FEATHER DEGRADING ACTINOMYCETES: A BIOTECHNOLOGICAL APPROACH TO ENVIRONMENTAL CLEAN-UP

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1. INTRODUCTION

FEATHERS – ***The Unutilized Renewable Bio-Resource***

Feathers which account for 5-7% of the total weight of mature chickens are produced in huge quantities as a waste by-product at commercial poultry processing plants as well as by small scale and local vendors. Considering that millions of tons of feathers are produced annually worldwide (Santos et al., 1996) and that they are made up primarily of keratin protein and dry matter (Table 1), this by-product represents potential alternative to more expensive dietary ingredients, as for example, for poultry production. However, it has long been recognized that feather protein is poorly digested by birds (Romoser, 1955). In addition the leather and fur plants, as well as slaughterhouse throw away considerable amounts of materials like wool, bristle, horns, feathers, hoofs, etc. containing mainly keratin. Till recent years, these materials together with other animal wastes were baked at high temperatures then milled to produce the so-called 'animal flour' and used as 'protein' supplement in the feed mixtures of domestic animals. It was established that this flour is the carrier of diseases like mad cow, swine fever, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease etc. As a result, the feeding of domestic animals with animal flour was

Table 1. Chemical composition of feather waste (Zerdani, et al., 2004)

Component	Percentage
Protein	81
Fat	1.2
Dry matter	86
Ash	1.3

strictly prohibited in the EU countries. Only now the incineration of animal wastes is considered as a reliable way for breaking the spread of prions. As incineration is connected with large expenses for transport, fuel, equipment, labor, etc., now these wastes are predominantly thrown away over controlled dung-hills. However, the latter create important ecological and sanitary problems. Thus, environmental friendly, economically sound and safe methods of decontamination and disposal are badly needed. Disposal of this bulk waste is a global environmental problem accounting to pollution of land and underground water resources.

Feathers are composed of over 80-90% pure keratin protein (Table 2) represent a potential alternative to expensive dietary ingredients for animal feedstuffs. The aminoacid composition of keratins from different avian species is very similar (O'Donnell and Inglis, 1974), as observed for different tissues of chicken feather (Harrap and Woods, 1964). 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 supercoiled polypeptide chain results in mechanical stability and resistance to proteolysis. In addition, cross linking of protein chains by cysteine bridges, disulfide and hydrogen bonds, and salt linkages confer high mechanical stability and resistance to digestive enzymes such as trypsin and pepsine

or other enzyemes (Kunert, 1973). Therefore, feather waste is utilized on a limited basis as a dietary protein supplement for animal feedstuff. The degradation of keratinous material is important medically and agriculturally. The use of keratinase to nutritionally upgrade feather meal has been described. A comparable growth rate was observed between chickens fed with isolated soybean and those fed with feather meal fermented with *Streptomyces fradiae* plus

Table 2. Aminoacid composition of chicken feather hydrolysate (Sangali & Brandelli, 2000)

Amino acid	Mol percentage
Alanine	5.42
Argenine	8.43
Asparagine	5.78
Cysteine	6.51
Glutamine	9.22
Glycine	5.96
Histidine	0.93
Isoleucine	16.28
Leusine	9.69
Lysine	2.41
Methionine	1.70
Phenylalanine	5.42
Proline	5.28
Serine	10.83
Thereonine	3.66
Tyrosine	3.29
Valine	8.56

(Values are the average of triplicate samples)

methionine supplementation (Elmayergi and Smith, 1971). The utilization of *Bacillus licheniformis* feather lysate with amino acid supplementation produced a growth curve identical to that of soybean meal (Williams et al., 1991). The use of crude keratinase significantly increased the amino acid digestibility of raw feathers and commercial feather meal (Lee et al., 1991). This enzyme increased the digestibility of commercial feather meal and could replace as much as 7% of the dietary protein for growing chicks (Odetallah et al., 2003). However, feathers are currently utilized on a limited basis as a dietary protein supplement for animal feed because feather meal production is an expensive processes, requiring significant amounts of energy.

Feathers hydrolyzed by mechanical or chemical treatment can be converted to feedstuffs, fertilizers, glues and foils or used for the production of amino acids and peptides. Because of environmental considerations use of microbial keratinolytic enzymes in the production of amino acids and peptides is becoming attractive for biotechnological applications. With the help of these enzymes feathers could be converted to defined products such as the rare amino acids serine, cysteine and proline. This enzyme processes is advantageous over commercial methods, as large amounts of salts, which needs to be separated from the end product, would not be produced. The production of keratinases is the domain of fungi, actinomycetes and some *Bacillus* species. These proteases are the most important group of industrial enzymes and certainly form a major portion of the worldwide sales (Huang et al., 2003).

Insoluble and hard-to-degrade animal proteins are ubiquitously present throughout animal bodies. Enormous amounts of these proteins are generated in the meat industry in a mixture of bones, organs and hard tissues, finally being converted

to industrial wastes, the disposal of which is tremendously difficult. Millions of tons of feathers are produced annually as a waste byproduct at poultry processing plants. If these feathers are not disposed off in time, their accumulation will lead to a serious environmental pollution and feather protein wastage (Onifade et al., 1998; Gousterova et al., 2005). Most animal proteins (feathers) are currently disposed off by incineration (Deydier et al, 2005). This method, however, has ecological disadvantages in terms of apparent energy loss and the production of a large amount of carbon dioxide. Thus, an innovative solution to these problems is urgently needed.

Currently, the methods of feather degradation include alkali hydrolysis, steam pressure cooking and biodegradation. However, due to feathers being almost pure keratin protein, alkali hydrolysis and steam pressure cooking will not only destroy the amino acids but also consume large amounts of energy (Cai et al., 2008). Therefore, the development of enzymatic and/or microbiological methods for the hydrolysis of feather into soluble proteins and aminoacids is extremely attractive. Furthermore, feather can be biodegraded to feather meal for animals, slow-release nitrogen fertilizers, glues and films or used for the production of the rare amino acids like serine, cysteine and proline (Gupta and Ramnani, 2006).

KERATINS - *The Tough Proteins*

Keratins are the largest and most complex family of cytoskeletal intermediate filament proteins of animal cells, particularly epithelia (Scott and Untereinen, 2004). Keratins are grouped into hard keratins (feather, hair, hoof, and nail) and soft keratins (skin and callus) according to the sulfur content (Gupta and Ramnani, 2006). Hard keratins are insoluble and resistant to degradation by common proteolytic enzymes,

such as trypsin, pepsin and papain because of their high degree of cross-linking by disulfide bonds, hydrogen bonding and hydrophobic interactions (Farag and Hassan, 2004).

Feathers are composed of over 90% of beta-keratin, rich in stacked beta-pleated sheets, held together by hydrogen bonding, hydrophobic interactions and highly cross-linked by disulfide bonds (Lin et al., 1999). Therefore, feathers are mechanically stable and difficult to be degraded by common proteases. Keratinous wastes are increasingly accumulating in the environment generated from various industries. To recycle such wastes, biotechnological alternatives are being developed (Gupta and Ramnani, 2006).

KERATINASES – *The Unique Proteases*

Keratinases [Ec 3.4.21/24/99.11] are enzymes that can hydrolyze both native and denatured keratin, which is an insoluble protein found in chicken feathers, human hair, animal horns and wool (Onifade et al., 1990). It is classified as a protease produced by keratin degrading microorganisms (Letourneau et al., 1998: Bressollier et al., 1999: Friedrich et al., 1999; Singh et al., 2001). The keratinolyic enzymes characterized to date all act as proteinases and are active on keratin. The gene (Ker A) encoding a keratinolytic protease of *Bacillus licheniformis* PWD-1 was sequenced and expressed in E. coli cells (Lin et al., 1995). It shares 97% sequence identity with the gene encoding subtilisin from *B. licheniformis* (Lin et al., 1995). This enzyme is widely used not only in chemical and pharmaceutical industries, but also for removing hair and fathers in poultry industry (Takami et al., 1992) and for improving the nutritional value of father meal in the feed industry (Lee et al., 1991) and basic

biological science (Ichida, 2001) and in newer fields like prion degradation for treatment of dreaded mad cow disease and biodegradable plastic manufacture etc. Properties of keratinase depend upon the producers. It is usually a serine protease (Bressollier et al., 1999: Riessen and Antranikian, 2001, Bockle et al., 1995). Occasionally, it has been found to be a serine protease with a cysteine protease (Sangali and Brandelli, 2000b) and a metallo protease (Lee et al., 2002). Its optimum temperatures and pH values were reported to be 40-80C and 6-10, respectively (Lin et al., 1992). Some reports have described thermoactive keratinolytic proteases produced by mesophilic microorganisms. These enzymes show keratinolytic activity at temperatures above 70°C, whereas most of the other keratinases from mesophilic bacteria and fungi are active at a rather alkaline pH but show optimal activity at lower temperatures.

The keratinolytic enzymes characterized to date all act as proteinases and are active on keratin. The gene (*Ker A*) encoding a keratinolytic protease of *Bacillus licheniformis* PWD-1 was sequenced and expressed in *E. coli* cells (Lin et al. 1995). Most of the reports on keratinases, group them as inducible enzymes; however few constitutive keratinases have also been reported (Gupta and Ramnani, 2006). Keratinolytic enzymes are widespread in nature, especially in microbial world. A vast variety of bacteria, actinomycetes and fungi are known to be keratin degraders (Wang et al., 2003; Letourneau et al., 1998; Santos et al., 1996). At present, keratinolytic microorganisms and their enzymes has become a subject of substantial scientific interest (Worapot et al., 2005; Nadir et al., 2008; Matsui et al., 2009).

