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Isolation of Keratinolytic Microorganism *Streptomyces chartreusis strain ISP 5085* from the Soil Sample: Characterization, Degradation and Microbial Identification using 16S rDNA Region

Jitendra Nayak[1] | Bhikhu More[2]

[1,2]Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.

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ABSTRACT

Keratinolytic microorganisms have a broad significance in feather waste degradation and it can be used for advancement of livestock feed and production of protein hydrolysates. Production of keratin into the environment in different forms is very high which leads to setback for its degradation. An approach for degradation of keratin based wastes through potential microorganism with higher efficiency can be attained. The study shows that maximum DFD% i.e. Degree of Feather (keratin) Degradation, through the isolates is achieved, when nutrient medium is kept at temperature 30 °C and pH-8.5 supplemented with dehydrated powdered form of chicken feathers as keratin source for 72 h – 120 h. Chicken feathers acts as a better substrate and source of keratin content. A molecular approach was necessary therefore; phylogenetic trees of the isolate were constructed with neighbour-joining method through the Microbial Identification using 16S rDNA Region, which showed that sequence exhibited a high level of homology with Streptomyces chartreusis strain ISP 5085. The present study deals with identification of isolates that play a significant role in the degradation of chicken feather waste as well as keratin degradation ability of the isolates and optimum conditions required for it.

KEYWORDS: *Keratinolytic microorganisms, Keratin, DFD%, 16S rDNA Region, Streptomyces chartreusis strain ISP 5085.*

INTRODUCTION

Annually several thousand tons of feather wastes are discharged into the surrounding environment as a by-product from commercial poultry processing industries which leads to many problems as they are affecting public health and the environment. Microorganisms could minimize regulatory problems of uncontrolled collection of feathers waste. This residue is almost pure keratin, which is not easily degradable by common proteolytic enzymes.[1]

Biodegradation processes of chicken feathers with the help of poultry farm isolates were noticed. Among the various microbial strainswith the best keratin degradation efficiency of 100% within 1 to 7 days are: *Bacillus sp., Kocuria sp., Pseudomonas sp.* and *Fervidobacterium sp.* Nowadays, certain microbial consortia or modified strains of microbes

are also used to enhance the efficiency and profitability of biodegradation.[2]

The amino acid composition of keratins from different avian species is almost similar. Keratins are the insoluble structural proteins from feathers, wool, hooves, scales, hair, nails etc. These proteins belong to the scleropeptides group. The tight packing of keratin chain in the α-helix (α-keratin) or β-sheet (β-keratin) into a super coiled polypeptide chain results in mechanical stability and resistance to proteolysis. In addition, cross linking of protein chains by cysteine bridges, di-sulfide and hydrogen bonds as well as salt linkages confer high mechanical stability and resistance to digestive enzymes such as trypsin and pepsin or other enzymes. Therefore, feather waste is utilized on limited basis as a dietary protein supplement for animal feedstuff. The degradation of keratinous material is pertinent medically and agriculturally because viscoelasticity and stiffness are the properties of keratin containing compounds. The use of crude keratinase significantly increases the amino acid digestibility of raw feathers and commercial feather meal.[1]

Feathers hydrolyzed through mechanical or chemical treatment can be converted to different forms for several means such as: feedstuffs, fertilizers, glues and foils or used for the production of amino acids and peptides. The methods of feather degradation include alkali hydrolysis, steam pressure cooking and biodegradation. Due to feathers being almost pure keratin based protein; alkali hydrolysis and steam pressure cooking will not only destroy amino acids but also consume large amounts of energy. Use of microbial keratinolytic enzymes in the production of amino acids and peptides is becoming attractive for biotechnological applications. This enzyme processes is advantageous over commercial methods, only drawback is requirements of large amount of salts, which needs to be separated from the end product. Feathers can be biodegraded to feather meal for animal, slow-release nitrogen fertilizers, glues and films or used for production of rare amino acids like serine, cysteine and proline.[1]

Keratin by virtue of its insolubility and resistance to proteolytic enzymes is not attacked by most living organisms. Keratin is the recalcitrant compound which does not accumulate in nature and therefore, biological degradation may be hypothesized to manage its removal. The common occurrence of microorganisms in nature is preferably due to their utilization capacity of keratinaceous substrates. Some microbes or their process or use of feather wastes can be exploited for better management.[1]

MATERIALS AND METHODOLOGY:

A. Sample collection:

Sample was collected from the "Kinariwala Poultry Farm" located in Olpad, Surat (India). Using a sterile spatula about 5 g surface soils were collected from the site. The sample were placed in a clean polythene bag and brought to laboratory for isolation of isolates for keratin degradation.

B. Collection and processing of feathers:

Fresh feathers were collected and washed with tap water 2-3 times then thoroughly washed with distilled water. Washed feathers were dried at 50°C for 3 h and stored at 4°C in powdered form until used.

C. Isolation of Keratin degrading microoganisms:

For isolation of keratin degrading microorganisms Glucose Aspargine Agar (GAA) supplemented with 0.05 mg/mL of rifampian and 0.05 mg/mL fluconazol to minimize bacterial and fungal growth was used. Asparagine was added after autoclave in the aseptic condition. Plates were incubated at 30 °C.

D. Inoculum Preparation:

The nutrient medium Glucose Asparagine Broth (GAB) was supplemented with Glucose- 10.0 g, Asparagine- 0.5 g, Yeast extract- 0.5 g, K_2HPO_4- 0.5 g, $MgSO_4.7H_2O$- 0.25 g dissolved in 1000 mL distilled water and adjusted to pH 7.2 followed by autoclaving. After autoclaving isolated colony was suspended in the prepared media. Flasks were incubated in incubator at 30°C at 120 rpm for 24-48 h. For fermentation medium the fresh overnight culture was used.

