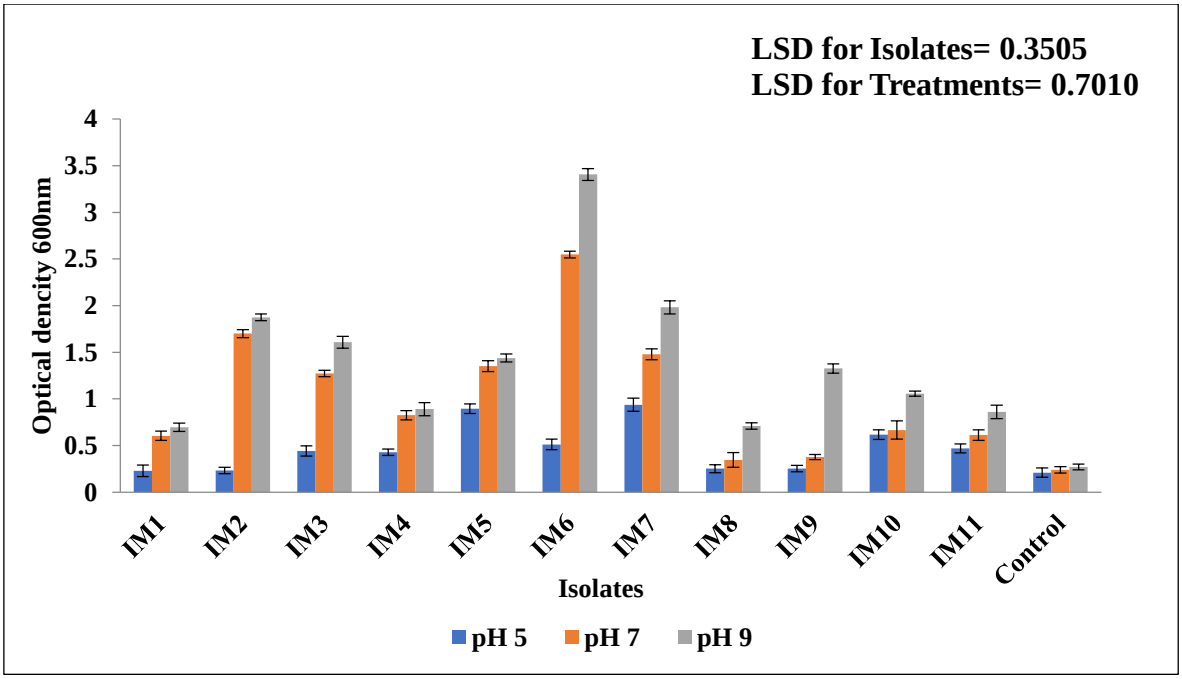

Figure 2: Effect of different pH (5, 7 and 9) on bacterial growth

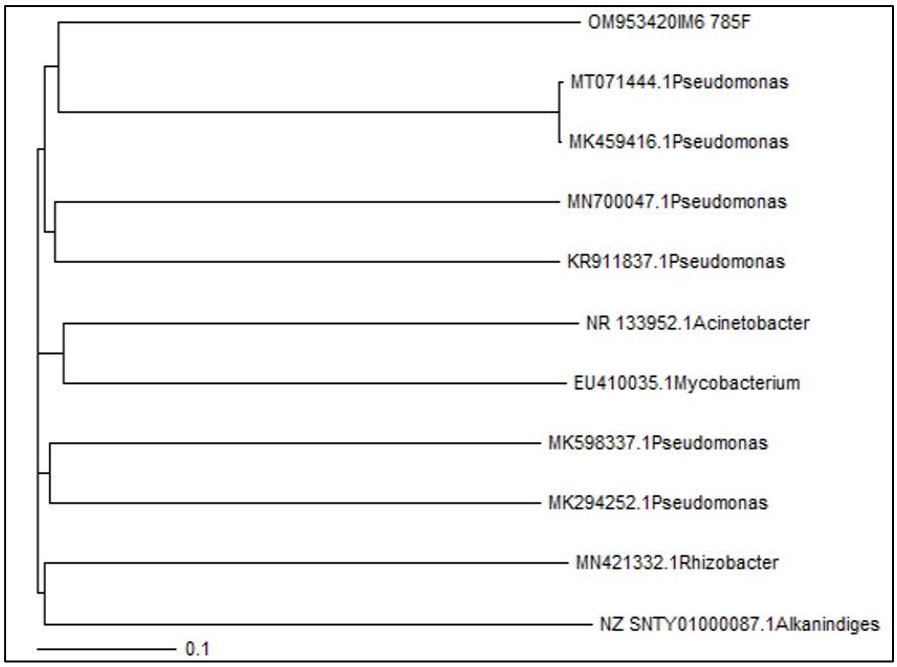

Figure 3: Phylogenetic tree (Saitou and Nei, 1987).