In recent years, several keratinases have been purified and characterized from different microorganisms, e.g. *Bacillus licheniformis* (Zerdani *et al.*, 2004; Ramnani *et al.*, 2005; Korkmaz *et al.*, 2004; Manczinger *et al.*, 2003; Williams *et al.*, 1990;),

Fervidobacterium pennivorans (Friedrich and Antranikian, 1996), *Burkholderia*, *Chryseobacterium*, *Pseudomonas*, *Microbacterium sp.* (Brandell and Riffel, 2006) and was studied with respect to different parameter. This enzyme is also produced by fungi, including the species of *Aspergillus*, *Onygena*, *Absidia* and *Rhizomucor* (Friedrich *et al.*, 1999), some species of dermatophytes, including *Trichophyton mentagrophytes, T. rubrum, T.gallinae, Microsporum canis* and *M. gypseum* (Wawrzkiewicz *et al.*, 1991). *Streptomyces* sp. (Bressollier et al., 1999; Montero-Barrientos et al., 2005). However, keratinolysis by microorganisms remains poorly understood.

The optimum activity at moderate temperature will be less energy consuming than the currently used thermophilic bacteria and therefore meets the increasing consciousness for energy conservation. Most thermoactive keratinases from mesophilc bacteria and dermatophytes producing keratinases are mostly pathogenic, they are undesirable for application. So it is necessary to evaluate a greater variety of mesophilic microorganisms producing characteristic proteases with broad substrate specificity and thermostable activity in order to establish the utility of mesophilic microorganisms for the degradation of hard proteins.

DEGRADATION

Keratin by virtue of its insolubility and resistance to proteolytic enzymes, is not attacked by most living organisms. Nevertheless, keratin does not accumulate in nature and, therefore, biological agencies may be presumed to accomplish its removal. Several insects, including cloths moth larvae, carpet beetles and chewing lice are known to digest keratin. The common occurrence in nature of microorganisms that readily and, in some cases, preferably grow on keratinaceous substrates has supported

the general belief that certain microorganisms can digest keratin (Noval and Nickerson, 1959). Such microbes or their process can be exploited for better management/use of feather wastes.

2. REVIEW OF LITERATURE

Actinomycetes are often responsible for spoilage of hay, straw, cereal grains, seeds, wool, paper, wood, hydrocarbon, rubber and plastics. In nature biodegradation by actinomycetes play an extremely useful role in waste removal and is an integral part of the recycling of materials. Most actinomycetes live in aerobic soils, where they degrade organic substrates. Number of actinomycetes living in soils often exceeds one million per gram. Given below is a brief review of work done on feather degradation by microorganisms.

ACTINOMYCETES

Nickerson (1959) tested 15 bacteria, 21 actinomycetes and 18 fungi for keratinolytic activity and found that *Streptomyces fradiae* was the most effective in the degradation of sheep wool. Bockle et al. (1995) isolated a serine protease from the keratin degrading *Streptomyces pactum*. The proteinase was optimally active in the pH range from 7-10 and at temperatures from 40-75°C. It showed a high stereo selectivity and secondary specificity with different synthetic substrates. The *S. pactum* proteinase was significantly more active than the various commercially available proteinases.

Letourneau et al. (1998) isolated a *Streptomyces* sp. from a naturally degraded feather produced a high keratinolytic activity when cultured on feather meal medium. Maximal keratin degrading activity in the supernatant of this organism was observed at 70°C and pH 10. Keratinolytic activity was only partially inhibited by EDTA or phenyl methyl sulphonyl fluoride (PMSF), suggesting that the overall keratinolytic activity was supported by different proteases.

Chitte et al. (1999) detected keratinolytic activity in the culture broth of feather-degrading thermophilic *Streptomyces thermoviolaceus* SD8. PAGE analysis of the enzyme indicated monomeric form with a molecular weight of 40kDa. The optimum pH and temperature for production of the enzyme were 8 and 55°C, respectively. The enzyme was stable at a pH range of 6.5-8.5 and up to 65 °C. The enzyme could hydrolyze fibrin, muscle, collagen, nail and hair and could produce leucine, threonine and tyrosine from feather. According to Bressollier et al. (1999) *Streptomycs albidoflavus* secreted at least six extracellular proteases when it was cultured on feather meal-based medium. A serine protease of molecular weight of 18000 kDa was optimally active at pH values ranging from 6-9.5 and at temperatures ranging from 40-70°C isolated from the culture broth. Its sensitivity to protease inhibitors, its specificity to synthetic substrates, and its remarkably high level of NH_2-terminal sequence homology with *Streptomyces griseus* protease B (SGPB) showed that the new enzyme, designated SAKase, was homologous to SGPB. However, activity of SAKase showed that its activity was very specific to keratinous substrates compared to SGPB and proteinase K.

A thermotolerant *Streptomyces graminofaciens* isolated from decomposing feather heap could digest chicken feather at 40°C very efficiently (Szabo et al., 2000). The majority of the fragments produced during feather digestion was in the range of

colloidal particles or even smaller. The maximum amount of feather in the medium which could be digested in one week in submerged culture was 10%. The procedure did not require previous mechanical disruption of the feather which makes the application fast and easy.

Al-Zarban et al. (2002) described two new species of keratinolytic actinomyctes from salt marsh soil in the southern part of Kuwait. One is a halotolerent *Nocardiopsis halotolrans* and the other one is a halophilic *Saccharomonospora halophile*. Both species showed keratinase activity in the culture filtrate in the presence of feather meal as the sole source of carbon and nitrogen. The keratinase activities of both species were repressed in the presence of 1% glucose in the medium. Al-Musallam et al. (2003) described a novel keratinolytic actinomycete strain D2, *Amycolatopsis keratiniphila* sp. nov., from Kuwait marsh soil.

Pettett and Kurtboke (2004) reported keratinolytic actinomycetes from poultry farm manure and used to augment the compost microflora. The inclusion of the organisms to poultry waste compost was successful in producing an odorless, pathogen free product with complete biological degradation of the feather waste in 17 days. The application of this system may improve the rapid disposal of poultry waste as well as the public and environmental health in the region.

Barrientos et al. (2005) described a novel keratinolytic actinomycete *Terrabacter terrae* sp. nov., from soil, in Spain. Gousterova et al. (2005) compared microbial degradation of indigenous keratin waste with a method of alkaline hydrolysis. Native sheep skin and wool were chosen as model mixture of collagen and keratin wastes discarded by the leather and fur industries. Nine thermophilic and ten mesophilic actinomycete stains isolated from Antarctic soil samples were screened

for keratinolytic activity in liquid Mineral salts medium containing wool waste (6g/l) (Gushterova et al., 2005). Two strains identified as *Streptomyces flavis* 2BG (mesophilic) *Microbispora aerata* IMBAS-11A (thermophilic), showed 9 fold and 6 fold increase in keratinase production respectively in the presence of starch.

Streptomyces sp. 594 produced protease in submerged and solid state fermentation with pH increase in both media. Protease production was lesser in SSF than in SF. It produced a mixture of proteases belonging to serine and metalloproteinase classes, were active over a wide range of pH (5-10) and high temperatures (55-80°C) (De Azeredo et al., 2006). The same authors in 2006 reported that protease synthesis by *Streptomyces* sp. 594 was markedly enhanced by corn steep liquor by approximately 86% and 39% in submerged fermentation and solid state fermentation respectively compared with the yeast extract used previously in the place of corn steep liquor.

A *Streptomyces* sp. was isolated from poultry plant wastewater, showed high keratinolytic activity when cultured on feather meal medium (Tapia et al., 2008). Optimum keratinolytic activity was observed at 40°C and pH 8.0. The enzyme also showed to be stable between 40°C and 60°C. The keratinolytic activity was not inhibited by EDTA, DMSO and Tween 80. On the other hand, $CaCl^2$, $ZnCl^2$, and $BaCl^2$ slightly inhibited the keratinolytic activity. The *Streptomyces* isolated might be useful in leather, keratin waste treatment, animal feeding industry, and also cosmetic industry.

Kansoh et al. (2009) screened 27 *Streptomyces* spp. isolated from soil for protease activity. Twenty one isolates with protease activity were screened for keratinolytic activity. Only 8 isolates exhibited keratinolytic activity, two of them

with intense activity were identified as *Streptomyces albidus* E4 and *S. griseoaurantiacus* E5. Galactose and ammonium nitrate supported maximum production of keratinase on 5th day of incubation in shake culture at 30^{o}C and pH 8.0. In addition to feathers both isolates utilized wool, nails and hair as well. Puhl et al. (2009) described *Actinomadura keratinolytica* sp. nov., a keratin degrading actinobacterium isolated from bovine manure compost.

Saha and Dhanasekaran (2010) screened 22 soil actinomycete isolates for proteolytic activity; ten isolates showed positive results. These ten proteolytic isolates were screened for keratinolytic activity. Three isolates (SD5, SD6, SD7) presented keratinolytic activity and completely degraded feathers within 20-25 days.

