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Taxonomic Studies of the Keratinase-Producing Isolate *Streptomycetes matensis* (MPLS-1)

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ABSTRACT

The actinomycetes were identified and characterized earlier in our laboratory, and used for the optimization of keratinase production. Production of keratinase (EC 3.4.4.25) by *Streptomyces matensis* PLS-1 optimized, which has importance in recycling wastes. The micro-morphology of the culture shows that the spore chain morphology appeared to be extended, flexuous, and belongs to the section "Rectiflexibiles" according to the *Streptomyces* classification. The spore surface is smooth while the spore mass is white. Mature spore chains are short with less than 10 spores per chain. The size of each elliptical spore was 1.26-1.75µm. The most important characteristics of the isolate MPLS-1 were summarized as follows: It grew well on all media. The aerial mycelium developed abundantly on all media and it was greyish white in color in all media (front side); on the reverseside, it was light brown in color on yeast extract-malt extract agar medium (ISP-2), nutrient agar medium, starch-casein agar medium and dark brown on Oatmeal agar (ISP-3) and Bennets medium**.** The evolutionary history was suggested using the Neighbor-Joining method. The tree is depicted to scale, with branch lengths measured in evolutionary distances that were utilized to determine the phylogenetic tree. The evolutionary distances were calculated with the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. technique (Kimura, 1980). A detailed survey of the literature indicated that, among *Streptomyces* species compared to our isolate MPLS-1 is related to *S. matensis*. The isolate MPLS-1 was sent to the Institute of Microbial Technology (IMTECH) for identification and it was identified as 94% similar to *Streptomyces matensis*. According to a recent review, there was no report of *Streptomyces matensis* which can produce keratinase from poultry litter soil in the Indian Peninsula.

INTRODUCTION:

Microbial keratinases target the hydrolysis of highly inflexible, strongly cross-linked structural polypeptides called "keratin," which are resistant to the widely used proteolysis enzymes trypsin, pepsin, and papain, they have gained significant biotechnological importance. When these substrates break down, keratinous materials such as hair, feathers, wool, nails, horns, etc. are formed in large quantities. Sulfitolytic and proteolytic systems work together to facilitate the intricate process of keratinloysis. The majority of keratinases are serine or metallo proteases, which are strong enzymes with a broad temperature and pH activity range. Microbial keratinases have become biotechnologically important since they target the hydrolysis ofhighly rigid, strongly cross-linked structural polypeptide “keratin” recalcitrant to the commonly known proteolysis enzymes trypsin, pepsin and papain. These enzymes are largely produced in the presence of keratinous substrates in the form of hair, feathers, wool, nail, horn etc. during their degradation. The complex mechanism of keratinloysis involves cooperative action of sulfitolytic and proteolytic systems. Keratinases are robust enzymes with a wide temperature and pH activity range and are largely serine or metallo proteases.

The keratinous wastes largely comprised of the insoluble structural protein “keratin” is increasingly accumulating in the environment mainly from various industries. Today, it is also becoming a part of solid waste management since it is tough to degrade due to the highly rigid structure rendered by extensive disulfide bonds and cross-linkages. Hence, there is a demand for developing biotechnological alternatives for recycling of such wastes. Despite the recalcitrance, keratin wastes can be efficiently degraded by a myriad of bacteria, actinomycetes and fungi due to the elaboration of keratinolytic proteases – keratinases. [8]

Identification and characterization of actinomycete isolate MPLS-1:

Identification and characterization of microorganisms plays a key role as it expands the scope for industrially important products. In the present investigation, criteria laid down by the International Streptomyces Project (ISP) were followed for the identification and characterization of the selected isolate.

To establish the novelty or otherwise of the present isolates, the various morphological, cultural and biochemical characteristics of the isolated organisms were compared with the descriptions of the numerous species of *Streptomyces* species cited in the literature.

This literature survey includes Bergey's Manual of Systematic bacteriology[23], Bergey's Manual of Determinative Bacteriology [2], the actinomycetes (Vol II) [19]. The International Streptomyces Project Report (ISP) [14][15][16][17] and the information collected from the following journals: *Journal of General Microbiology*, *Journal of Bacteriology*, *The Journal of Antibiotics (Japan)*, *International Journal of Systemic Bacteriology*, *Hindustan Antibiotics Bulletin*, *Indian Journal of Microbiology*, *Journal of Biotechnology and Bioengineering*, *Biological Microbiological abstracts and Chemical abstracts*.