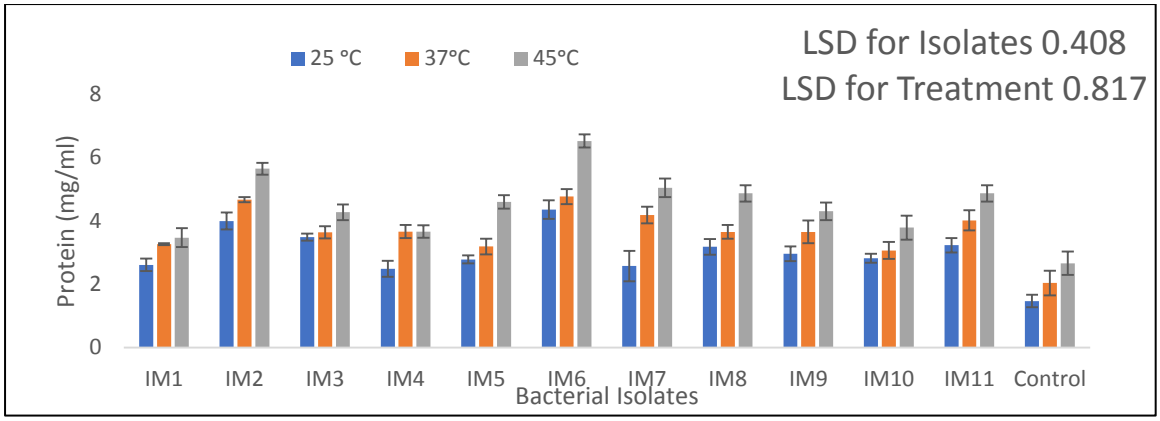

Figure 4: Protein content estimation at temperatures 25˚C, 37˚C and 45˚C.

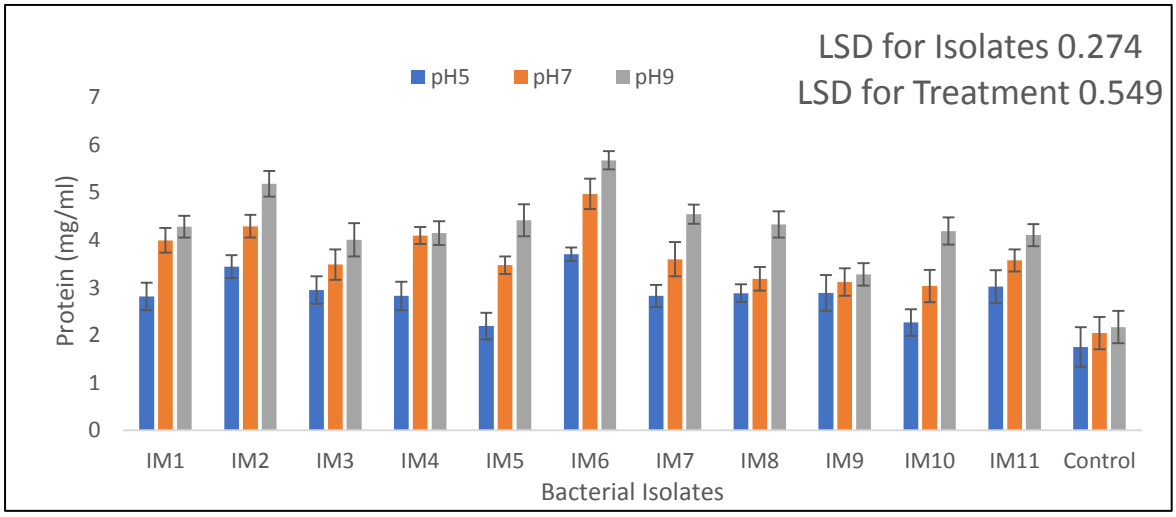

Figure 5: Protein content estimation on pH 5.0, pH 7.0 and pH 9.0.

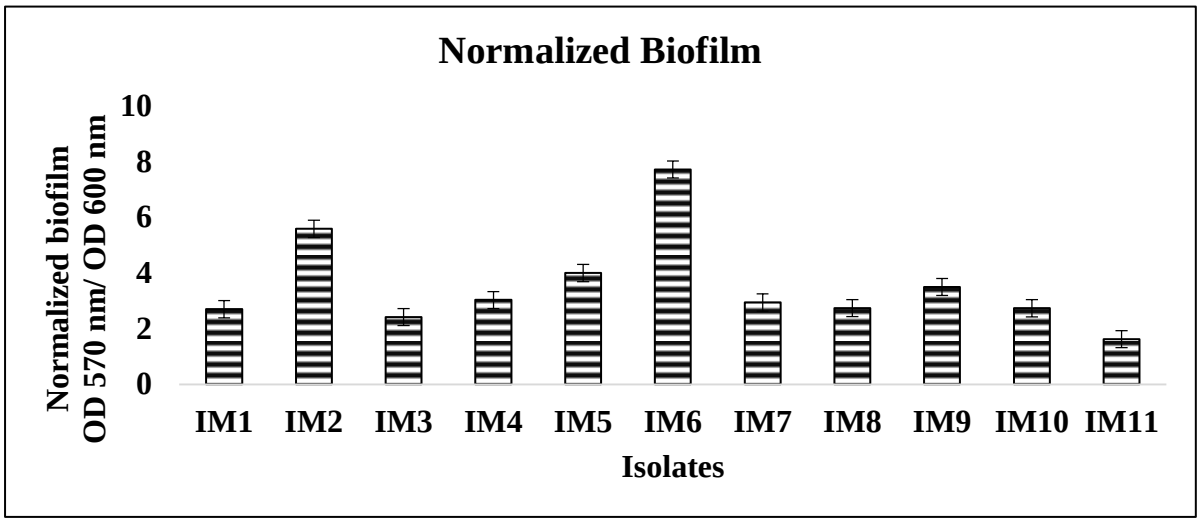

Figure 6: Optimized bacterial Isolates forming Biofilm from *Gallus domesticus*.