BACTERIA

Williams et al. (1990) isolated a feather-hydrolytic Gram variable, endospore forming, motile, rod shaped bacterium by enrichment culture method. Electron microscopy of the isolate showed internal crystals. The bacterium was identified as *Bacillus licheniformis* PWD-1. The isolate demonstrated facultative growth at thermophilic temperatures with optimum at 45-50^{o}C and pH 7.5. The most efficient conditions for feather fermentation occurred during the incubation of 1 part feathers to 2 parts *B. licheniformis* PWD-1 culture (10^{-7} cells/ ml) as evidenced from the free amino acids released in the fermentation medium. Lin et al. (1992) isolated a keratinase from the culture medium of feather degrading *B. licheniformis* PWD-1. The purified keratinase was monomeric with a molecular mass of 33 kDa. The purified keratinase hydrolyzed a wide range of substrates and displayed higher proteolytic activity than most proteases. Friedrich and Antranikian (1996) evaluated nineteen strains of anaerobic extremely thermophilic and hyperthermophilic anaerobic

archaea and bacteria belonging to the orders *Thermococcales, Thermoproteals, Thermotogales* and *Clostridials* for their abilities to grow on feathers and produce heat-stable keratinolytic enzymes. Of the 19 investigated extremely thermophilic microorganisms, 18 strains were unable to attack feathers. The feather degrading bacterial isolate was identified as *Fervidobacterium pennavorans* sp. novo. Interestingly the keratinase from *F. pennavorans* is catalytically active at high temperature (80ºC) and pH (10.0). The thermo stability of the enzyme is of advantage for converting poultry feathers in to peptides and rare aminoacids at around 70 ºC and the risk of contamination can be minimized.

Nitisinprasert and Keawsompong (1997) isolated two aerobic bacterial isolates (K6 and K82) from feather waste and soil. These isolates showed high feather digestibility at wide pH ranges and high temperature up to 50ºC. The two isolates differed in their ability to degrade the feathers and in releasing free aminoacids. The isolate K6 showed high feather digestion but released low aminoacid. On the other hand the isolate K82 exhibited low feather digestion but released high amount of aminoacid.

Nitisinprasert et al. (1999) studied the synergistic action of culture filtrates of two feather degrading bacteria - *Bacillus licheniformis* (KUB-K0006) and *B. pumilis.*(KUB-0082). The crud enzyme of *B. lichniformis* and *B. pumilis* in the ratio of 5:1 showed higher amount of amino acid released than they were used individually.

Studies conducted by Burtt and Ichida (1999) revealed that occurrence of feather degrading bacilli are wide spread in the plumage of birds. They sampled bacteria from the plumage of 1,588 individuals of 83 species of birds. Bacteria capable of extracting energy and nutrients by breaking up B-keratin were isolated

from 134 individuals in 32 species. They occurred most frequently on ground-foraging species and least frequently on aerial-foraging species. Nine of 11samples of feather degrading bacteria were identified as *Bacillus licheniformis*, one as *B. pumilus*, and one as *Bacillus* of undetermined species. The researchers suggested that the natural occurrence of keratinolytic bacteria in the plumage of birds may contribute to the deterioration of feathers and be a selective force in the evolution and timing of molt.

A *Vibrio* sp. strain Kr2 isolated from poultry industry waste showed high keratinolytic activity (Sangali and Brandelli, 2000). The bacterium grew with an optimum at pH 6.0 and 30 °C, where maximum feather degrading activity was also observed. Keratinse production was similar at both 25°C and 30°C, while maximum concentration of soluble protein was reached at 30°C. Reduction of disulphide bridges was also observed, increasing with cultivation time. Vidal et al. (2000) reported keratinolytic activity of a strain of *Kocuria rosea*, a soil isolate. In batch culture, the optimum temperature for feather degradation, bacterial growth and protease secretion was at 40°C. No organic acids were detected in the fermentation broth in significant amount.

Several thermophilic anaerobic bacteria with keratinolytic activity growing at temperatures between 50°C and 90°C were isolated from soil samples form islands of Portugal (Riessen and Antranikian, 2001). One of the isolate identified as *Thermoanarobacter kratinophilus* is the first member of the genus *Thermoanaerobacter* that has been described for its ability to degrade native keratin. The strain was shown to possess intra cellular and extracellular proteases optimally active at 60°C, pH 7, and 85°C, pH 8, respectively. The extra cellular protease was identified as serine protease with a molecular mass of 135 kDa.

A *Flavobacterium* sp. isolated from a poultry industry waste completely degraded raw feathers (Riffel and Brandelli, 2002). The proteolytic activity was assessed in the presence of specific protease inhibitors. The crude enzyme showed mainly metalloprotease character. Two newly described actinomycetes, isolated from salt marsh soil in the southern part of Kuwait, are reported to degrade feather keratin (Al-Zarban et al., 2002). One is a halotolerant *Nocardiopsis* sp. and the second is halophilic *Saccharomonospora* sp. Both species showed keratinase activities in the culture filtrate in the presence of feather meal as the sole source of carbon and nitrogen. The keratinase activities of both the species were repressed when 1% glucose was included in the medium. A *Flavobacterium* sp. producing a high keratinolytic activity was isolated from a poultry industry after growth on selective feather meal agar (Riffel and Brandelli, 2002). The proteolyic activity was assessed in the presence of specific protease inhibitors. The crud enzyme showed mainly metalloprotease character. Allpress et al. (2002) screened nine bacterial isolates with known proteolytic activity for keratinase activity. Of these *Lysobacter* sp. exhibited the highest keratinolytic activity. Optimum activity occurred at 50°C; no inhibition was demonstrated by PMSF, but inhibition by EDTA was demonstrated indicating that the keratinase is a metalloprotease. Nam et al. (2002) isolated a feather degrading thermophilic anaerobe from a geothermal hot stream in Indonesia. The isolate AW-1, identified as a member of the species of *Fervidobacterium islandicum,* was shown to degrade native feathers (0.8%, w/v) completely at 70°C and at pH 7.0. After 24 h culture, feather degradation led to an increase in free amino acids such as histidine, cysteine and lysine. Moreover, nutritionally essential amino acids such as tryptophan and methionine, which are rare in feather keratin, were also produced as microbial

metabolites. A homomultimeric keratinolytic protease exhibited activity towards soluble keratin optimally at 100°C and pH 9, and had a half-life of 90 min at 100°C.

Riffel et al. (2003) isolated a novel feather degrading bacterium from poultry waste, producing a high keratinolytic activity when cultured in broth containing native feather. The bacterium was identified as *Chrysobactrium* sp. strain kr6. Maximum keratinase production was reached at 25°C. The keratinase activity was inhibited by 1,10-phenanthroline, EDTA, Hg^{2+} and Cu^{2+} and stimulated by Ca^{2+}.

Korkmaz et al. (2004) isolated a highly keratinolytic strain of *Bacillus licheniformis* HK-1 from poultry waste. Complete feather degradation was achieved at 60°C in 3days in culture supernatant. Maximum keratinase production was observed at 50°C and pH 8. The enzyme was active over a wide range of pH (with optimum 11.0) and temperature (with optimum 60°C) and was relatively heat stable (up to 60°C). Keratinolytic activity was partially inhibited by EDTA, NaCl and PMSF and completely inhibited by $ZnCl_2$, On the other hand activity was increased by DMSO, TritonX-100, SDS, $CaCl_2$ and sodium sulphite. Eight strains of *Bacillus* isolated from natural composting waste were screened for growth and protein digestion in feather medium (Zerdani et al., 2004). Results showed that two of the isolates, *B. licheniformis* and *B. subtilis,* were the most efficient in feather hydrolysis. A new strain of *Microbacterium* sp. isolated from a poultry plant presented optimum growth and high feather degrading activity at pH 7.0 and 30°C. The partially purified enzyme was optimally active at pH 7 and 55°C . The enzyme was inhibited by1-10, phenanthroline, EDTA, p-chloromercuribenzoic acid, 2-mercaptoethanol and metal ions like Hg^{2+}, Cu^{2+} and Zn^{2+}, suggesting that it is metaloprotease -type keratinase (Thys et al., 2004). Brandelli and Riffel (2005) studied the effect of temperature, pH

and media composition on protease production by a keratinolytic bacterium *Chryseobacterium* sp. The enzyme was produced between 25-37ºC with maximum activity and yield at 30ºC and pH 6-7. Addition of carbohydrates or surfactants to feather broth resulted in decrease in keratinolytic activity.

Suntornsuk et al. (2005) investigated the properties of keratinase produced by a strain of *Bacillus lichenifomis* isolated from Thai soil. The purified enzyme exhibited a high specific activity (218 U mg^{-1}). The optimum pH and temperature for the enzyme were 8.5 and 60ºC respectively. The enzyme activity was significantly inhibited by PMSF and partly by EDTA, but was stimulated by metal ions.

A mutant strain KD-N2 of *Bacillus subtilis* produced inducible keratinase 2.5 times more than the wild strain in different substrates of feather, hair and silk and wool under submerged cultivation. The optimal conditions for keratinase production include initial pH of 7.5, inoculum size of 2% (v/v), age of inoculum of 16 h, and cultivation at 23ºC, after 30 h. Essential aminoacids like threonine, valine, methionine as well as ammonia were produced when fathers were used as substrates (Cai et al., 2008). Deivasigamani and Alagappan (2008) isolated a keratinolytic *Bacillus* sp. from the soil samples collected from slaughter house and poultry farm area. Highest keratinase activity (122.5 KU/ml) was observed at pH8, and on 4^{th} day in submerged culture condition. The molecular weight of this keratinase was 32 KDa by SDS-PAGE. Gosh et al., (2008) reported production of a minor extracellular protease by *Bacillus cereus* DCUW, and its involvement in feather degradation. The protease was found to have broad substrate specificities that include keratin, casein, collagen, fibrin and gelatin. The purified protease had a molecular weight of 80 kDa and showed pH and temperature optima of 8.5 and 50 ºC.