E. Fermentation medium and its Optimization:

The nutrient broth was supplemented with glycerol and powdered dried feather (1g) as substrate and it was used as fermentation medium by adjusting the medium in different flasks to pH 8.0, pH 8.5 and pH 9.0 respectively. The active suspension of 5 mL was inoculated in 100 mL production medium and kept at temperature viz., 30 °C, 37 °C and 45 °C respectively; pH 8.0, pH 8.5 and pH 9.0 as well as incubation period of 72 h – 120 h were selected and flasks were kept at 120 rpm.

F. Determination of keratinase activity:

The keratinase activity was measured by modified method, after 72 h – 120 h of incubation. The cultured broth was filtered and centrifuged at 4°C, 10000 rpm for 10 minutes to obtain crude enzyme of different flasks. The reaction mixture for Absorbance was taken at 660 nm and keratinase activity estimated by standard enzymatic activity formula. The concentration of enzyme was estimated using L-tyrosine standard.

G. Preparation for Standard graph: L-Tyrosine

Stock solution of 1 mg/mL Tyrosine was prepared. Different concentrations ranging from 10-50 µg/mL was prepared from stock solution. To this 5 mL of 0.44 M sodium carbonate buffer and 0.5 mL FC (FolinCiocalteu) reagent was added. Final volume of the different concentration tubes was made up to 10 mL with distilled water. The mixture was incubated at room temperature for 30 minutes. Absorbance was taken at 660 nm. For Blank absorbance, solution was made with distilled water and FC reagent.

H. Determination of Degree of Feather Degradation (DFD):

keratinase activity contained 1 mL of enzyme and 10 mg of powdered feather in 2 mL 0.05 M Tris-HCl buffer having pH 8.5. The reaction mixture was incubated at 37°C for 1 h and the reaction was terminated by adding 1 mL of 10% trichloroacetic acid. The debris was removed by filtration and the

Residual feather was washed, dried and scaled to calculate DFD:

$$DFD\ (\%) = (TF - RF) \times 100/\ TF$$

Where, TF = Total feather

RF = Residual feather

I. 16S rDNA Sequencing of Potential Isolate:

Isolates preserved on Glucose Asparagine Agar plate (as aforementioned supplemented with agar) were sent to Saffron Lifescience, Bilimora, District-Navsari, Gujarat (India) for 16S rDNA sequencing and for homology searching with BLAST; and data was used for confirm identification of the isolate VV1. The phylogenetic tree of VV1 isolate was constructed with neighbour-joining method. Following figure-1 represent the protocol for the Microbial Identification using 16s rDNA Region:

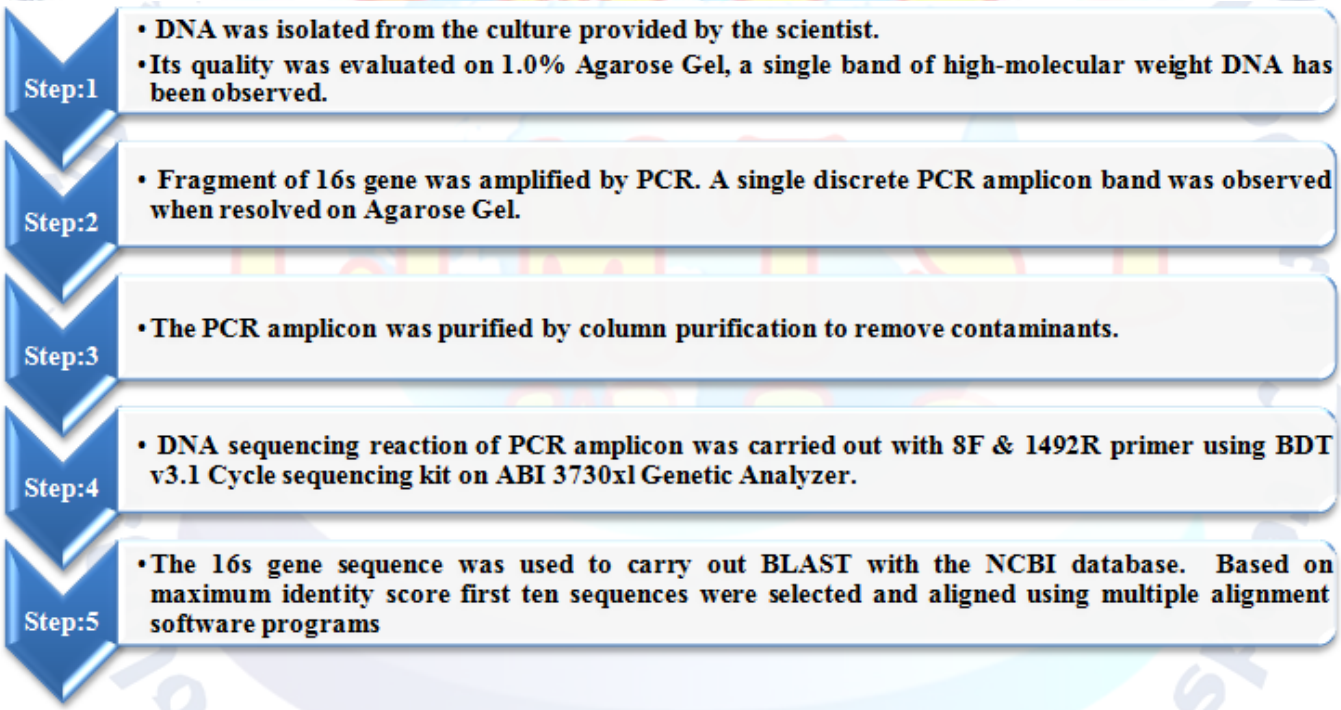


Figure-1 Protocol for the Microbial Identification using 16s rDNA Region

RESULT AND DISCUSSION:

Isolation:

The isolates VV1 isolated from soil sample were collected from the near poultry farm of Surat, Gujarat; by using Glucose Asparagine Agar (GAA). Antifungal (Fluconazole) and Antibacterial (Rifampicin) were used to avoid contamination during the process of isolation of isolates. Isolated colonies were observed for physical morphology on Glucose Asparagine Agar (GAA) plate, Sabouraud Dextrose Agar (SDA) plate, L-Tyrosine Agar plate and Adenine Agar plate which is shown in figure-2(a), 2(b), 2(c), 2(d) respectively.