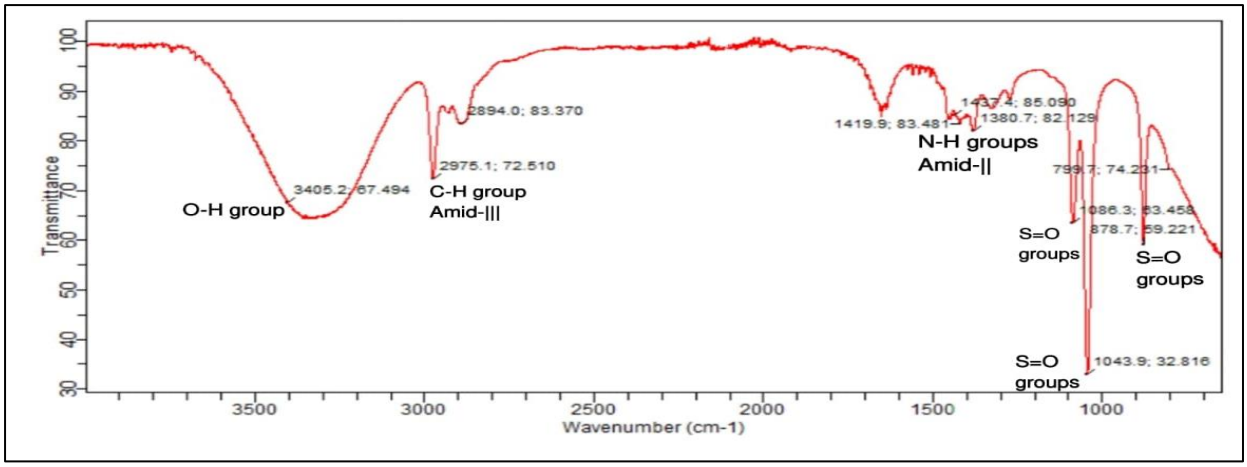

Figure 7: Fourier Transform Infrared analysis of Keratin hydrolysate from *Gallus domesticus* feathers.

Table 1: Plant growth parameters with bacterial inoculation

Isolates	Root lengths in cm	Shoot lengths in cm	Plant lengths in cm	Germination %
Triticum aestivum				
IM1	1.388 ± 0.39	0.177 ± 0.03	1.565 ± 0.16	66.6 ± 0.60
IM 2	3.244 ± 0.18	0.277 ± 0.01	3.521 ± 0.17	75.0 ± 0.88
IM 3	1.933 ± 0.43	0.133 ± 0.03	2.066 ± 0.24	58.3 ± 0.66
IM 4	2.109 ± 0.26	0.344 ± 0.07	2.453 ± 0.25	66.6 ± 0.61
IM 5	2.221 ± 0.10	0.944 ± 0.16	3.165 ± 0.19	50.0 ± 0.50
IM 6	2.541 ± 0.33	1.075 ± 0.33	3.616 ± 0.37	83.3 ± 0.71
IM 7	2.497 ± 0.17	0.377 ± 0.03	2.874 ± 0.27	41.6 ± 0.49
IM 8	1.653 ± 0.37	1.788 ± 0.21	3.411 ± 0.26	66.6 ± 0.94
IM 9	2.333 ± 0.54	0.655 ± 0.10	2.988 ± 0.28	66.6 ± 0.43
IM 10	1.777 ± 0.14	0.888 ± 0.11	2.665 ± 0.26	41.6 ± 0.53
IM 11	1.722 ± 0.09	0.588 ± 0.05	2.310 ± 0.29	58.3 ± 0.66
Control	2.451 ± 0.22	0.605 ± 0.06	3.155 ± 0.22	58.3 ± 0.73
LSD for treatment 0.59, LSD for Isolates 0.34				
Zea mays				
IM 1	1.966 ± 0.38	1.563 ± 0.28	3.502 ± 0.24	66.6 ± 0.42
IM 2	3.225 ± 0.59	1.455 ± 0.44	4.680 ± 0.14	83.3 ± 0.45
IM 3	2.220 ± 0.30	1.995 ± 0.42	4.215 ± 0.21	75.0 ± 0.83
IM 4	2.109 ± 0.18	1.886 ± 0.35	3.995 ± 0.22	50.0 ± 0.88
IM 5	3.012 ± 0.21	1.139 ± 0.51	4.151 ± 0.24	75.0 ± 0.31
IM 6	3.371 ± 0.22	1.411 ± 0.66	4.782 ± 0.53	91.6 ± 0.94
IM 7	2.587 ± 2.50	1.075 ± 0.46	3.662 ± 0.48	58.3 ± 0.55
IM 8	2.022 ± 0.20	2.566 ± 0.42	4.588 ± 0.38	41.6 ± 0.40
IM 9	2.555 ± 0.16	1.241 ± 0.48	3.796 ± 0.44	75.0 ± 0.25
IM 10	2.364 ± 0.34	1.511 ± 0.27	3.875 ± 0.70	66.6 ± 0.70
IM 11	2.452 ± 0.42	1.366 ± 0.16	3.818 ± 0.57	58.3 ± 0.62
Control	2.677 ± 0.40	1.328 ± 0.26	3.995 ± 0.48	66.6 ± 0.50
LSD for treatment 0.57, LSD for Isolates 0.32				