A strain of *Bacillus licheniformis* K-19, isolated from feather dumping site produced a large amount of thermostable keratinase (224 U/ml) at 37°C when cultured for 72 h in broth containing feather meal with initial pH 7.5. The enzyme was active over a range of temperatures (0-90°C) and pH values (pH 6-10) and active in broad range of pH. It was optimal at 60°C and pH 7.5-8 respectively (Bo et al., 2009). Cao et al. (2009) isolated a father degrading bacterium from decomposing poultry feathers. The bacterium was identified as *Stenotrophomonas maltophilia*. Maximum growth and feather degrading activity of the bacterium were observed at 40°C and initial pH ranging from 7.5-8.0. The enzyme was purified and had a molecular weight of 35.2 kDa and was completely inhibited by a serine protease inhibitor, PMSF.

Prasad et al. (2010) screened eight soil bacterial isolates for keratinolytic activity, only one isolate showed keratinolytic activity. The isolate identified as *Bacillus* sp. showed better activity at pH7 an temperature 30°C. A feather degrading bacterium with high keratinase activity was isolated and identified as *B. licheniformis* YJ4 (Lin and Yin, 2010). After 72 h incubation in a feather mal medium at 37°C , 2 keratinases (keratinase I and keratinase II) with molecular weight of 30.5 and 32.5 kDa were purified. The enzymes were stable at pH 6-10 and 10-50°C. Based on the effect of metal ions, inhibitors and reducing agents the keratinases I and II were designated as cysteine and serine proteases respectively. Similarly *Bacillus licheniformis* isolated from poultry soil (Vigneshwaran et al., 2010) completely degraded feather after 7days incubation at 40°C at 150 rpm. The enzyme was stable at temperatures 30-40°C and active between pH 6-8. Among the various metal ions tested zinc and magnesium enhanced enzyme activity whereas mercury, copper, cadmium, phenanthroline and EDTA completely inhibited the enzyme activity. Under submerged fermentation condition with continuous agitation (180 rpm) *Bacillus* sp.

JB 99 produced high level of keratinse at 45ºC and pH 10. The presence of carbon source in feather medium suppressed the enzyme production, while 0.1% yeast extract enhanced enzyme production. The purified enzyme showed maximum keratinase activity at temperature 65ºC and pH 10. The enzyme may be a serine protease as it was completely inhibited by PMSF. Presence of metal ions such as Ca^{2+}, Mg^{2+}, Co^{2+} and Ba^{2+} stimulated enzyme activity, while Hg^{2+}, Pb^{2+}, Zn^{2+} and Fe^{2+} decreased the activity (Pushpalata and Naik, 2010). Eight strains of *Bacillus*, isolated from decomposing feathers were tested for feather degradation. Among these strains *Bacillus cereus* KB043 showed maximum activity and caused 78% degradation with a significant release of soluble protein (1206.15µg/ml) and cysteine (20.63 µg/ml) in the cultivation fluid (Nagal and Jain, 2010). Tork et al. (2010) isolated 23 bacterial strains from poultry industrial wastes (feather, soil, water, fodder) on feather meal agar plates. Out of 23 isolates seven isolates that showed promising proteolytic activity were screened for keratinolytic activity. The best keratinase producing isolate was identified as *Pseudomonas* sp. The optimum temperature and pH were determined to be 37ºC and pH 8.0 respectively. Agrahari and Whadha (2010) isolated three keratinolytic bacteria from soil samples collected from Ghazipur poultry waste. These bacteria showed optimum activity at pH 7.5 and temperature 30ºC.

FUNGI

Jain and Agrawal (1980) reported that among 34 fungi tested for keratin hydrolysis, the most active in peacock feather degradation were *Verticillium tenuipes*, *Trichophyton equinum* and *T. mentagrophytes*. In another study out of 21 fungi tested for chicken feather degradation, *Trichophyton simii* was reported to be effective (Singh et al., 1995).

Santos et al. (1996) found that the opportunistic pathogen *Aspergillus fumigatus* can utilize chicken feather keratin as its sole carbon and nitrogen. The feather degrading activity was induced by keratin but repressed by low molecular weight nitrogen and carbon sources. Hydrolysis was optimal at pH 9 and at 45°C. Kaul and Sumbali (1997) demonstrated that species of *Chrysosporium, Malbranchea, Scopulariopsis, Microascus*, and *Gliocladium* isolated from poultry farm soils could grow on human hair and degrade it releasing sulphur containing aminoacids and polypeptids in to the medium. Keratinase activity also changed pH of the medium towards alkalinity and species with strong keratinolytic ability rendered the culture medium more alkaline than those that were less keratinolytic. Friedrich et al. (1999) screened 293 common fungi isolated from soil and air for synthesis of extracellular keratinases. About 54% of the fungi grew on agar plates with soluble keratin and excreted keratinolytic enzymes. Some representatives of *Fusarium*, *Acremonium* and *Geotrichum* were the most active. However, in submerged culture other fungi proved to be potent. *Aspergillus flavus* was the most powerful producer of extracellular kertinases, followed by *Alternaia radicina, Trichurus spiralis* and *Stachybotrys atra.*

Al-Sane et al. (2002) isolated keratin degrading fungi and actinomycetes from Kuwait soils using baiting technique. The preponderant fungal species isolated was *Chrysosporium*, however, *Fusarium, Aspergillus* and *Acremonium* and three actinomycetes were also isolated. Among the microbes tested one isolate of actinomycete *Streptomyces* sp. K, and two fungal species - *Chrysoporium panicola* and *C. zonatum* showed higher activity and produced multicomponent keratinases and manipulating some extraneous factors increased their production.

Kim (2003) screened soil samples from ten different poultry farms for prevalence of keratinolytic fungi. Fourteen species of feather associated fungi

belonging to ten genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Monascus, Mucor, Penicillium* and *Verticillium* were recovered from feathers and assessed for their keratinolytic activity. Five species of aspergilli ie. *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans,* and *A. terreus* could utilize keratin of chicken feather and degrade it producing sulphydryl containing compounds detected as keratinase, cysteine and total proteins. These five fungi also changed the pH of the medium more alkaline than those that were less keratinolytic.

Scott and Untereiner (2004) employed azure dye impregnated sheep's wool keratin (keratin azure) based culture media assay to study keratin degradation by 58 fungal taxa including 49 members of the Arthrodermataceae, Gymnoascaceae and Onygenaceae (Order Onygenales). The results were comparable to measures of keratin utilization reported in studies using tests based on the perforation of human hair in vivo. Kim (2005) immobilized keratinase produced by *Aspergillus flavus* and compared the activity of immobilized and free enzyme. The immobilized keratinase showed proteolytic activity against soluble azo-keratin and insoluble feather keratin. Heat stability and pH tolerance of keratinase were greatly enhanced by immobilization.

Among 106 filamentous fungi isolated from poultry farm waste, 13 species belonging to seven genera (*Aspergillus, Acrmonium, Alternaria, Beauvaria, Curvularia, Paecilomyces, and Penicillium*) were able to grow and produce keratinase in stationary culture using poultry feather powder. Out of the 13 keratinolytic fungi highest keratinolytic activities were recorded after 4-6 days of cultivation in submerged conditions for *Beauvria bassiana* (62.8 U/ml) followed by *Curvularia*

brachyspora. Acrmonioum hyalinulum. and *Alternaria tenuissima* (Marcondes et al., 2008).

Saber et al. (2010) screened 82 fungi, isolated from poultry industry waste, for feather degradation on feather meal agar plates. Twenty seven isolates showed weak to strong keratinolytic activity. Two fungi - *Alternaria tenuissima* K2 and *Aspergillus nidulans* K7 produced higher levels of keratinase of 53.4 and 55.8 U/ml on 6^{th} and 5^{th} day of incubation respectively. Addition of maltose and starch enhanced keratinase production at pH 7.5, 35ºC and 7.5% inoculum ratio. Addition of the enzyme brought about 70% hydrolysis of chicken, duck, goose and turkey feather after 24h incubation. Goat hair, sheep wool and buffalo horn showed lower response towards keratinolytic hydrolysis.

The brief review on feather degradation reveals that the ability to colonize and breakdown the complex and recalcitrant feathers is wide spread among the soil microbial population. Though a limited microbes are involved they seem to be present in all types of soil environment but are more in polluted environments.

3. PRESENT PROBLEM

Every year millions of tons of chicken feathers are generated as waste byproduct by the poultry farms and poultry processing industries (Plate 1a & b). Major poultry processing units, however, recycle the feather wastes as 'feather meal' after physical and chemical hydrolysis of feathers, an energy consuming process. However, medium and small scale sellers especially the local farms simply dump the feathers in abandoned/waste lands and on road sides (Plate 2 I-V). This results in environmental pollution and social nuisance. Since feather contains about 90% keratin protein it can be converted into nitrogen rich compost using naturally occurring microorganisms. Or can be used to produce rare aminoacids and other value added products. Therefore screening naturally occurring microbes for keratinolytic activity assumes great importance and need of the hour. Though several bacteria and fungi have been reported keratinolytic, work on actinomycetes is very limited. Therefore in the present investigation actinomycetes from a feather dumping site and a sacred grove were evaluated for feather degradation and their ability to compost chicken feathers.