The detailed methods used for taxonomic characterization of the isolate MPLS-1 was carried out and the results are discussed.

METHODS

Selection of media for growth:

Cultural media used for the characterization and identification of species consists of both synthetic and organic forms. Organic media are utilized to gather further cultural data, while synthetic media are widely used in the research of the morphology, physiology, and cultural characteristics of the organism.

Media used for characterization:

Waksman [18] and others recommended the inclusion of the following media for the characterization of actinomycetes:

1. At least three synthetic media, preferably sucrose nitrate salt agar or sucrose ammonium salt agar, glucose or glycerol asparagine agar, and malate or citrate agar.

2. Two or three organic media such as nutrient agar, yeast extract malt extract agar, potato glycerol glutamate agar or oat meal agar.

3. Three or four complex natural media such as potato plug, gelatin and milk.

4. Peptone iron yeast extract for H_2S production.

5. Tyrosine medium for tyrosine reaction.

6. A synthetic medium for carbohydrate utilization.

Experimental procedure:

In the present work, morphological studies and color determination of the selected isolates were determined by following the International Streptomyces Project (ISP) procedures of Shirling and Gottlieb [17].

The following media as recommended by ISP were used for morphological studies and color determinations for the isolate MPLS-1 asYeast extract malt extract agar (ISP-2),Oat meal agar (ISP-3), Inorganic salts- starch agar medium (ISP-4), Glycerol- asparagine agar medium (ISP-5). Special morphological features like spore morphology and spore surface ornamentation were determined by scanning electron microscope.

Medium used: Yeast extract- malt extract agar (ISP-2)

Method used: Coverslip method [21]

Further, the following biochemical tests and others were determined by employing the prescribed media for the isolate MPLS-1 such as Melanin formation, H_2S production, Tyrosine reaction, Gelatin hydrolysis [23] [5], Coagulation and peptonization of milk [12], Starch hydrolysis [12], Nitrate reduction [12] and Cell wall composition.

Preparation of inoculum:

In general, agar media which favor abundant sporulation are those with a high C/N ratio such as jowar starch agar, oat meal agar (ISP-3) and starch-casein agar medium.

In the present study, yeast extract malt extract agar was used for isolation. These slants were inoculated from the stock cultures and incubated at 28 °C for 7-10 days to get maximum sporulation. Spore suspension was prepared for transferring loop of spores from these slants into sterile distilled water and shaking thoroughly. For gelatin liquefaction, starch hydrolysis and casein hydrolysis, a loop full of spores taken from the stock cultures was used for inoculation. For all the other tests, spore suspensions prepared as above were used employing equal volumes of the suspension in each case.

Morphological and cultural studies:

The color of aerial mycelium, substrate mycelium and soluble pigment when grown on different media were observed and recorded. The macro and micromorphological features of the colonies and the color determination of the aerial mycelium, substrate and soluble

pigment were examined every 24 h for 7-14 days of incubation. Macro morphology was noted by the naked eye and observation with magnifying lens.

Micro morphology (spore morphology):

To study the aerial mycelium and its sporulation characteristics, the following two methods were used.

A. Direct method:

Very thin layers of the respective solidified media in petri plate were inoculated each with 0.05 mL of the spore suspension. This was placed near the edge of the plate to serve as a pool of inoculum. Using a sterile loop, four or five equally spaced streaks were made. A number of plates were inoculated in this manner to facilitate observations on different days. Observations were recorded from next day onwards up to 14 day.

Inclined cover slip method: [22]

Sterile coverslips were placed at an angle of 45° into solidifying agar medium in petri plate such that a part of the cover slip was in the medium. The inoculum was dispersed at the edge where the medium and cover slip's upper surfaces met. Following complete sporulation, the cover slips were taken out and closely inspected under a microscope.

Scanning electron microscopy for isolate MPLS-1:

For microscopic studies cells of the isolate MPLS-1 were transferred each to vials and fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C and post-fixed in 2% in aqueous osmium tetroxide for 4 h and dehydrated in series of graded alcohols and dried to critical point drying with CPD unit. The processed sample was mounted over the stubs with double-sided carbon conductivity tapes. A thin layer of gold coat was applied over the samples using an automated sputter coater (Model- JOEL- JFC- 1600) and observed under scanning electron microscope (SEM- Model- JOEL- JSM- 5600) at the necessary magnifications in accordance with protocol. The scanning electron microscope was provided by RUSKA Laboratory's College of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India.