Figure-2(a) Isolated colonies on Glucose Asparagine Agar (GAA)

Figure-2(b) Isolated colonies on Sabouraud Dextrose Agar (SDA)

Figure-2(c) Isolated colonies on L-Tyrosine Agar

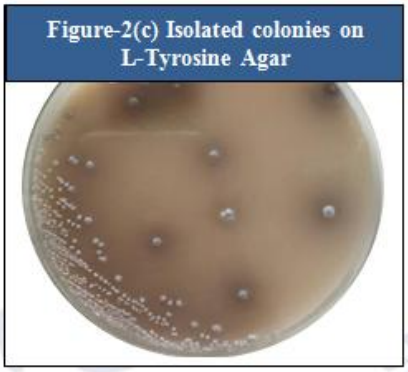
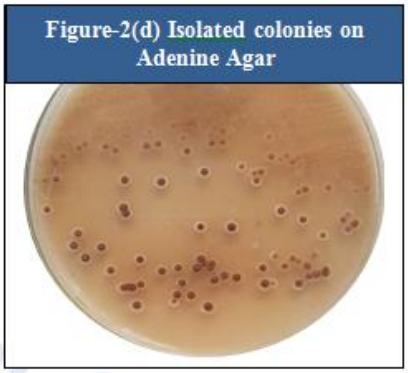


Figure-2(d) Isolated colonies on Adenine Agar



Cultural Characterization:

The isolates VV1 showed distinguishing gross morphologies when cultured on different media through macroscopic appearance for their identification as mentioned in Table-1.

Characteristic	GAA plate	SDA plate	L-Tyrosine plate	Adenine plate
Size	Big	Big	Big	Big
Shape	Round	Round	Round	Round
Edge	Entire	Erose	Erose	Erose
Elevation	Umbonate	Umbonate	Umbonate	Flat
Consistence	Moist	Moist	Moist	Moist
Texture	Smooth	Smooth	Smooth	Smooth
Pigmentation	White	White	White	Dark brown
Opacity	Cretaceous	Opaque	Cretaceous	Opaque

Table-1 Cultural Characteristic of selected isolate organism VV1 on different media

Catalase Test:

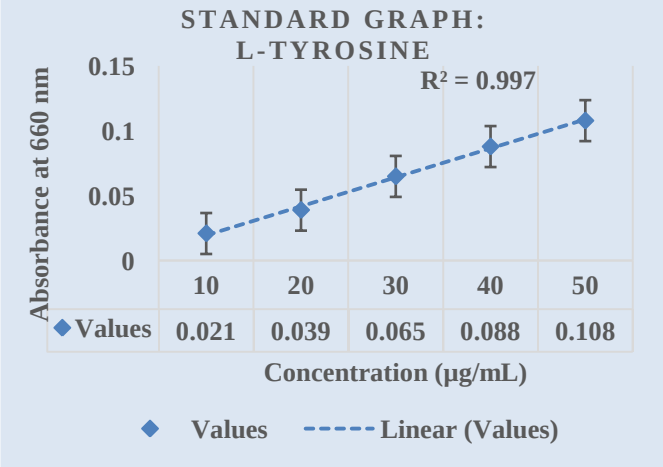
Isolates were checked for their catalase activity as shown in figure-3. So, when 30% H_2O_2 added to the slant containing well developed colonies, bubbles were formed which shows catalase test to be positive.

Figure-3 Catalase test for the selected isolate

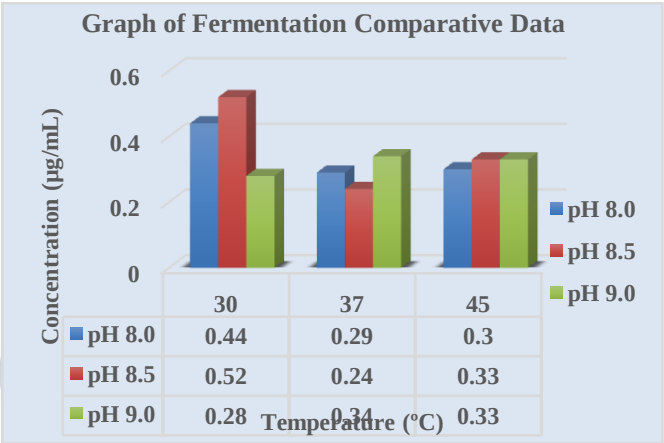
Determination of Degree of Feather Degradation (DFD%) and Keratinase activity:

With the help of standard graph-1 the keratinase activity was estimated, the highest degree of chicken feather degradation (DFD%) of 54.3% with keratinase activity of 0.0042 µmol./min./mL was optimized to be at temperature 30 °C and pH 8.5 kept at 120 rpm for 72 h – 120 h in nutrient medium containing 1% dried powdered chicken

feather as shown in graph-2 and Table-2. The highest crude keratinase content estimated to be 0.52 µg/mL as mentioned in Table-2.



Graph-1 Standard Graph of L-Tyrosine



Graph-2 Fermentation Comparative Data

Table-2 Degree of degradation of feathers and keratinase activity for selected isolate VV1

No.	Temperature (°C)	pH	Turbidity at 400 nm	DFD (%)	Enzyme activity (µmol./min./mL)	Concentration (µg/mL)
1	30	8.0	0.512	48.20%	0.00210	0.44
2	37	8.0	0.222	21.90%	0.00110	0.29
3	45	8.0	0.123	16.65%	0.00160	0.30
4	**30**	**8.5**	**0.873**	**54.30%**	**0.00420**	**0.52**
5	37	8.5	0.225	27.20%	0.00170	0.24
6	45	8.5	0.123	18.40%	0.00140	0.33
7	30	9.0	0.017	25.00%	0.00082	0.28
8	37	9.0	0.014	14.95%	0.00062	0.34
9	45	9.0	0.101	8.95%	0.00059	0.33

A. Fermentation Medium and its Optimization:

It is known that temperature and pH is the most critical parameter which has to be controlled in bioprocess, which greatly affects enzyme production. The effect of different incubation temperature on keratinase production was evaluated and it was found that 30˚C and at pH 8.5 was the most favourable temperature and pH for keratinase production by the isolate VV1 as shown in graph-2 and Table-2.