i. 16S rRNA Sequencing

Isolate (IM6) showed the best results and further passed through 16S rRNA gene sequence (1491 bp) which showed closest (99 %) similarity to *Pseudomonas* strain DSM 5425 and it was identified as Pseudomonas sp. with assigned gene accession number as ACCESSION OM953420 from gene bank NCBI.

Phylogenetic tree

A Phylogenetic tree was developed based on 16S rRNA gene's partial sequences of bacterial strains using the Neighbor-Joining method with the help of Clustal w software (Saitou and Nei, 1987). Clustering of bacterial cells in the groups, subgroups, sections and subsections showed their common ancestral origin (Figure 3).

i. Protein Estimation of Keratin on Different Temperatures and pH

The protein estimation of the keratin from feathers of *Gallus domesticus* showed that the amount of protein content (mg/ml culture) was high at high temperatures and alkaline conditions. The protein content of *Gallus domesticus* feathers at 45 ℃ and pH 7 was 6.52 mg/ml from bacterial isolates IM6 (Figure 4). Protein content at 45 ℃ and pH 9.0 was 5.67 mg/ml for bacterial isolates IM6 (Figure 5).

ii. Biofilm Assay

Bacterial Isolates obtained from *Gallus gallus domesticus* showed positive biofilm formation in 4 days at temperature 45 ˚C and pH 9.0. However, the significantly highest ($p > 0.05$) optical density was recorded by bacterial isolate IM6 (Figure 6).

iii. Fourier Transform Infrared (FTIR)

iv. Plant Microbes Interaction

Plant microbe interaction was studied and it was observed that in various parameters such as root, shoot, plant length and percentage of germination for *Triticum aestivum* and *Zea mays* seed, a significant increase was found. However, a pronounced increase was noticed particularly in the case of IM6 isolate (Table 1).

v. Keratin as Fertilizer in Controlled Condition and in the Field

Keratin was used as a fertilizer and studied both for control and field conditions.

Effect of Keratin as Fertilizer on Length Parameters in Controlled Conditions

Keratin as a fertilizer was used at the dilution of 10, 20, 30, 50, and 70x against *Triticum aestivum* and *Zea mays*. A significant increase in root, shoot and plant length was recorded for *Triticum aestivum* with all the dilutions. While, a general increase was noticed for shoot and plant length in *Zea mays*. An insufficient increase was noticed for 10x, 30x, and 50x dilution of keratin for root length except with other dilutions (Table 2).

Effect of Keratin as Fertilizer on Fresh Weight and Dry Weight in Controlled Condition

Keratin as a fertilizer was used at the dilution of 10, 20, 30, 50, and 70x against *Triticum aestivum* and *Zea mays*. A significant increase (< 0.05) in fresh weight and dry weight was noticed for both the plants with all the dilutions (Table 3).

Effect of Keratin Hydrolysate as Fertilizer on Length Parameters in Field Conditions

Keratin hydrolysate was also used as a fertilizer only for 50, and 70x against *Triticum aestivum* and *Zea mays*. A

significant increase (< 0.05) in length was noticed for both the plants with 50x and 70x dilutions (Table 4).

Table 2: Effect of keratin as fertilizer on Length parameters in controlled conditions

Dilutions	Root lengths in cm	Shoot lengths in cm	Plant lengths in cm
Triticum aestivum			
10x	16 ± 0.35	29 ± 0.91	45 ± 0.67
20x	15 ± 0.35	28 ± 0.54	43 ± 0.60
30x	17 ± 0.70	25 ± 0.49	42 ± 0.40
50x	18 ± 0.38	31 ±0.79	49 ± 0.74
70x	16 ± 0.70	29 ± 0.86	45 ± 0.38
Control	11 ± 0.53	14 ± 0.66	25 ± 0.70
LSD for treatment 2.34			
Zea mays			
10x	7.5 ±0.21	19 ± 0.70	26.5 ±0.40
20x	15 ±0.45	16 ± 0.42	31.0 ± 0.66
30x	6.5 ±0.61	13 ± 0.70	19.5 ± 0.81
50x	07 ± 0.88	14 ± 0.82	21.0 ± 0.91
70x	14 ± 0.67	17 ± 0.87	25.0 ± 0.98
Control	07 ± 0.31	13 ± 0.70	20.0 ± 0.62
LSD for treatment 4.97			
10x (keratin0.5 mg/ml) 20x (0.25 mg/ml) 30x (0.016mg/ml) 50x (0.1 mg/ml) 70x (0.07 mg/ml)			

Table 3: Effect of keratin on fresh weight and dry weight in controlled condition