Physiological and biochemical characterization:

A. Gram staining:

Smear of the isolate MPLS-1 was stained by Gram's stain technique and observed under microscope.

B. Effect of temperature on growth:

The ability of the isolates to grow at different temperatures were studied at 10°C, 15°C, 20 °C, 28 °C, 37 °C, 42 °C and 50 °C. Selected isolates were streaked on starch-casein agar slants and incubated at different temperatures as mentioned above [20].

C. Biochemical characterization:

The following biochemical tests were performed for the selected isolate MPLS-1 using the prescribed media.

1. Melanin production: [13]

The chromogenicity is so significant that it has long been regarded as an important characteristic for the identification and classification of *Streptomyces*. Melanin is a dark pigment produced by the organisms on tyrosine-containing medium and other proteinaceous media.

The following ISP media were used for testing melanin production.

A. Tryptone yeast extract broth (ISP-1)

B. Yeast extract iron agar (ISP-6)

C. Tyrosine agar (ISP-7)

Freshly sub-cultured and well sporulated MPLS-1 isolate was inoculated on media mentioned above and incubated at 28 °C for 6-7 days. Using uninoculated batch of the media as a control, color change was observed after 2 days and 4 days for production of melanin. Greenish brown, brown to black diffusible pigments was considered as melanin positive and the absence of brown to black color or total absence of diffusible pigment was considered as melanin negative.

2. Hydrogen sulphide production:

Hydrogen sulphide may be produced by the reduction of organic sulphur present in the amino-acids (cysteine and methionine) or by the reduction of inorganic sulphur compounds [thiosulfates ($S_2O_3^{2-}$), sulfates (SO_4^{2-}), or sulfites (SO_3^{2-})]. Therefore, the hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}), or lead (Pb^{2+}) ion as hydrogen sulfide indicator to a nutritive culture medium with sodium thiosulphate and cysteine acting as the sulfur substrates.

This was carried out by stabbing of the test isolate MPLS-1 on peptone yeast extract iron agar slant (ISP-6) and incubated at 28 °C for 7 days. Following incubation, observation was made at 2^{nd}, 4^{th} and 7^{th} day. The presence of greenish brown, bluish black or black coloring along the line of stab was recorded as positive. The inoculated tube was compared with un-inoculated control.

3. Tyrosine production:

This test was performed on ISP-7 medium slants. The inoculated tubes were incubated at 28 °C and observations were recorded on 2^{nd} and 4^{th} day. Isolate which showed greenish-brown, brown, brown to black, black were considered as positive.

4. Gelatin hydrolysis:

Gelatin as a nutritional source is questionable (it is an incomplete protein lacking the essential amino acid tryptophan); its value in identifying bacterial species is well established. Gelatin is a protein produced by the hydrolysis of collagen, a major component of connective tissue and tendons in humans and other animals. Microorganisms can produce gelatinase that can hydrolyze gelatin to amino acids.

This test represents the ability of microorganisms to hydrolyze gelatin. It was carried out by streaking the isolate MPLS-1 on gelatin agar and incubation at 28 °C for 7 days. Following incubation, the plates were flooded with 10 mL of mercuric chloride solution and observed zone of hydrolysis [5].

Mercuric chloride solution was prepared as follows:

Mercuric chloride	15.0 g
Conc. HCl	20.0 g

Distilled water up to	1000 mL

The extent of the hydrolysis was noted comparing the width of the clear zone around the growth. Measured and noted was the difference between the growth's width and the width of the hydrolyzed zone surrounding it.

5. Peptonization and coagulation of milk:

Milk coagulation and peptonization test were carried out with skim milk. The skim milk tubes were inoculated with MPLS-1 each and incubated at 28 °C for 7 days. The extent of coagulation and peptonization was recorded on 3rd and 7th day [12].

6. Casein hydrolysis:

Proteins that are connected by peptide bonds to form amino acid subunits make up casein, the main protein found in milk. Before their assimilation into the cell, proteins must undergo step-by-step degradation into peptones, polypeptides, dipeptides and finally to amino acids. This process is called peptonization or proteolysis, and it is mediated by extracellular enzymes called proteases.