B. Microbial Gene Finding and Annotation of Potential Isolate:

A molecular approach was necessary to support unambiguous identification of isolate at species level. Classification of gene sequences with accuracy to a distinct genus or species requires analysis with a high-quality, comprehensive reference library. Many useful databases are there like MicroSeq, GenBank, Ribosomal Database Project, Ribosomal Differentiation of Microorganisms, and SmartGene, but each database has distinctive strengths and limitations. For example, GenBank is a large, general database with >200,000 named 16S rDNA sequences, with the constraint of short oversight resulting in reference sequences of poor quality. Microseq(Applied Biosystems) is a popular database, commercially available reference sequence library with ~2000 16S rDNA sequences obtained from different types of microbial strains, which may yield higher-quality sequence data but should be used carefully, because use of a single type strain of microorganism to represent entire taxa is often incompetent.

Nucleotide sequences of microorganisms are usually reported in terms of “parent identity”, which refers that total number of identical nucleotide bases shared by the query and reference sequences divided by the number of nucleotide

bases sequenced; figure-4(a), 4(b), 4(c), 4(d), 4(e), 4(f), and 4(g) represents report generated for the isolate VV1. Based on the results of the physiological, biochemical and the phylogenetic tree derived from 16S rDNA sequences of isolate VV1 is presented in figure-5(c) which classified as that sequence alignment statistics exhibited a high level of homology of 100% identity as shown in figure-5(a) and 5(b) to be ***Streptomyces chartreusis strain ISP 5085***. Therefore, the isolate VV1 was designated as ***Streptomyces chartreusis strain ISP 5085***.

Sample: 0318_734_017_PCR_VV1_8F

AGTAACCAGCCTATAGTGTGAGCCTCTGCTCTCAGAAACATCTTCAGTGGGGACTAGTAGGCGTAACGGGTGAGTAACAC
GTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCCTGTCCTCCTGGACG
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AGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGGCCCGCACAA
ACAGCCGGAGCATGTGGCTTTATTTCGACGCAACGCGAAGAAACCTTACCAAGGCTTGACATACACCCGCAAACGTCTGG
AGACCAGCGCCCCCCTTGTGGTCGGTGTACAGGT

Figure-4(a) 16S rDNA sequence of isolate VV1- *Streptomyces chartreusis strain ISP 5085*.

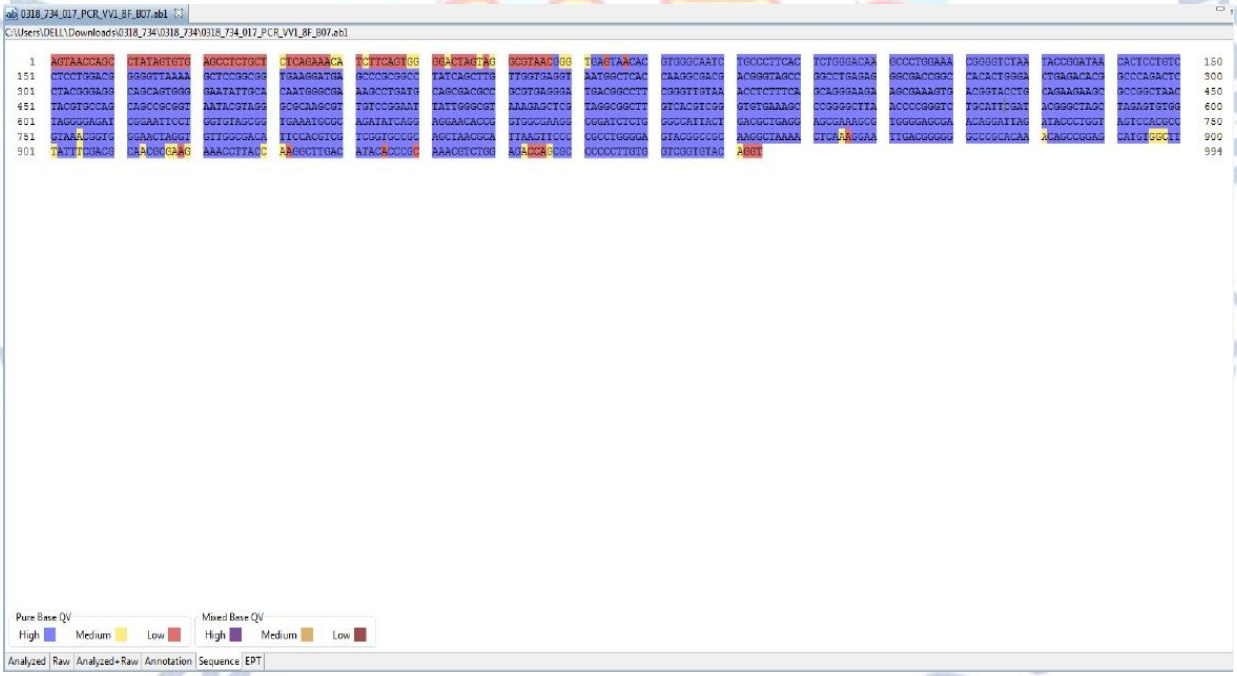

Figure-4(b) Graphical illustration of a MultipleSequence Alignment analysis of isolate VV1