4. MATERIALS AND METHODS

1. Soil sample collection

Soil samples collected from two different sites were used in this study.

Site 1: Soil sample was collected from a road side feather dump site, where chicken feathers were found in half decomposed state. Using a sterile spatula soil samples were collected from under the decomposing feathers. About 200 g surface soils (0-10cm depth) were collected from within the site at different points. All the subsamples were placed in a clean polythene bag and a composite sample was produced and brought to laboratory for isolation of actinomycetes. In the laboratory the soil sample was passed through a 2 mm sieve to remove feather fragments and other organic debris and stones. Then the soil sample was dried overnight in a hot-air oven at 40^{o}C to reduce bacterial contamination in isolation plates.

Site-2: The second soil sample was collected from a sacred grove near Puducherry. Using a sterile spatula leaves on the soil surface were removed and the underlying soil samples were collected. About 300 g surface soils (0-10cm depth) were collected from the sacred grove from different points. All the subsamples were placed in a clean polythene bag and mixed well and brought to laboratory for isolation of

actinomycetes. In the laboratory the soil sample was passed through a 2 mm sieve to remove organic debris. Then the soil sample was dried overnight in a hot-air oven at 40°C to reduce bacterial contamination in isolation plates.

2. Isolation of actinomycetes

2.1. Serial dilution: Ten gram soil sample from each site was transferred to two 250 ml conical flasks containing 90 ml sterilized distilled water and shaken vigorously for 10 minutes to dislodge the microbes adhering on the soil particles. This suspension was serially diluted by transferring 10 ml aliquots successively to six 250 ml conical flasks containing 90 ml sterilized water. Up to seven dilutions were made viz. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, $10^{-6,}$ and 10^{-7}. The last three dilutions were used for inoculation.

2.2. Pour plate method: One milliliter aliquots from each of the three dilutions (10^{-5} to 10^{-7}) were aseptically transferred to sterilized pertiplates (90mm dia.). To this was added 15-20 ml molten (40°C) Starch casein agar (Kuster and Williams, 1964) and swirled gently for uniform distribution of the inoculum with the medium. Five replicates were prepared for each dilution and for each soil sample. All the plates were incubated in dark up to 2-4 weeks. The plates were observed for actinomycete colonies. Selected colonies from each plate were sub-cultured on PDA slants and incubated for 7-10 days before screening for keratinolytic activity.

Starch Casein Agar (Kuster and Williams, 1964)

Starch	10.0 g
Potassium nitrate	2.0 g
Sodium chloride	2.0 g
Di-Potassium Hydrogen Phosphate	2.0 g
Magnesium sulphate	0.05 g
Calcium carbonate	0.02 g

Ferrous sulphate .....0.01 g
Casein .....0.30 g
Agar .....15.0 g
Distilled Water .....1000 ml
pH 7-7.2

3. Screening of actinomycetes for keratinolytic activity (Whole feather fermentation)

Based on the colony morphology and colour of aerial spore mass, diffusible pigments 25 actinomycete isolates were selected for screening for keratinolytic activity from site-1 and 220 isolates from site-2. All the actinomycetes were tested for feather degradation in submerged culture. Chicken feathers were collected from a local chicken processing shop. Structure of a typical feather is shown in Plate 3. The feathers were washed in tap water to remove blood, tissues and other dirt, rinsed 3 times with distilled water, dried overnight at 35°C. A single feather weighing 45-50 mg (5 cm length) was placed in a boiling tube (25 X 150 mm) containing 15ml mineral medium as described by Williams and Shih (1989) and sterilized by autoclaving at 121°C for 15 min. A loop full of spores and mycelium from each isolate was used to inoculate two replicate tubes. Uninoculated tubes served as control. All the tubs were incubated under laboratory conditions (28±2C) for 4-5 weeks. The tubes were agitated gently every alternate day and visually examined for growth and colonization on feather pieces. Strains that showed visible growth on the feathers were considered to be potentially keratinolytic / keratinophilic.

Ammonium chloride (NH_4 Cl)	0.5g
Sodium chloride (Na Cl)	0.5g
Dipotassium hydrogen phosphate (K_2HPO_4)	0.3g
Potassium dihydrogen phosphate (KH_2PO_4)	0.4g
Megnesium chloride (Mg Cl_2)	0.1g
Yeast extract	0.1g
Chicken feathers	10.0g
pH	7.5
Distilled water	1000ml

Two isolates one from each sample site with strong keratinolytic activity were selected for further studies.

4. Shake vs. still culture condition on feather degradation

Eight 250 ml flasks containing 50 ml mineral medium (pH 7.2) with 1% whole feather was prepared. Four flasks were inoculated with spores and mycelia of the selected isolates Ktn-1 and another four flasks with spores of SG-10. Two flasks were incubated on a rotary shaker (180 rpm) and two flasks were incubated in still conditions under laboratory conditions. The flasks were observed at 24 h intervals for lysis of feather. The flasks were photographed.

5. Feather degradation by cell free extract (crude enzyme)

Fifty milliliter culture filtrate of Ktn-1 and SG-10 from the above experiment (shake culture) was centrifuged at 10000 rpm for 10 minutes. The clear supernatant was used as crude keratinase enzyme (pH 8.1 & 8.3 respectively). Two 20 ml portions of each of the crude enzymes were aseptically transferred to four sterile boiling tubes. Then a single pre-sterilized feather (5cm length, weighing 45-50 mg) was introduced into each tube. All the tubes were incubated on a rotary shaker under laboratory conditions. The time required for complete lysis was visually recorded.

6. Effect of pH on feather degradation

Effect of medium pH on feather degradation by the two isolates was evaluated. pH of 200 ml portions of the mineral medium was adjusted to 4, 5, 6, 7, 8, 9, 10 and 11 before sterialization. Each of the 200 ml medium was equally distributed into four 250 ml conical flasks and 0.5 gram feather was added to each flask and

sterilized for 15 min at 15psi. All the flasks were inoculated with a loop full of spores and mycelium removed from 10days old cultures growing on PDA plates. All the flasks were incubated at 30°C in an orbital shaker at 180 rpm for 7 and 8 days. At the end of the incubation period pH of the culture filtrate was recorded and the keratinase activity (Takiuchi et al. (1982) and protein contents (Lowry et al.,1951) were estimated.

6.1. Keratinase assay

Prior to analysis, the fermentation broth was filtered. The filtrate was used as the crude enzyme. Keratinolytic activity was determined by a modified method used by Takiuchi et al. (1982). Keratin powder (20mg) (Himedia) was incubated with 3ml phosphate buffer (0.028 mol l^{-1}, pH 7.8) and 2ml culture filtrate for 1 hr at 35°C in a shaken water bath at 160 rev/ min. The enzyme reaction was stopped by adding 2ml 10% trichloroacetic acid (TCA) and the samples were put in a refrigerator at 4°C for 30 min. and then centrifuged for 15 min. at 10000 rpm. The absorption of the supernatant fluid was measured in a spectrophotometer at 280 nm towards the blank. The blank was treated in the same way except for the addition of TCA which was accomplished before the enzyme reaction. An increase of 0.01 in absorbance was taken to indicate one unit of enzyme activity. The results are taken as an average of three replicates.

6.2. Determination of protein concentration

The cell free supernatant fluid of each feather culture was used for the determination of soluble protein by the Folin phenol reagent method (Lowry et al., 1951) with bovine serum albumin as standard protein. To 1 ml of sample (culture

filtrate) 5 ml of freshly prepared alkaline copper sulphate was added and incubated for 10 min at room temperature. To this 0.5 ml of folinciocalteau's reagent was added and incubated for 20 min at room temperature and the absorbance was measured at 660 nm. The blank was prepared using the same procedure without sample. The protein content was estimated by calibration with the standard graph.

7. Effect of temperature on feather degradation

Effect of temperature on feather degradation by the two isolates was determined as detiled below. Twenty 50 ml portions of the mineral medium was transferred to 250 ml conical flasks and 0.5 gram feather was added to each flask and sterilized for 15 min at 15psi. Ten flasks were inoculated with a loop full of spores and mycelium removed from 10 days old Ktn-1 culture growing on PDA plates. The remaining 10 flasks were inoculated with the spore suspension of SG-10. Two flasks for each temperature range and for each culture were incubated in an orbital shaker at 180 rpm for 7 and 8 days. At the end of the incubation period pH of the culture filtrate was recorded and the keratinase activity and protein content were determined as mentioned above.

8. Characterization of the keratinase enzyme

8.1. Enzyme production

The two organisms were cultivated for 8 days in 50 ml whole feather medium pH 9 at 30C. At the end of eight day the culture filtrates were centrifuged separately at 5000 rpm for 5 min and the supernatants were used as crude enzyme.

8.2. Effect of temperature on enzyme activity

The optimal temperature for keratinase activity was determined by addition of 2ml crude enzyme to 3ml phosphate buffer (pH 7.5; 50mM/l) containing 15mg powdered keratin, and incubated at 20, 25, 30, 35, 40°C for 60 min. and the residual activity was determined.