Dilutions	Fresh weight (g)	Dry weight (g)
Triticum aestivum		
10x	0.2677 ± 0.001	0.0547 ± 0.002
20x	0.3644 ± 0.001	0.0984 ± 0.005
30x	0.3867 ± 0.001	0.1890 ± 0.010
50x	0.2603 ± 0.002	0.0780 ± 0.020
70x	0.2533 ± 0.001	0.0507 ± 0.003
Control	0.2133 ± 0.019	0.0493 ± 0.002
LSD for treatment 0.05		
Zea mays		
10x	1.616 ± 0.007	0.461 ± 0.02
20x	1.720 ± 0.090	0.501 ± 0.05
30x	1.425 ± 0.020	0.370 ± 0.17
50x	1.491 ± 0.009	0.403 ± 0.01
70x	1.504 ± 0.010	0.491 ± 0.01
Control	0.853 ± 0.008	0.289 ± 0.01
LSD for treatment 0.29		
10x (keratin 0.5 mg/ml) 20x (0.25 mg/ml) 30x (0.016mg/ml) 50x (0.1 mg/ml) 70x (0.07 mg/ml)		

Table 4: Effect of keratin hydrolysate as fertilizer on Length parameters in field conditions

Dilutions	Root lengths in cm	Shoot lengths in cm	Plant lengths in cm
Triticum aestivum			
50x	21 ± 0.35	35 ± 0.53	56 ± 0.88
70x	21 ± 0.70	31 ± 0.70	52 ± 0.88
Control	19 ± 0.35	24 ± 0.17	43 ± 0.53
LSD for treatment 5.57			
Zea mays			
20x	23 ± 0.63	40 ± 0.28	63 ± 0.91
70x	19 ± 0.60	31 ± 0.31	50 ± 0.91
Control	17 ± 0.70	23 ± 0.40	40 ± 0.30
LSD for treatment 8.13			
20x (0.25 mg/ml), 50x (0.1 mg/ml), 70x (0.07 mg/ml)			

Effect of Keratin Hydrolysate as Fertilizer on Fresh and Dry Weight in Field Conditions

Keratin hydrolysate was also used as a fertilizer only for 50, and 70x against *Triticum aestivum* and *Zea mays*. A significant increase (< 0.05) in fresh and dry weight was noticed for both the plants with 50x and 70x dilutions (Table 4).

i. Proline Estimation from Plants in Field

A general increase in the proline concentration was also noticed. Plants treated with 50x and 70x dilutions showed significant increase in the concentration of proline as compared to that of control (Figure 8, 9).

Table 5: Effect of keratin hydrolysate on fresh and dry weight in field conditions

Dilutions	Fresh weight (g)	Dry weight (g)
Triticum aestivum		
50x	0.996 ± 0.194	0.1920 ± 0.285
70x	1.127 ± 0.303	0.2504 ± 0.353
Control	0.713 ± 0.410	0.1798 ± 0.458
LSD for treatment 0.268		
Zea mays		
20x	7.745 ± 0.253	2.4203 ± 0.202
70x	6.338 ± 0.278	1.6251 ± 0.176
Control	5.422 ± 0.376	1.3224 ± 0.325
LSD for treatment 0.909		
20x (0.25 mg/ml), 50x (0.1 mg/ml), 70x (0.07 mg/ml)		