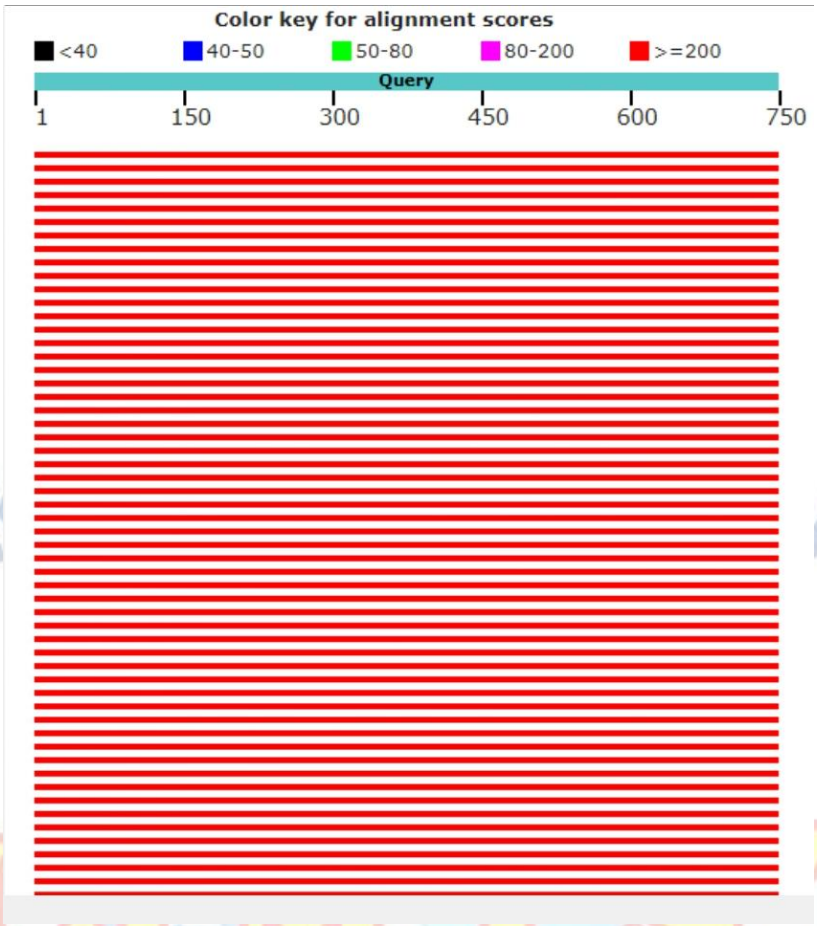

Figure-4(c) Graphical output of the BLAST of isolate VV1 sequence

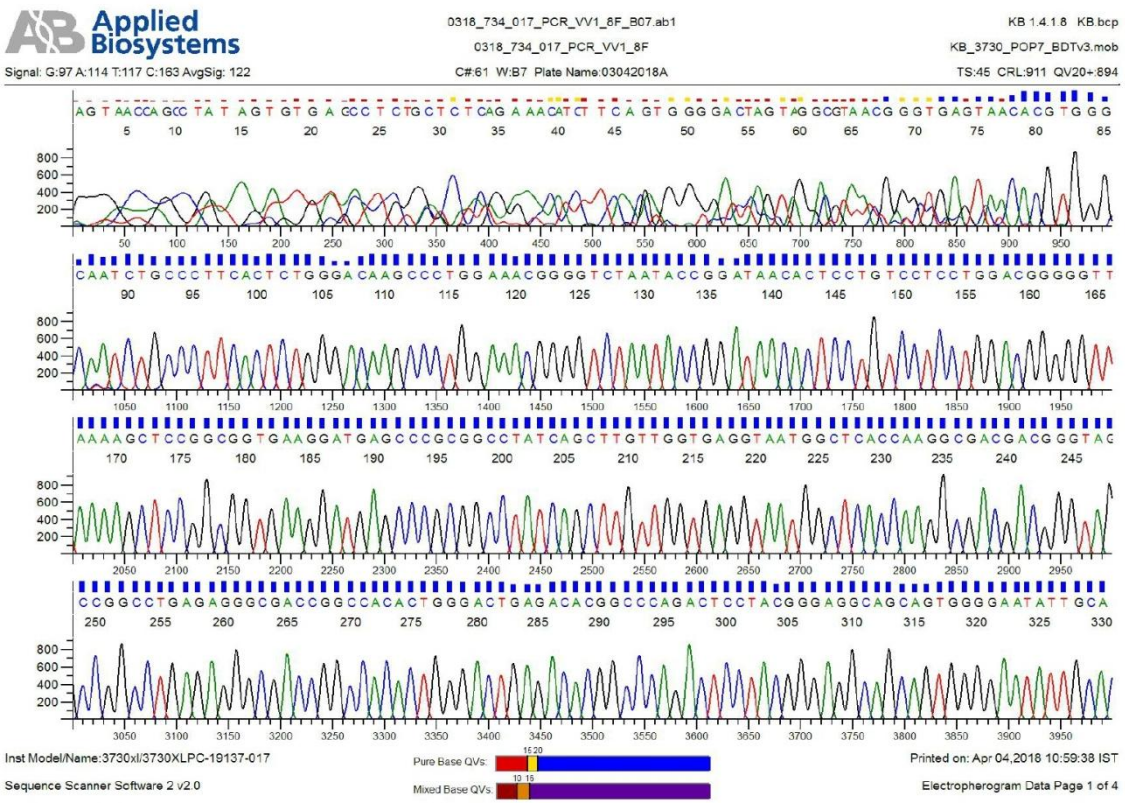

Figure-4(e) Representative Electropherograms Data of 16S rDNA sequence of isolate VV1