8.3. Effect of pH on enzyme activity

The optimum pH was determined at 35°C using the following buffers (50 mMol/L): sodium phosphate buffer (pH 6.0~7.5), Tris-HCl buffer (pH 7.5~9.0), and Glycine/NaOH buffer (pH 9.0~10.0). The pH rang used were pH 5-11.

8.4. Effect of enzyme inhibitors on enzyme activity

The effects of enzyme inhibitor, and an organic solvent, mercaptoethanol, on keratinase activity were studied by assaying the enzyme activity as described above after pre-incubation with each chemical for 30 min at room temperature. The concentrations of the enzyme inhibitors are as follows: 5.0 mMol/L of phenylmethanesulfonyl fluoride (PMSF); 10.0 mmol/L ethylene diamine tetra acetic acid (EDTA); 1% (v/v) of mercatoethanol.

9. Feather degradation under optimized conditions

Two 250 ml conical flasks containing 50ml mineral medium, pH 9 and another two flasks containing 50 ml mineral medium, pH 10 were prepared. To all the flasks whole feathers, 5cm length, were added to get 1% final concentration. The flasks were sterilized at 15 psi for 20 min. Two flasks with medium pH 9 were inoculated with a loop full of spore suspension of SG-10 and the other two flasks with medium pH 10 were inoculated with the spore suspension of Ktn-1. All the flasks were incubated on a rotary shaker at 180 rpm for 10 days. After 10 days the

extent of feather degradation was visually recorded. The contents were then transferred to petriplates and photographed.

10. Determination of free aminoacids present in the feather lysate

The feather lysates from the above experiment was analyzed for free aminoacids released during feather degradation. Amino acids analysis was performed in an amino acid analyzer A-200 (Knauer, Germany) after hydrolysis of the samples in 6 N HCl for 24 h at 105°C.

11. Feather degradation under exposed condition on soil surface

The two selected isolates were tested for their ability to degrade the chicken feathers on soil surface. Feathers measuring around 5 cm were surface sterilized by immersing in 4% sodium chlorite for 4 minutes, washed in sterile distilled water and dried in oven for 5 min at 35C. Then spore suspension of each isolate was prepared by flooding 10 days old plate cultures with 20 ml of 1% starch solution containing 0.1% tween 20. The plates were gently scraped with inoculation needle to dislodge spores and mycelium. This spore suspension was used for infesting the feathers. Required number of feather of 5cm length were immersed in the spore suspension of Ktn-1 and SG-10 for 30 min. and placed on soil surface in petriplates. The edges of the plates were covered and sealed with parafilm to maintain the moisture content of the soil at 60% WHC and incubated under laboratory conditions. Control plates received uninfested feathers. All the treatments were replicated thrice. The plates were observed for colonization and degradation of feathers at weekly intervals up to 30 days.

12. Feather composting using keratinolytic actinomycetes

The two keratinolytic actinomycetes were used as inoculum for composting experiments. Six 250 g portions of garden soil were sterilized in 500 ml conical flasks for 30 min. at 20psi for 20 min. To this 2.5 g clean chicken feathers (treated with tween 20 for 10 min. then washed in sterile tap water) was added to each flask. Then 10 ml spore suspension of Ktn-1 was added to two flasks and SG-10 was added to two flasks along with required volume of sterile water to maintain 60% water holding capacity of the soil. The uninoculated flasks served as control. All the flasks were incubated for 30 days under laboratory conditions. The soil content in the flasks was mixed well by agitating at 5 d intervals.

13. Pasteurization of the compost

For pasteurization of the end product (compost), after 30 days of composting the two compost samples from the flasks were transferred to two polythene bags, labeled and exposed to solar radiation for 3 days. Control was also treated similarly. Compost core temperature during this period was monitored at midday to ensure achieving the temperature ranges above 50C. Each compost sample was turned regularly to ensure a homogeneous mixture during pasteutization.

14. Test for phytotoxic effect of the feather compost

Whether the compost has any phytotoxicity on seed germination and seedling growth was tested as follows. Ten uniform sized green gram seeds were surface sterilized by immersing in 4% sodium hypochlorite for 2 min., rinsed two times in sterile water and sown in plastic cups filled with the compost soil and control soil. Six seed were sown in each cup. The cups were watered regularly (60% water holding capacity) and maintained outdoors. For 10 days. After 3 days % seed germination

was recorded. After 10 days five seedling from each treatment including control were carefully removed from the cups and their root shoot length was recorded and the average was calculated for each parameter.

15. Scanning electron microscopy

The colony micro-morphology of both the keratinolytic actinomycetes was examined using light microscope as well as scanning electron microscope. For electron microscopy agar blocks ($2mm^2$) of ten days old cultures grown on PDA plates were cut out, fixed in 2.5% (w/v) glutaraldehyde in 0.1M phosphate buffer (pH 7.2) and left overnight. The sample was then dehydrated by passing through a series of increasing concentration of ethanol (10, 30, 50, 70, 90 and 100%). The preparations were then dried, mounted on an aluminum stub, sputter coated with gold, then observed with a scanning electron microscope (S3400, Hitachi, Tokyo, Japan) at 15kV at Pondicherry University, Pondicherry, South India.

16. Colony characterization

Methods and media described by the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966) were used to determine most of the cultural and physiological characteristics of the isolates Ktn-1 and SG-10. Spore chain and sporophore morphology of a mature colony was determined under light microscope. Colour determinations were made for (1). mature sporulating aerial surface growth, (2). colour of substrate mycelium as viewed from the reverse side, and (3). diffusible (soluble) pigments, other than melanin. Whenever diffusible colours other than brown or black were produced on any medium, the colour was recorded in simple terms (red, orange, yellow, green, blue and violet). Production of melanoid pigments on tyrosine agar (ISP-7) and tryptone-yeast extract agar (ISP-1) was also examined. A greenish

brown to brown or black diffusible pigments or a distinct brown pigment, modified by other colour, were recorded as positive. Absence of brown or black colours was recorded as negative for melanoid pigment production. The various media used and their composition (g/L) is given below:

ISP 1 - Tryptone-Yeast extract agar

Tryptone	5.0 g
Yeast Extract	3.0 g
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

ISP 2 - Yeast extract- malt extract agar

Yeast Extract	4.0 g
Malt Extract	10.0 g
Dextrose	4.0 g
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

ISP 3 - Oatmeal Agar

Oatmeal	20.0 g
Trace salts soution	1.0 ml
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

ISP 4 - Inorganic salts-starch agar

Soluble starch	10.0 g
Di-potassium hydrogen phosphate	1.0 g
Magnesium sulphate	1.0 g
Sodium chloride	1.0 g
Ammonium sulphate	2.0 g
Calcium carbonate	2.0 g
Trace salts solution	1.0 ml
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

ISP 5 - Glycerol-asparagine agar

L-asparagine	1.0 g
Glycerol	10.0 g
Di-potassium hydrogen phosphate	1.0 g
Trace salts solution	1.0 ml
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

ISP 7 - Tyrosine agar

L-asparagine	1.0 g
L-Tyrosine	0.5 g
Glycerol	15.0 g
Magnesium sulphate	0.5 g
Sodium chloride	0.5 g
Ferrous sulphate	0.01 g
Di-potassium hydrogen phosphate	1.0 g
Trace salts solution	1.0 ml
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

Benett's agar

Glucose	10.0 g
Yeast extract	1.0 g
Beef extract	1.0 g
N-Z-Amine A (Casein)	2.0 g
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

Czapek's agar

Sucrose	30.0 g
Sodium nitrate	2.0 g
Magnesium sulphate	0.5 g
Potassium chloride	0.5 g
Ferrous sulphate	0.01 g
Di-potassium hydrogen phosphate	1.0 g
Trace salts solution	1.0 ml
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

Potato dextrose Agar

Potato (peeled and sliced)	200 g
Dextrose	20 g
Agar	15 g
Distilled Water	1000 ml
pH	6.5

Nutrient agar

Peptone	10.0 g
Beef extract	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
pH	7 ± 0.1
Distilled water	1000 ml

Trace salt solution

Ferrous sulphate	0.1 g
Manganese chloride	0.1 g
Zinc sulphate	0.1 g
Distilled water	100 ml

17. Enzyme activity

Amylase

The medium was dispensed into required number of plates and allowed to solidify. The plates were then streaked with spores of Ktn-1 and SG-10 and incubated at room temperature for 10 days. Then the plates were flooded with 2% KI solution. A clear zone around the colony indicates amylase activity.

Medium composition:	
Peptone	5.0 g
Yeast extract	1.5 g
Beef extract	1.5 g
Sodium chloride	5.0 g
Soluable starch	10.0 g
Agar	16.0 g
Distilled water	1000 ml
pH	7.0

Gelatinase:

The cultures were streak inoculated on agar surface in Petri plates, incubated at 28±2°C. After 7 days plates were flooded with 15% mercuric chloride dissolved in 20% HCl for 5 min. and the reagent was discarded. A clear zone around the colony against milky white background indicates gelatinase activity.

Medium composition

Peptone	5.0 g
Yeast extract	1.5 g
Beef extract	1.5 g
Sodium chloride	5.0 g
Agar	16.0 g
Gelatin	40.0 g
Distilled water	1000 ml
pH	7.0

Urease

5 ml of 40% urea solution (filter sterilized) was added to sterilized molten medium, made into 100 ml and poured into test tubes and slanted. Spores of Ktn-1 and SG-10 was streaked on to the slants, incubated at room temperature for 48 hr. Change in colour of the medium from orange to deep pink indicates production of urease.