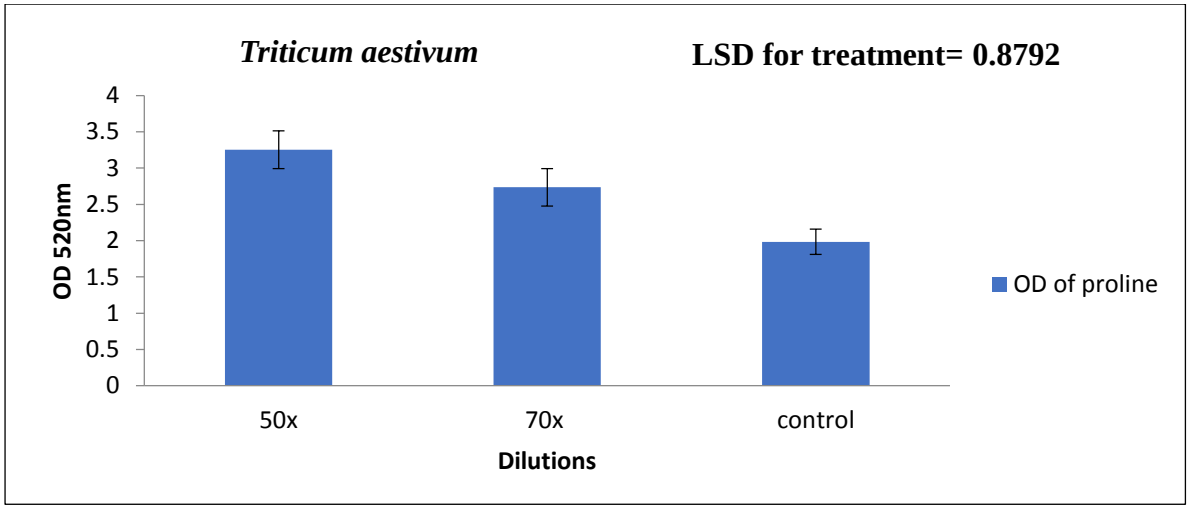

Figure 8: Optical density for proline content estimated from *Triticum aestivum*

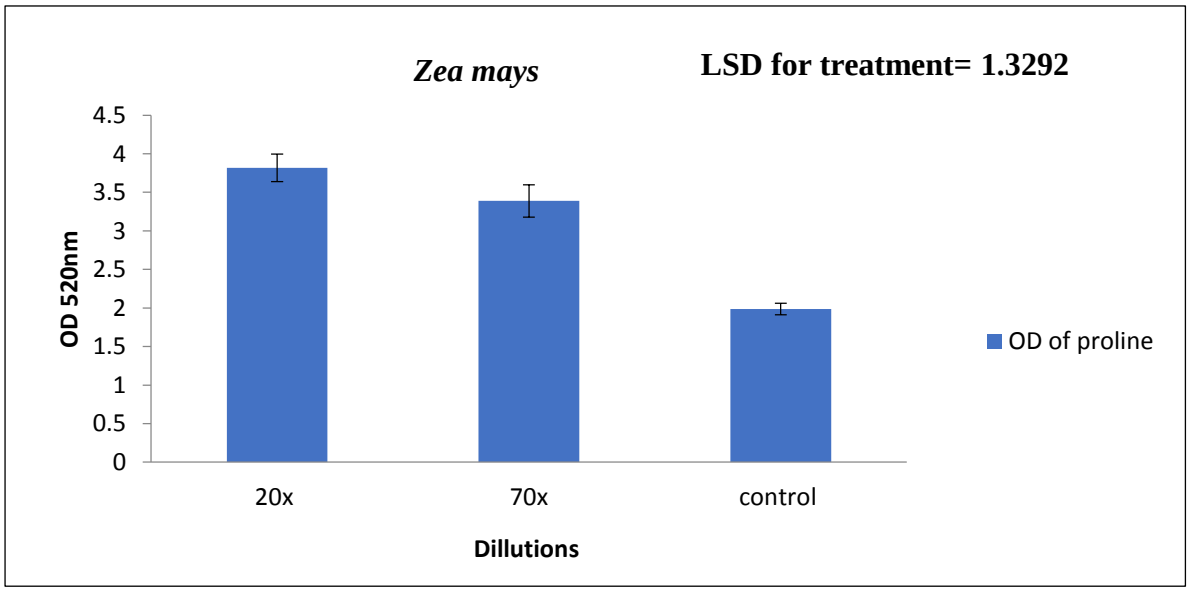

Figure 9: Optical density for proline content estimated from *Zea mays*

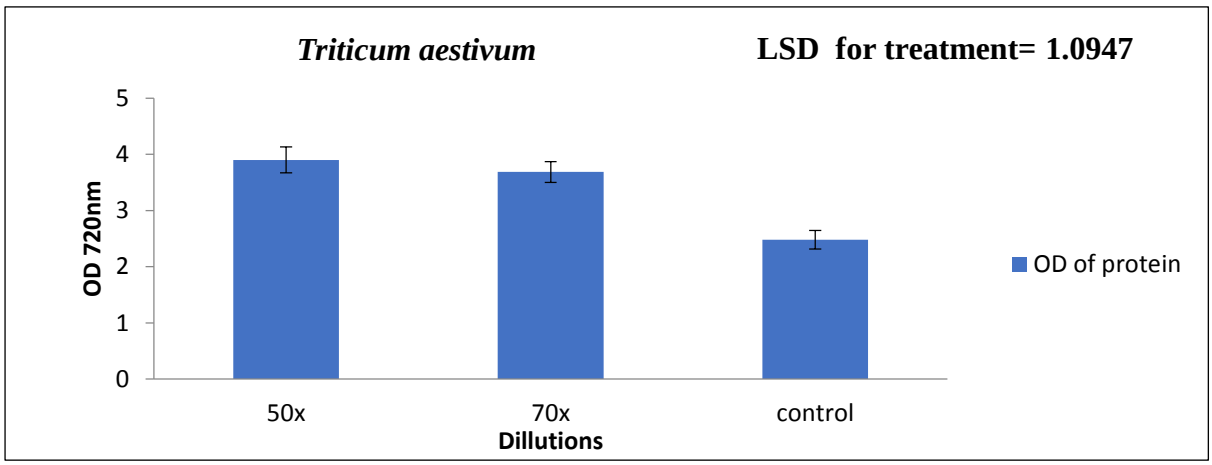

Figure 10: Optical density protein content estimated from *Triticum aestivum*

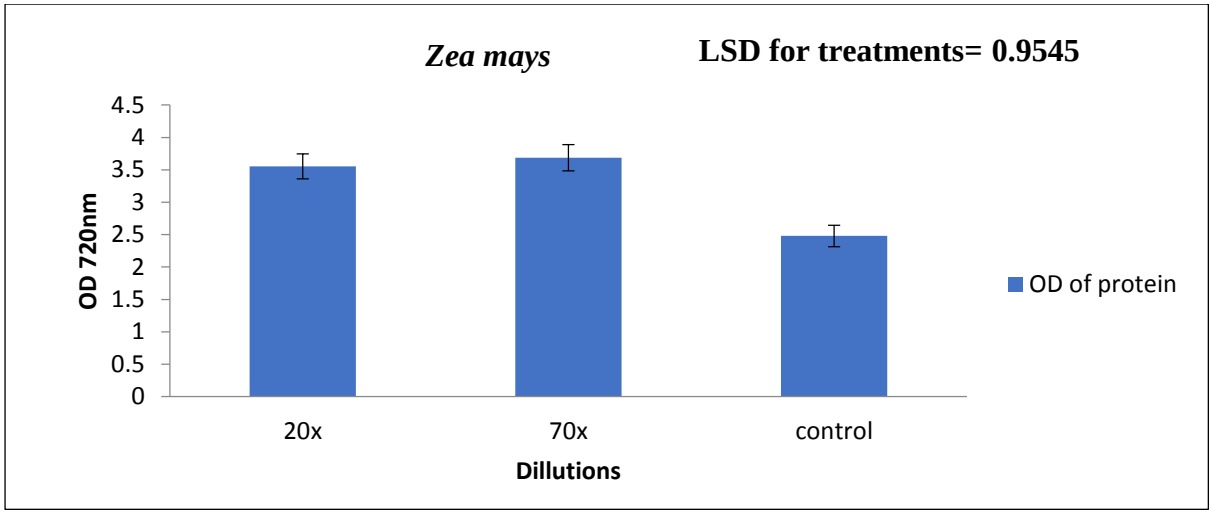

Figure 11: Optical density protein content estimated from *Zea mays*

i. Protein Estimation from Plants in Field

The protein content of *Triticum aestivum* plants treated with 50x was significantly high as compared to control plants (Figure 10). The protein content of *Zea mays* plants treated with 20x increased significantly when compared to control plants (Figure 11).

DISCUSSION

Soil is an important source of microbes that can play a significant role in degradation of keratin protein. Biodegradation can cause the recycling of important elements that are locked in chicken feathers (Mishra et al., 2016). Due to diverse microflora, keratin–utilizing bacteria can degrade feathers that are commonly present in the soil. This produces a large amount of keratin in the soil. This renders the poultry farm soil sample an ideal source of feather-degrading bacteria (Gopinath, 2015; Alahyaribeik and Ullah, 2020). Bacterial isolates that degrade keratin hydrolyze it into soluble peptides and amino acids. The stereo microscopy of poultry feathers was carried out at the start and end of the degradation process. Feathers were completely degraded into powder in 35 to 40 days in case of control. However, this process was enhanced by inoculation of the bacterial streak and degradation was completed before the 4th week. This method of the degradation of feathers was described by Kumar et al., (2016) and also reported Xu et al., (2009). Keratinolytic bacterial colonies were isolated by sub-culturing on skim milk agar plates. Clear zones exhibited the highest proteolytic activity similar to that reported by Sherma et al., (2016).