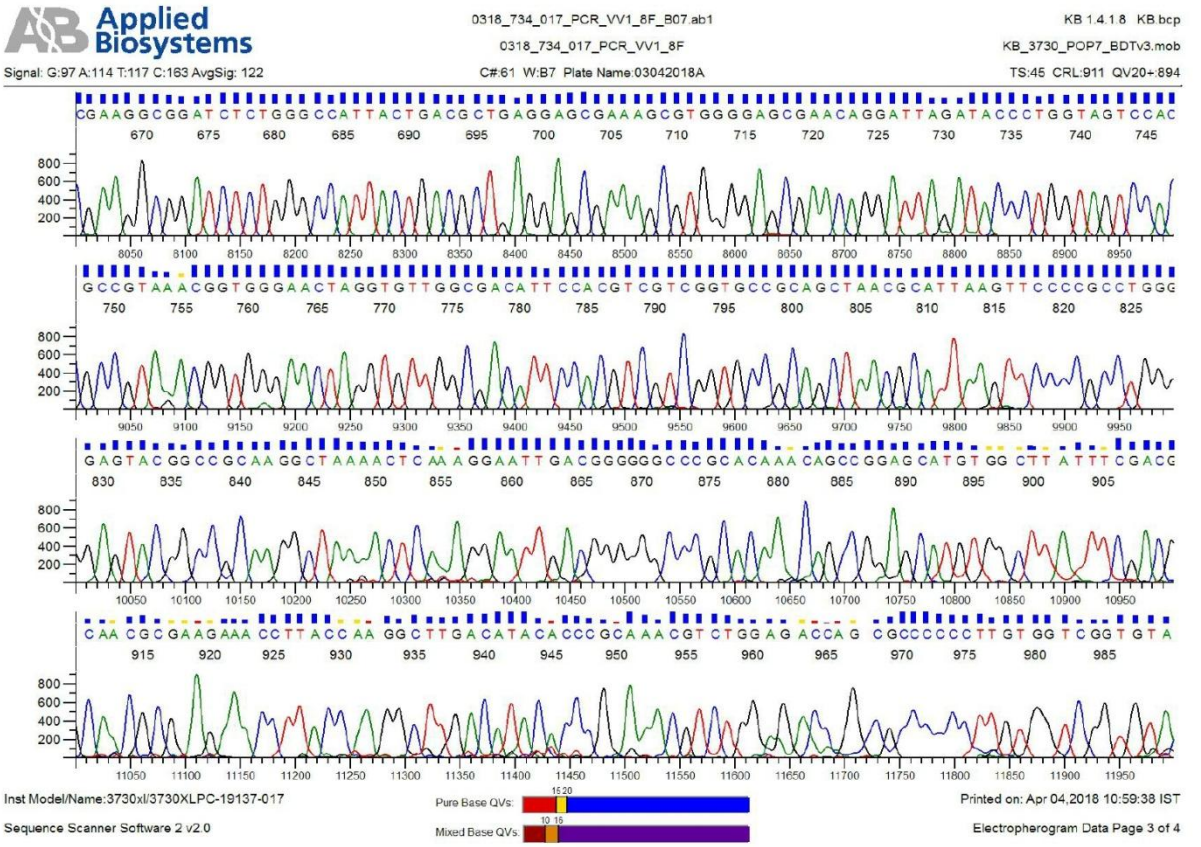

Figure-4(f) Representative Electropherograms Data of 16S rDNA sequence of isolate VV1