Christensen's Urea agar

Peptone	1.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Di-potassium hydrogen phosphate	2.0 g
Phenol red	0.12 g
Agar	18.0 g
Distilled water	1000 ml
pH	8.0

Lipase

The basal medium (99ml) and 5ml of Tween 20, were autoclaved separately. Then 1ml of Tween 20 was added to the molten medium mixed and poured in to five

plates. The plates were streaked with the spores of isolates Ktn-1 and SG-10 and incubated for three to eight days. Clearing or precipitation around the actinomycete colony indicates lipolytic activity.

Basal Medium

Glucose	1.0 g
Peptone	15.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	950 ml
pH	7.5

Catalase

A loop full of spores and mycelium of Ktn-1 and SG-10 was transferred to a sterile glass slide; a drop of 3% hydrogen peroxide was added on to the culture immediately and observed for effervescence. Evolution of effervescence indicates production of catalase.

Protease

Protease activity was determined using the above medium but gelatin was replaced by casein. A clear zone around the colony indicates protease activity.

Cellulase

A loop full of the cultures were inoculated on cellulose amended medium and incubated for seven days. The plates were flooded with a mixture of 0.1ml HCl and five ml of 2% potassium iodide for three to five minutes and observed for clear zone around the colony against reddish brown back ground for cellulase activity.

Sodium nitrite	2.0 g
Di-potassium hydrogen phosphate	1.0 g
Magnesium sulphate	0.05 g
Potassium chloride	0.05 g
Ferrous sulphate	0.01 g
Cellulose	10.0 g

Agar	16.0 g
Distilled water	1000 ml

Citrate utilization

The two isolates (Ktn-1, SG-10) were streaked on to Simmon's citrate agar slants and incubated at room temperature for 48 hr. Change in colour of the medium from green to Persian blue indicate the ability of the strain to utilize citrate.

Simmons citrate agar

Ammonium hydrogen phosphate	1.0 g
Di-potassium hydrogen phosphate	1.0 g
Sodium chloride	5.0 g
Magnesium sulphate	0.2 g
Bromothymol blue	0.08 g
Sodium citrate	2.0 g
Agar	18.0 g
Distilled water	1000 ml
pH	6.8

Indole production

A loop full of the culture of Ktn-1and SG-10 was used to inoculate the test tubes containing 20 ml indole broth and incubated at room temperature for 7 to 10 days. Production of indole derivatives by the isolate was determined by the addition of Kovac's reagent. Formation of a red color ring in the tubes indicates a positive reaction.

Peptone	20.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml
pH	7.0 – 7.2

Kovac's reagent

P-dimethlyamino benzaldehyde	5.0 g
Iso amyl alcohol	75.0 ml
Con. HCl	25.0 ml

Phosphatase

Phosphatase activity was determined on Pikovaskaya's agar plates streaked with the spore suspension of Ktn-1and SG-10 and incubated for eight to ten days at 28°C (Pikovaskaya, 1984). Appearance of clear zone around the colonies indicates phosphatase activity.

Pikovskaya's agar medium

Yeast extract	5.0 g
Dextrose	10.0g
Calcium phosphate	5.0g
Ammonium sulphate	0.5g
Potassium chloride	0.2g
Magnesium chloride	0.1g
Manganese sulphate	0.0001g
Ferrous sulphate	0.0001g
Agar	15.0g
Distilled water	1000 ml
pH	7.0

Nitrate reduction activity

The two isolates was grown in tryptone nitrate broth for 48 hr. to which 0.5 ml sulphanilic acid and α-napthylamine reagents were added. Development of red colour indicates ability of the organism to reduce nitrate.

Tryptone nitrate broth

Tryptone	2.0 g
Dextrose	1.0 g
Potassium nitrate	1.0 g
Agar	1.0 g
Distilled water	1000 ml
pH	7.2

Reagents

Solution-A

Sulphanilic acid	0.008 g
5N acetic acid	10.0 g

Solution-B

α-napthylamine	0.005 g
5N acetic acid	10.0 g

18. Growth in different temperatures

Growth in different temperatures was tested by incubating PDA slants inoculated with spore suspension of Ktn-1and SG-10 at the following temperatures 20, 25, 30, 35, 40, and 45°C in a BOD incubator. Observations were recorded after 8-10 days.

19. Sodium chloride tolerance

For salt tolerance studies Glucose-yeast extract-malt extract agar (ISP-2) was used as the basic medium. The NaCl concentrations (W/V) used were: 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16% and 18%. The slants were inoculated by streaking the agar surface with a loopful of spore suspension of the isolate Ktn-1. The inoculated tubes were incubated at 25±2°C. The growth response was recorded after 15 days.

Medium composition

Glucose	4.0 g
Yeast extract	4.0 g
Malt extract	10.0 g
Agar	15.0 g
Distilled water	1000ml
pH	7.2

20. Utilization of different carbon sources

Ability of isolates Ktn-1 and SG-10 to use 20 different sugars as sole carbon sources for energy and growth was examined in carbon utilization medium (ISP-9) suggested by Shirling and Gottlieb (1966). The various carbon compounds were added to 5 ml liquid medium as discs (Himedia) impregnated with the respective compound to the final concentration of 1%. The tubes were inoculated with 100 µl of spore suspension of Ktn-1 and SG-10. All the inoculated tubes were incubated at

25±2°C for 10-15days. The test strain was also inoculated in the basal medium without glucose as a negative control and medium with glucose (1%) as positive control. A positive result was recorded when growth was greater than that in the negative control and that equal to or less than that in the negative control as negative. Composition of the medium is given below:

A. Basal mineral salts broth

Ammonium sulphate	2.46 g
Potassium di-hydrogen phosphate	2.38 g
Di-potassium hydrogen phosphate	5.56 g
Megnesium suphate	1.0 g
Pridham & Gottlieb trace salts solution (B)	1.0 ml
Distilled water	1000 ml
pH	7-7.2

B. Pridham & Gottlieb trace salts

Copper sulphate	6.40 mg
Ferrous sulphate	1.10 mg
Manganese chloride	7.90 mg
Zinc sulphate	1.50 mg
Distilled water	100 ml

21. Susceptibility to antibiotics

Susceptibility to antibiotics was tested as follows: Discs (diameter, 6.5mm) impregnated with antibiotics (Himedia) were laid on potato dextrose agar (PDA) plates which had been surface inoculated with the spore suspension of Ktn-1 and SG-10. The following ten antibiotic discs (mg/disc) were used: amoxycillin (25 mg), ampicillin (25 mg), chloramphenicol (25 mg), clindamycin (10 mg), erythromycin (15 mg), kanamycin (30 mg), rifamycin (30 mg), streptomycin (25 mg), tetracycline (30 mg) and vancomycin (10 mg). After 24-48 hours inhibition zones were recorded.

22. Molecular characterization of the isolate Ktn-1

In addition to the cultural characteristics of Ktn-1 and SG-10, the taxonomic identity of the isolates was further analyzed at molecular level by partial sequencing the 16S rRNA gene.

22.1. Genomic DNA isolation

The total genomic DNA from Ktn-1 and SG-10 was isolated by following standard methods (Boudjella et al. 2006). Briefly, the strain was grown in tryptic soy broth (TSB) for five days and the mycelium was separated by centrifugation and washed thrice with distilled water. Approximately 200 mg of mycelium was suspended in 800 µl of lysis solution (100 mM Tris HCl, pH 7.5, 20 mM EDTA, 250 mM NaCl, 2% SDS, 1 mg/ml lysozyme); to the cleared lysate, 5 µl of RNase (50 mg/ml) was added and incubated at 37°C for 3 hr. Then, 10 µl of proteinase K solution (20 mg/ml) was added and incubated at 37°C for 1 hr. The lysate was extracted with an equal volume of phenol : chloroform (24:1), then centrifuged to obtain the aqueous phase. DNA was precipitated by adding 2 volumes of 95% ice-cold ethanol to the aqueous phase. After centrifugation, the DNA pellet was washed twice with 70% ethanol and suspended in 50 µl of TE buffer (10 mM Tris HCl pH 7.4 and 1 mM EDTA pH 8). The DNA was tested for purity and quantity by spectrophotometer at 260 and 280 nm.

22.2. Amplification of 16S rRNA amplicon

Genomic DNA was used as template for polymerase chain reaction (PCR) amplification. For 16S ribosomal RNA (rRNA) gene amplification, the following primers- forward primer:- 5'- A G A G T T T G A T C C T G G C T C A G -3' and reverse primer- 5'- A C G G C T A C C T T G T T A C G A C T T-3' were used. The

PCR cocktail (50 µl) contained 50 pM of primer, 50 ng of genomic DNA, 1X *Taq* DNA polymerase buffer, 1 U of *Taq* DNA polymerase (Promega, Madison, USA), 0.2 mM of each dNTPs, and 1.5 mM $MgCl_2$. Amplification was performed in a DNA thermal cycler (2400 Cycler, Perkin Elmer International, Rotkreuz, Switzerland) at 95°C for 3 min followed by 30 cycles consisting of denaturation (1 min at 95°C), annealing (1 min at 58°C) and extension (2 min at 72°C), with a final extension at 72°C for 5 min.