In this experiment procedure, the proteolytic activity was studied using milk casein agar (g/L: Peptone 1.0, sterile skimmed milk 100, agar 20.0). Test isolate MPLS-1 was streaked and incubated at 28 °C for 7 days. Following incubation organisms secreting protease enzyme will exhibit a zone of proteolysis, which is demonstrated by a clear zone surrounding the bacterial growth. This lack of opacity is indicative of a positive reaction that occurs from a hydrolytic process that produces soluble, non-colloidal amino acidsIn the absence of protease activity, the medium surrounding the growth of the organism remains opaque, which is negative reaction; the width of the hydrolyzed zone around the growth versus the width of growth was measured and recorded [12].

7. Starch hydrolysis:

Glycosidic linkages bind glucose molecules together to form starch, a high molecular weight, branching polymer. The degradation of this molecule first requires the presence of the extracellular enzyme amylase for its hydrolysis into shorter polysaccharides, namely dextrins, and ultimately into maltose molecules. The final hydrolysis of this disaccharide, which is catalyzed by maltose, yields low molecular weight, soluble glucose molecules that can be transported into the cell and used for energy production through the process of glycolysis.

Test isolate MPLS-1 each were streaked on starch agar plates and incubated at 28 °C for 7 days. Then the plates were flooded with an iodine solution. Starch in the presence of iodine will impart a blue-black colour to the medium indicating the absence of starch splitting enzymes and representing a negative result. However, the presence of clear colorless zone surrounding the growth of the organism represents a positive result for starch hydrolysis. The width of the hydrolyzed zone around the growth versus the width of growth was measured and recorded [12].

8. Nitrate reduction test:

A loopful of test isolate MPLS-1 were inoculated into 5 mL of nitrate broth and incubated as a control. Following 7 days of incubation of the culture, an organism's ability to reduce nitrates to nitrites was determined by the addition of two reagents: Solution A, which is sulfanilic acid, followed by Solution B, which is α-naphthylamine. Following reduction, the addition of solution A and B will produce an immediate cherry red color which is recorded as positive for nitrate reduction. Culture not producing color change can be due to one of the following two reasons: - (i) nitrites were not reduced by the organisms, or (ii) Nitrates were quickly reduced by the organism's powerful nitrate reductase enzymes from nitrites to ammonia or even molecular nitrogen. To confirm whether or not nitrates were reduced past the nitrite stage, a small amount of zinc powder was added to the colourless cultures. Zinc reduces nitrates to nitrites. The development of red colour therefore verifies that nitrates were not reduced to nitrites by the test isolates. This result is therefore, recorded as nitrate reduction negative. However, if the addition of zinc does not produce a color change, the nitrates in the medium were reduced beyond nitrites to ammonia or nitrogen gas. This was recorded as positive for nitrate reduction [3].

Reagents: Solution A: α-Naphthylamine test solution

α-naphthylamine	5.0 g
Conc. H_2SO_4	8.0 mL
Distilled water	1000.0 mL

α-Naphthylamine was added to the diluted sulphuric acid and stirred until the solution effected.

Solution B:	Sulphanilic acid	8.0.g
	Conc. H_2SO_4	48.0 mL
	Distilled water	1000 mL

A 500 mL solution of sulfuric acid was added. The Sulphanilic acid was added, followed by water to make the fluid volume.

9. Cell wall composition:

The chemical composition of cell wall has been accepted as a criterion for the classification of aerobic actinomycetes [7]. Therefore, the cell wall composition; amino-acids and whole cell sugar patterns of the isolate MPLS-1 were determined by the following methods [1] [7].

Procedure:

Isolate MPLS-1 were sub-cultured in yeast extract malt extract agar (ISP-2) each and incubated at 28 °C for 7 days. Well sporulated slants were aseptically transferred using 5 mL sterile distilled water into 75 mL of yeast extract malt extract broth containing in 250 mL Erlenmeyer flask. Flask was kept for incubation, on a rotary shaker at 28 °C for 7 days. Culture was centrifuged and the supernatant was discarded while the cell mass was washed 3 times with distilled water and each sample was divided into screw-capped tubes. One of the tubes is for amino acids, the other for whole sugar analysis.