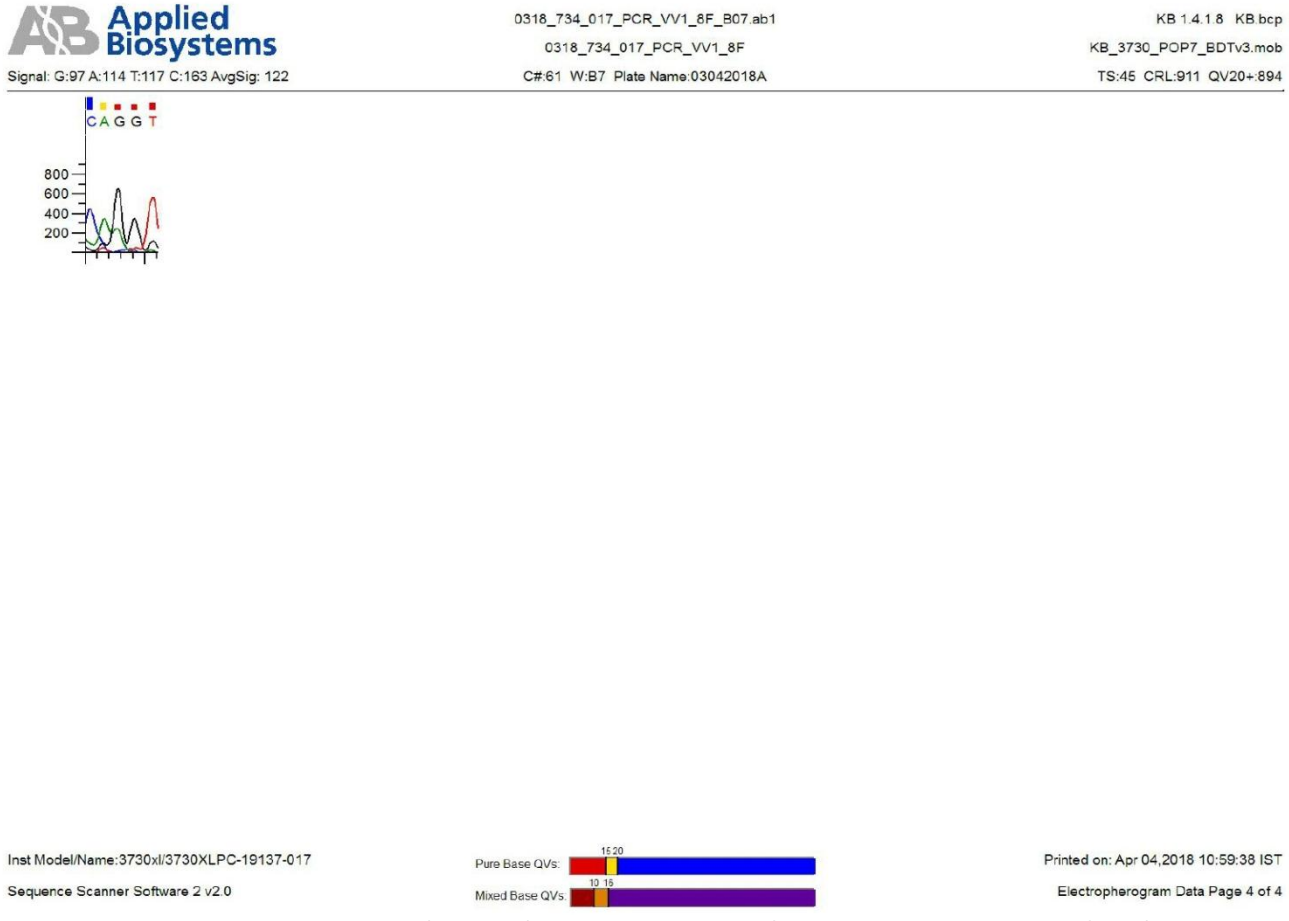

Figure-4(g) Representative Electropherograms Data of 16S rDNA sequence of isolate VV1

Streptomyces chartreusis strain ISP 5085 16S ribosomal RNA gene, partial sequence

Sequence ID: NR_114825.1 Length: 1446 Number of Matches: 1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1386 bits(750)	0.0	750/750(100%)	0/750(0%)	Plus/Plus

```
Query  1    TGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCCTGTC  60
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Sbjct  65   TGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCCTGTC  124

Query  61   CTCCTGGACGGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  125  CTCCTGGACGGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTG  184

Query  121  TTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  185  TTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGC  244

Query  181  CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  245  CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA  304

Query  241  CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA  300
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Query  301  ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAAC  360
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Query  361  TACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGT  420
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Query  721  TTAAGTTCCCCGCCTGGGGAGTACGGCCGC  750
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Sbjct  785  TTAAGTTCCCCGCCTGGGGAGTACGGCCGC  814
```

Figure-5(a) Sequence Alignment Statistics for selected isolate VV1

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces chartreusis strain ISP 5085 16S ribosomal RNA gene, partial sequence	1386	1386	100%	0	100%	NR_114825.1
Streptomyces chartreusis strain NBRC 12753 16S ribosomal RNA gene, partial sequence	1386	1386	100%	0	100%	NR_041216.1
Streptomyces osmaniensis strain OU-63 16S ribosomal RNA gene, partial sequence	1375	1375	100%	0	99%	NR_116760.1
Streptomyces bobili strain NBRC 16166 16S ribosomal RNA gene, partial sequence	1358	1358	100%	0	99%	NR_112584.1
Streptomyces bobili strain NBRC 13199 16S ribosomal RNA gene, partial sequence	1358	1358	100%	0	99%	NR_041121.1
Streptomyces plumbiresistens strain CCNWHX 13-160 16S ribosomal RNA gene, partial sequence	1352	1352	100%	0	99%	NR_044518.1
Streptomyces galilaeus strain NBRC 13400 16S ribosomal RNA gene, partial sequence	1352	1352	100%	0	99%	NR_112389.1
Streptomyces resistomycificus strain NBRC 12814 16S ribosomal RNA gene, partial sequence	1352	1352	100%	0	99%	NR_112287.1
Streptomyces galilaeus strain JCM 4757 16S ribosomal RNA, partial sequence	1352	1352	100%	0	99%	NR_040857.1
Streptomyces alboniger strain DSM 40043 16S ribosomal RNA gene, partial sequence	1347	1347	100%	0	99%	NR_043228.2

Figure-5(b) Representation of identity scores from the Ribosomal Differentiation of Microorganisms database. The query isolate VV1 shares 100% identity with *Streptomyces chartreusis strain ISP 5085.*

Evolutionary Relationships of taxa: The evolutionary history of the isolated strain was concluded using the Neighbor-Joining method.[3] The optimal phylogenetic tree with the sum of branch length = 0.06437924 is shown in figure-5(c). The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test 500 replicates is shown next to the branches.[4][5] The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to conclude the phylogenetic tree for the microorganisms. The evolutionary distances of branch lengths were calculated using the Maximum Composite Likelihood method[6] and are in the units of the number of base substitutions per site.

Estimates of Evolutionary Divergence between Sequences: The number of base substitutions per site from between sequences is shown in distance matrix figure-5(d). Standard error estimate(s) are shown in figure-5(d) the diagonal and were obtained by a bootstrap procedure (500 replicates). Analysis were conducted using the Maximum Composite Likelihood model.[6] The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions of nucleotide sequences containing gaps and missing data were eliminated. There were a total of 962 positions in the final dataset