Temperature and pH have a significant impact on feather degradation. In the present study, it was observed that the proteolyic activity of keratinolytic bacteria and its efficiency increased with a slight elevation in pH. Alkaline conditions are known to be conductive to the production of ammonia through the deamination of amino acids, peptides and proteins during degradation of keratin as reported by Lakshmi et al., (2013) .This slight increase in pH also promotes the action of keratin enzymes, thereby enhancing keratinolysis.

Next to pH, high temperature can also serve an indicator of the highest keratinolytic activity of bacterial strains as reported by Lateef et al., (2010) and similar results were noticed in the present study. The highest proteolytic activity was recorded at pH 9 and 45°C temperatures. It was noticed that keratinolytic bacterial strains could withstand high temperatures

like 50˚C. Change in pH was observed as the degradation moves toward completion it increases and media becomes alkaline despite adjusted pH and temperatures.

Cultural, microscopic and morphological characteristics were checked for selected isolates. Many of isolates were gram negative, and also showed negative result for spore staining tests as well as capsule staining test. By identification based on morphological, cultural, and microscopic characteristics, the identity of the isolates was determined to be *Pseudomonas aeruginosa* and this coincides with the results of Dhiva et al., (2020).

In a previous study, Moonnee et al., (2021) and Han et al., (2012) reported that the isolates were recognized as *Pseudomonas aeruginosa* by sequencing of 16S rRNA gene produced maximum enzymes for keratinolytic activity from *P. aeruginosa* YK17 and *P. aeruginosa* C11 by using 2% chicken feather. Bhuyar et al., (2018) observed keratinolytic activity by isolating keratinolytic bacteria from poultry waste, identifying them as Pseudomonas species. They produced alkaline keratinase enzyme. Maximum keratinolysis activity showed at 7 pH and 37°C. The most keratinolytic bacteria which have been previously reported to be gram-negative, rod-like, and motile, were *Pseudomonas aeruginosa* that could withstand the highest temperature and alkalinity of its substrate. Therefore, gram positive bacteria are less efficient as compared to gram-negative bacteria in synthesizing keratinase. A few studies reported the production of keratinase through gram-negative bacteria, especially *P. aeruginosa* (Moonnee et al., 2021; Han et al., 2012; Bach et al., 2011)