Amino acid analysis:

Five mL of 6 N HCl was added to each sample. These samples were placed in tightly screw capped tubes at 100 °C in a boiling water bath for 2 h. The liquid hydrolysates were filtered and the solid material on the filter paper was washed with 3 drops of distilled water. The liquid hydrolysate was transferred to watch glass and dried for 3 consecutive times on a steam bath to remove HCl. Samples were dissolved in 0.3mL of distilled water for chromatography. The solvent system used for amino acids is n-butanol: acetic acid: water (4:1:5) and descending chromatography was followed. Developing reagent used for amino acid analysis was 0.2% w/v ninhydrin in 95% ethanol sprayed followed by heating for 5 min at 100 °C. The developed spots were compared with meso and L-DAP and glycine was taken as standards.

Sugar analysis:

Five ml of 2 N HCl was added into each sample. Samples were tightly capped and placed at 100 °C in a boiling water bath for 2 h. They were then dried as described above. The solvent system used for sugar analysis was n-butanol: pyridine: water: toluene (5:3:3:4) and descending chromatography was used. A developing reagent for sugar analysis was taken, 0.1 mL of aniline in 100 mL of 0.1 N oxalic acid. After spraying paper was dried at 65 °C for 2-5 min and spot was compared with standards.

Molecular identification of the isolate MPLS-1:

Molecular identification and characterization of the isolates MPLS-1 were carried by 16S rRNA gene sequencing analysis of *Streptomyces* taxonomy to investigate the relationships at genus, species and strain level. This was performed at the Institute of Microbial Technology (IMTECH), Chandigarh.

Enzymatic assay of keratinase:

About 20 mg of chicken feather powder was suspended in 4 mL of 0.05 M glycine/NaOH buffer pH 10.09 to which 1 mL of culture filtrate was added. The reaction mixture was incubated at 60 °C for 1h. After incubation, the reaction was terminated by adding 4 mL of 5% trichloroacetic acid. The feather and insoluble residue were removed by filtration and the filtrate was centrifuged at 3000 rpm for 5 min. Proteolytic products in the supernatant were determined by reading the absorbance at 280 nm. A control sample was prepared by adding TCA to the reaction mixture before incubation [4] [10].

Unit definition: Under the specified test conditions, one unit of keratinase activity was defined as the amount of enzyme needed to cause an absorbance rise of 0.01 [4].

RESULTS AND DISCUSSION

Identification of the Isolate MPLS-1:

Micro morphology:

Data on the micro-morphology of the culture shows that the spore chain morphology appeared to be extended, flexuous, and belongs to the section “Rectiflexibiles” according to the *Streptomyces* classification by Pridham *et al*., 1956-1957. The data on cultural characteristics, physiological and biochemical properties are given in Tables 1 and 2. The

scanning electron microscopic images of the isolate MPLS-1 show that the spore surface is smooth while the spore mass is white. Mature spore chains are short with less than 10 spores per chain. The size of each elliptical spore was 1.26-1.75µm.

Table 1: Cultural characteristics of the isolate MPLS-1.

Agar medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Starch-casein agar	Abundant, spreading, powdery	grey or white	Light greyish brown	None
Yeast extract-malt extract agar (ISP-2)	Abundant, spreading, powdery	grey or white	Light brown	None
Oatmeal agar (ISP-3)	Abundant, spreading, powdery	light grey	White/grey	None
Inorganic salt starch agar (ISP-4)	Moderate spreading, powdery	grey or white	Light greyish brown	None
Glycerol aspargine agar (ISP-5)	Moderate spreading, powdery	white	White	None
Nutrient agar	Abundant, spreading, powdery	light grey	Light greyish brown	None
Bennets agar	Abundant, spreading, powdery	white	Grey/brown	None

The most important characteristics of the isolate MPLS-1 were summarized as follows: It grew well on all media. The aerial mycelium developed abundantly on all media and it was greyish white in color in all media (front side); on the reverse side it was light brown in color on yeast extract-malt extract agar medium (ISP-2), nutrient agar medium, starch-casein agar medium and dark brown on Oat meal agar (ISP-3) and Bennets medium**.** (Table 1; Fig 1 and 2).

Fig 1: Growth of the isolate MPLS-1 on Starch-casein agar medium-front side.