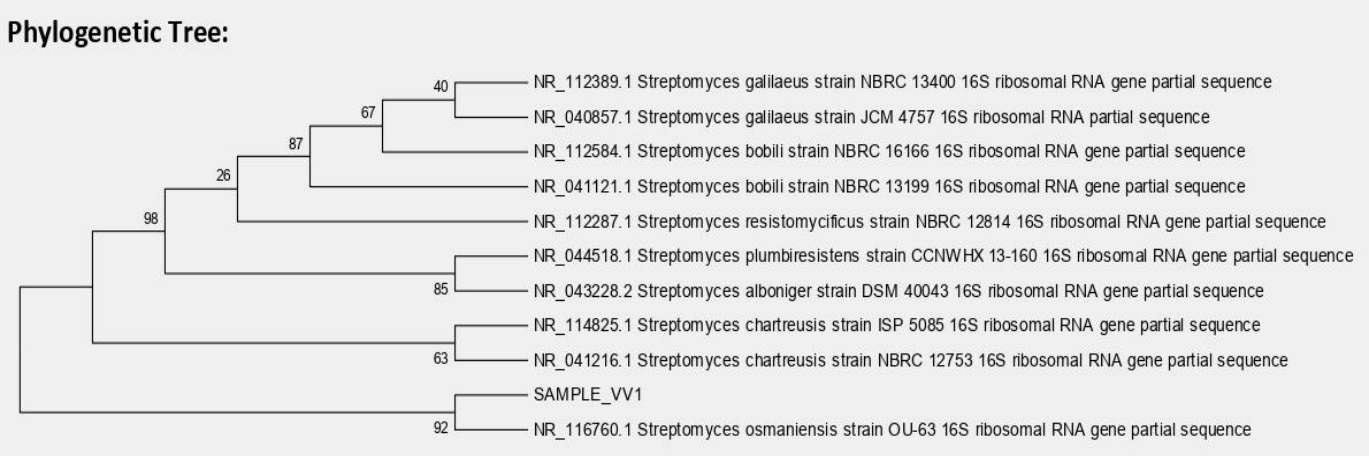

Figure-5(c) Molecular Phylogenetic analysis of the isolate VV1 based on BLAST results

Distance Matrix:

	1	2	3	4	5	6	7	8	9	10	11
1. SAMPLE_VV1		0.0067	0.0067	0.0079	0.0081	0.0079	0.0102	0.0082	0.0082	0.0084	0.0098
2. NR_114825.1 Streptomyces chartreusis strain ISP 5085 16S ribosomal RNA gene partial sequence	0.0233		0.0000	0.0038	0.0030	0.0028	0.0052	0.0031	0.0031	0.0033	0.0050
3. NR_041216.1 Streptomyces chartreusis strain NBRC 12753 16S ribosomal RNA gene partial sequence	0.0233	0.0000		0.0038	0.0030	0.0028	0.0052	0.0031	0.0031	0.0033	0.0050
4. NR_116760.1 Streptomyces osmaniensis strain OU-63 16S ribosomal RNA gene partial sequence	0.0277	0.0115	0.0115		0.0058	0.0056	0.0079	0.0059	0.0058	0.0061	0.0078
5. NR_112584.1 Streptomyces bobili strain NBRC 16166 16S ribosomal RNA gene partial sequence	0.0298	0.0084	0.0084	0.0200		0.0009	0.0047	0.0014	0.0023	0.0010	0.0042
6. NR_041121.1 Streptomyces bobili strain NBRC 13199 16S ribosomal RNA gene partial sequence	0.0287	0.0073	0.0073	0.0190	0.0010		0.0049	0.0010	0.0021	0.0014	0.0042
7. NR_044518.1 Streptomyces plumbiresistens strain CCNWHX 13-160 16S ribosomal RNA gene partial sequence	0.0386	0.0168	0.0168	0.0287	0.0147	0.0158		0.0052	0.0047	0.0049	0.0056
8. NR_112389.1 Streptomyces galilaeus strain NBRC 13400 16S ribosomal RNA gene partial sequence	0.0298	0.0084	0.0084	0.0200	0.0021	0.0010	0.0168		0.0024	0.0009	0.0039
9. NR_112287.1 Streptomyces resistomycificus strain NBRC 12814 16S ribosomal RNA gene partial sequence	0.0298	0.0084	0.0084	0.0201	0.0052	0.0042	0.0147	0.0052		0.0026	0.0042
10. NR_040857.1 Streptomyces galilaeus strain JCM 4757 16S ribosomal RNA partial sequence	0.0308	0.0094	0.0094	0.0211	0.0010	0.0021	0.0158	0.0010	0.0063		0.0039
11. NR_043228.2 Streptomyces alboniger strain DSM 40043 16S ribosomal RNA gene partial sequence	0.0374	0.0168	0.0168	0.0286	0.0137	0.0137	0.0190	0.0126	0.0137	0.0126	

Figure-5(d) Estimates of Evolutionary Divergence between Sequences

CONCLUSION:

The present study clearly demonstrates that feathers which act to be the tough, rigid, insoluble structural protein – keratin, can be decomposed by specialized group of microbes by producing proteases named as keratinases, this proteases occupy an important niche among the group of proteases. Such microbes can be isolated and can be used to digest the waste feather into high-protein feather meal which can be used as feed supplements having a high nutritive value. Chicken feather are potential raw material to obtain by-products of high economical and scientific value. This is due to its low cost, large availability and chemical composition. The biotechnological processes for the degradation of feather possess some advantages over the physicochemical methods like their low energy costs and its being environment friendly. The isolate VV1 was isolated from the soil and used for chicken feather degradation in this study. In experimental conclusion, the highest degree of chicken feather degradation (DFD%) of 54.3% with keratinase activity of 0.0042 µmol./min./mL was

optimized to be at temperature 30 °C and pH 8.5 kept at 120 rpm for 72 h – 120 h in nutrient medium containing 1% dried powdered chicken feather. Based on the results of the physiological, biochemical and the phylogenetic tree derived from 16S rDNA sequence and sequence alignment statistics with homology of 100% identity, the isolate VV1 is confirmed to be ***Streptomyces chartreusis strain ISP 5085***. Utilization of this potential keratin degrader will definitely find biotechnological use in various industrial processes involving keratin hydrolysis, in leather industry, in upgrading feed, detergents and pharmaceuticals. Bioconversion of feather wastes and dead chickens could be a safe method of recycling these organic materials. This isolate would be able to solve the waste disposal problem of poultry waste and with limited resources recycling of keratinous waste would be beneficial both financially and environmentally. This microbial process of keratin degradation not only will resolve economic and environmental problems but at the same moment it generates value added bio-products with prospective industrial and organic farming application. The isolate VV1-*Streptomyces chartreusis ISP 5085* may also evaluate in the treatment of other kinds of wastes, but such complex process of keratinolysis is necessary to be well understood.

Abbreviations: **DFD**: Degree of Feather Degradation; **FC**: FolinCiocalteu; **GAA**: Glucose Aspargine Agar, **GAB**: Glucose Aspargine Broth; **SDA**: Sabouraud Dextrose Agar.

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