The protein content of *Gallus gallus domesticus* feathers at 45 ˚C was 6.52 mg/ml from bacterial isolate IM6. At 9.0 pH protein content was 5.67 mg/ml for bacterial isolate IM6. FTIR spectroscopy was applied to understand the chemical nature of keratin protein extracted by keratinolytic bacteria from *Gallus gallus domesticus*. The results confirmed that no significant chemical changes occurred and the keratin maintained most amide bonds even in alkaline treatment (Alahyaribeik et al., 2020; Sharma and Gupta, 2016). The broad transmission from region 3500-3100 attributed a stretching vibration of -OH and -NH group (Amide-A). The peak fell to 3000-2800 assigned to the stretching of the C-H bond (Amide-III). The weak transmission bond between 1500-1300 represented the –N-H bending and – C-H stretching of bonds. Peaks at 799, 1086, 1043 cm-1 ranging between 1100-750 cm1 showed S=O weak bond stretching represented cysteine (Sharma et al., 2016; Sharma et al., 2017; Pavia et al., 2008). All these bonds confirm the presence of keratin extracted from feather wastes.

The plant microbial interaction experiments were carried out to check the efficacy of isolates in germination on the seed of *Triticum aestivum* and *Zea mays*. The root, shoot and plant lengths were analyzed. Seedlings that were given isolates showed an increase in the root, shoot and plant lengths significantly as compared to the control treatment. So, there is a general trend of increase of the root, shoot, and plant lengths of both plants with few exceptions that were less than the control treatment. For *Triticum aestivum* IM2 and IM6 we observed an increase in root length and plant length, while IM6 and IM8 showed an increase in shoot length. For *Zea mays* IM2, IM5, IM6 showed an increase in root length, while IM3, IM4, IM6, IM8 showed an increase in shoot length and IM2, IM6, IM8 showed an increase in plant length.

Plants help to stimulate the survival and action of bacteria, which results in the degradation of pollutants such as chicken feathers. The root system of bacteria helps bacteria to penetrate the soil so they can upgrade the quality of the soil itself. The inoculation of degrading bacteria on the seeds of plants can improve the efficacy of keratin degradation from feathers which

itself helps in plant growth (Kuiper et al., 2004). *Pseudomonas aeruginosa* strain NJ-15 has exhibited the potential to assist plant growth. Gram-negative Pseudomonas spp. is a disease suppressive agent in a moist environment (Bano and Musarrat, 2003; Sitaraman, 2015).

Keratin as fertilizer, at 10x, 20x, 30x, 50x, and 70x, showed positive results for *Triticum aestivum.* All the factors including root lengths, shoot lengths and plant lengths increased significantly. In the case of *Zea mays*, there was a general increase in all the factors including root lengths, shoot lengths, and plant lengths, but keratin as fertilizer, at 20x and 70x, showed positive results and a significant enhancement in length of root. 20x revealed an enhancement in the length of shoot significantly, and 20x, 50x, and 70x showed an increase in the plant lengths significantly.

In *Triticum aestivum*, 20x, 30x, 50x and 70x dilutions showed significant fresh weight increase as compared to that of control, while 50x and 70x dilution showed a significant increase in dry weight in comparison with the control value. In *Zea mays*, all the dilutions i.e., 10x, 20x, 30x, 50x, and 70x showed increase in fresh weight, while 20x dilution showed a sufficient increase in dry weight in comparison to that of control value. In future, the centrifugation of keratin hydrolysate will ensure the remark of viable cells in hydrolysate that will ensure mitigation of any bio-risks.

Keratin from feathers was over 90 % extracted in an aqueous solution. It was observed that the coated fertilizers had increased nitrogen content, compared to the untreated sample. The first fertilizer with keratin coating released the highest N content, 0.3 %, almost three times higher than the uncoated fertilizer. Mihăilă et al., 2020; Berechet et al., (2020) used keratin hydrolysate as organic fertilizer. The results showed that 5 % keratin led to the increase of the wheat stem lengths by 10.7 % compared to the control treatment. The future implication of this study indicate the potential utilization of keratin-degrading bacteria for the degradation of keratin and the recycling of poultry feather waste into value-added products, which could be used as fertilizers.

CONCLUSION

It was concluded in the study that poultry farms are a rich source of indigenous bacteria that are involved in the biodegradation of keratin. There is a need to introduce keratin-degrading hdrolysate as a value-added product that could be used as fertilizer and can help in the removal of poultry waste from the environment All isolates have the potential to degrade waste but tremendous results were recorded for protein contents (6.52 mg/ml) at 45 ℃ and pH 7 for bacterial isolate IM6 that was later identified as *Pseudomonas aeruginosa* strain by using 16S rRNA sequencing. Fourier transform infrared (FTIR) was performed and confirmed the presence of strong Amide-A, Amide-III, and disulfide bonds in the degraded protein. Moreover, the keratin was used as a fertilizer for plant microbial interaction and a significant increase in the root, shoot and plant lengths of *Triticum aestivum* and *Zea mays* were recorded as compared to control. The future implication of this study shows the high potential of keratin degrading bacteria for the overall improvement of the environment and use as a value-added product for the poultry industry.

CONFLICT OF INTEREST

This paper is from M.Phil thesis research work of Imran Ahmad. Authors declare there is no conflict of interest.

AUTHOR'S CONTRIBUTION

Aisha Waheed Qurashi: Supervisor, planned and supervised the student.

Imran Ahmad: Conducted the research work. Roheela Yasmeen: Facilitate in paper write up,, and submission.

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