Fig 2: Growth of the isolate MPLS-1 on Starch-casein agar medium-reverse side.

Table 2: Physiological and biochemical characteristics of the isolate MPLS-1.

S.No.	Character	Observation	Result
1	Melanin production: ISP-1 ISP-6 ISP-7	 Red/violet color Red/violet color Red/violet color	 Positive Positive Positive
2	Nitrate reduction	Cherry red was observed	Positive
3	Starch hydrolysis	Hydrolyzed zone was observed	Positive
4	Casein hydrolysis	Hydrolyzed zone was observed	Positive
5	Gelatin liquefaction	No hydrolyzed zone	Negative
6	Hydrogen sulfide (H_2S) production	No Browning of the medium	Negative
7	Tyrosine production	No Browning of the medium	Negative
8	Citrate utilization	Prussian blue was not observed	Negative
9	Methyl red test (MR)	No reddish brown was observed	Negative
10	Voges-prauskouer (VP)	The red colour was not observed	Negative
11	Indole production	No red or pink coloured ring	Negative
12	Oxidase utilization	Blue color was observed	Positive
13	Urea	No deep pink was observed	Negative
14	Cell wall composition	LL-DAP, Glycine, Chemo type-I	Positive
15	Gram staining	Stained to violet colour	G-Positive
16	Spore staining	No staining observed	Negative

Physiological and biochemical properties of isolate MPLS-1 show that red/violet melanin pigment was observed in ISP-1, ISP-6 and ISP-7 (Table 2); the isolate did not create any other soluble pigment and was non-chromogenic, lacking any distinctive diffusible pigment.

Citrate utilization, Methyl red test, Voges-Proskauer, Indole production, Hydrogen sulphide production, Gelatin liquefaction, Tyrosine reaction and urea reaction were not observed. Starch hydrolysis, Casein hydrolysis, Nitrate reduction and oxidase utilization were positive (Table 9.2). Only LL-Diaminopimelic acid and glycine were detected in whole cell hydrolysates, no diagnostic sugars were present, indicating MPLS-1 has a chemo type I cell wall and can be grouped under the genus *Streptomyces*.

Molecular identification and characterization of the isolate MPLS-1 based on 16 S rRNA sequence:

ACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGT
GAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGG
TCTAATACCGGATACTGATCNTCTTNGGCATCNNRGNTGNTCGAAAGCTCCGGCG
GTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAATGGCTCACCAA
GGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGA
AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCT
CTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA
TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGG
CTTACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATC
GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGC
GAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCG
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGGTGT
GGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGG
GGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
CGGCCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTT
GACATACACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GCGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCCTTGTGGTGCTGGGGACTCA
CGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCA
TGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGC
GATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGT
CTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCT
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCG

GTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTG GGACTGGCGATTGGGACGAAGTCGT.

Evolutionary relationships of strain with the closely related taxa:

The Neighbor-Joining approach was used to infer the evolutionary history [10]. With branch lengths expressed in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is depicted to scale. The evolutionary distances were computed using the Kimura 2-parameter method [6] and are in the units of the number of base substitutions per site. A gamma distribution was used to model the rate variation between locations (shape parameter = 1). Gene bank accession number for all the type strains considered in the analysis.

The isolate PLS-1 was sent to the Institute of Microbial Technology (IMTECH) for identification and it was identified as 94% similar to *Streptomyces matensis*.

Table 3: Production of keratinase by various isolates from poultry litter soil (PLS)

S.No.	Isolate	Keratinase activity (IU/mL)
1.	**PLS-1**	**36.0**
2.	PLS-2	8.0
3.	PLS-3	10.0
4.	PLS-4	6.4
5.	PLS-5	6.3
6.	PLS-6	5.2
7.	PLS-7	4.5

Out of all the isolates, PLS-1 showed highest keratinolytic activity (36 IU/mL) as shown in Table 3.

Conclusion:

The objective of the present study was to identify and characterize the keratinase-producing actinomycetes from chicken feathers, since it is rich in keratin. The keratinolytic Streptomyces matensis (PLS-1) has the potential to be a viable option for both the dehairing process in the leather industry and the keratin degradation of feathers. This isolate could be successfully employed in the large-scale commercial synthesis of enzymes and could be used to generate keratinase for biotechnological applications.

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