22 .3. Agarose gel electrophoresis, purification and sequencing of 16S rRNA

A 5-µl aliquot of each amplification product was electrophoresed on a 0.7% agarose gel in 1x TAE buffer at 50 V for 45 min, stained with ethidium bromide, and the PCR products were visualized on UV transilluminator. PCR products were purified using quick PCR purification kit (Bangalore Genei, Bangalore, India). Purified PCR products were sequenced in an automated ABI Prism 377 DNA Sequencer.

5. RESULTS

Isolation of actinomycetes

A variety of actinomycetes were isolated from the soil samples collected from feather decomposing site as well as from the sacred grove by soil dilution technique. Actinomycete colonies started to appear on the isolation plates from 4th day onwards. However, plates were incubated for 2-4 weeks. The number of colonies increased with incubation time (Plate 4). A total of 820 colonies were counted in all the isolation plates in sample 1. From this, 25 colonies with different colony morphology (colony size, margin of colony, color of spore mass, diffusible pigment, reverse color etc.) were selected and subcultured on PDA slants and incubated under laboratory conditions until visible growth and sporulation occurred (Plate 5). Similarly 1020 colonies were counted in sacred grove soil plates and 220 were selected for screening. Then all the isolates were numbered and stored in refrigerator until used.

Screening for keratinolytic activity

The selected actinomycete isolates were screened for keratinolysis in submerged culture. Screening results of keratinolytic activity of the actinomycetes is presented in Table 3. The first sign of feather degradation was evident on the 4th day after inoculation. Such potential isolates multiplied faster than the non-keratinolytic

Table 3. Summary of screening for keratinolytic activity of actinomycetes from feather dumping site and sacred grove

Source of soil sample	Activity	Feather degradation within			Total No. of Isolates
		10 d	20 d	30 d	
Feather dumping site	Complete lysis	2	1	1	4
	Partial lysis	-	2	2	4
	Non-keratinolytic	-	-	-	17
Sacred grove soil	Complete lysis	6	10	11	27
	Partial lysis	-	15	25	40
	Non-keratinolytic	-	-	-	153

isolates. Some isolates showed little growth others did not show much growth or minimal. Among the 25 isolates 17 isolates were non-keratinolytic. Only four isolates completely lysed the feather (Isolates Ktn-1, Ktn-8, Ktn-10 and Ktn-16) (Plate 6). Additional four isolates showed partial degradation (Isolates Ktn-3, Ktn-6, Ktn-9 and Ktn-20). Among the four isolates which showed complete lysis, the time taken for complete degradation varied. The isolate Ktn-1 was faster and degraded the feather within 15 days, Isolates Ktn-8 and Ktn-16 took 20 days and Ktn-10 took more than 25 days (Plate 6). Similarly those isolates which showed incomplete degradation also varied in their ability. Therefore the Isolate Ktn-1, which lysed feathers rapidly, was selected for further study from sample-1. Out of 220 isolates tested for keratinolytic activity from sacred grove soil 67 isolates (30.45%) degraded feather. In this, 27 isolates exhibited complete lysis and 40 isolates were partial degraders (Plate 7a &b). The remaining 153 isolates were non-keratinolytic. However, most of the non-keratinolytic forms also showed considerable growth in the feather medium, indicating that they are keratinophilic. They grew with the little nutrients available on

the surface of the feathers. From the sacred grove, one isolate SG-10 with strong activity was selected for further study. Pure culture of the two isolates is shown in Plate 8.

Effect of still and shake culture conditions on feather degradation by the Isolates Ktn-1 and SG-10 was evaluated. Feather degradation in shake culture was accomplished in 7-8 days, whereas in still culture the process was slow and took 13-15 days (Table 4). The cell free culture filtrate (crude enzyme) (from shake culture) also brought about complete dissolution of the feather within 9 days in shake condition whereas in still condition took longer and was completed in 14 days.

Table 4. Influence of growth conditions on feather degradation

Isolate	Culture condition	Time taken for lysis	Final pH of the medium	keratinolytic activity(U/ml)
Ktn-1	Still culture (28±2°C)	15 days	8.1	87
	Shake culture (180 rpm; 28±2°C)	8 days	8.6	115
SG-10	Still culture (28±2°C)	13 days	8.3	91
	Shake culture (180 rpm; 28±2°C)	7 days	8.7	122

Effect of temperature and pH on feather degradation

Feather degradation by the isolates Ktn-1 and SG-10 in different pH and temperature was evaluated. Feather degradation efficiency of the isolate Ktn-1 in terms of keratinase activity and protein accumulation in the culture filtrate in different pH is presented in Figure 1 & 2. Keratinase activity was recorded in all the pH intervals investigated. The enzyme activity gradually increased from pH 5 and reached maximum in pH 10 (Plate 9a) and then decreased. A similar trend was noticed in protein content also. Protein content in the culture filtrate slowly increased

from pH 5 and reached maximum at pH 10 then declined. Effect of different temperatures on feather degradation by Ktn-1 is presented in Figure 3 & 4. Temperature exerted profound influence on feather degradation. Keratinase activity was lowest at the temperature 20°C, but gradually increased and reached maximum at 35°C and then declined. The protein content in the culture filtrate also followed the same pattern in the temperature ranges tested. The protein level was low at 20°C then gradually increased up to 35°C and then decreased.

Influence of medium pH on feather degradation by SG-10 and protein accumulation in the culture broth is presented in Fig. 5 & 6. Though feather lysis was evident from pH 5 onwards, maximum activity was recorded at pH 9 as evidenced by complete degradation of feathers (Plate 9b). From pH 9 to 11 complete degradation of feathers occurred. Effect of different temperatures on feather degradation by SG-10 is presented in Figure 7 & 8. Feather degradation was greatly affected by incubation temperature. Keratinase activity was lowest at the temperature 20°C, but gradually increased and reached maximum at 35°C and then declined. The protein content in the culture filtrate also followed the same pattern in the temperature ranges tested. The protein level increased from 20°C up to 35°C and then decreased.

Degradation under optimized conditions and aminoacid analysis

In this experiment the two keratinolytic actinomycetes were again evaluated for keratinolytic activity under optimized conditions. pH 9 for SG-10 and 10 for Ktn-1, temperature 25□C for SG-10 and 35□C for Ktn-1 under shake condition (180 rpm) for 8 days. Almost complete disintegration of feathers was achieved at the optimized conditions (Plate 10). The feather lysates were analyzed for free aminoacids released during feather degradation. In the present study, 20 aminoacids

were detected in the feather hydrolysate and quantified. The amount of individual aminoacids varied with the two isolates used in this study. Sixteen aminoacids were found to be higher in the feather lysate of SG-10 than the feather lysate of Ktn-1 (Table 5). Only four aminoacids were higher in the feather lysate of Ktn-1. In the case of SG-10 histidine (803mg/100ml) was present in the highest concentration, followed by tyrosine (643mg), aspartic acid (634mg), glutamic acid (592mg) and thereonine (519mg) and others. The amount of arginine (119mg) was the lowest in the fermentation broth.

Table 5. Free aminoacids released during feather degradation by Ktn^{-1} and SG-10

Sl.No	Aminoacid	Ktn-1 **(mg)**	SG-10 **(mg)**
1	Alanine	193	335
2	Arginine	222	119
3	Aspargine	202	219
4	Aspartic acid	495	634
5	Cysteine	305	494
6	Glutamic acid	314	592
7	Glutamine	291	194
8	Glycine	119	304
9	Histidine	193	803
10	Isoleucine	134	305
11	Leucine	205	483
12	Lysine	119	194
13	Methionine	193	294
14	Phenylalanine	119	355
15	Proline	205	335
16	Serine	315	404
17	Thereonine	305	519
18	Tryptophan	309	204
19	Tyrosine	293	643
20	Valine	201	193

In the case of Ktn-1 aspartic acid was present in the highest concentration (495mg) followed by serine (315mg) glutamic acid (314mg), tryptophane (309mg),

cysteine and thereonine (305mg) and others. Glycine, lysine and phenylalanine (119mg) were the least. These values are higher than the previous reports on aminoacid released during feather degradation. Presence of other essential aminoacids like leucine and isoleucine indicates that feather treatment with the keratinolytic actinomycetes Ktn-1 and SG-10 resulted in a feather meal with rich nutrient value. The present study clearly indicates that the feather degradation by SG-10 and Ktn-1 is not only economical but also a viable process for better utilization of the much neglected feather wastes.

Characteristics of the keratinase produced by isolate Ktn^{-1} and SG-10

Partial characterization of the extra cellular keratinase produced by the isolates Ktn-1 and SG-10 is presented in Table 6. The enzyme showed optimum temperature

Table 6. Characteristics of the keratinase produced by the actinomycete isolates Ktn-1 and SG-10

Treatment	Residual Activity (%)	
	Ktn-1	SG-10
20	32	25
25	49	64
30	45	50
35	48	41
40	35	30
5	24	40
6	29	42
7	36	76
8	44	84
9	62	89
10	81	81
11	71	78
EDTA 5mM/L	70	86
PMSF 10mM/L	25	38
Mercaptoethanol 1%	96	111

PMSF: Phenylmethylsulphonyl fluoride,
EDTA: Ethylenediaminetetraacetic acid

of 35°C and pH 10. Among the two enzyme inhibitors tested PMSF produced strong inhibition while EDTA brought about partial inhibition suggesting that the keatinase could be a serine protease. The reducing agent mercapto ethanol enhanced keratinase activity. The keratinase produced by SG-10 had optimum temperature of 25C and pH 9. The enzyme inhibitor PMSF strongly inhibited the keratinase activity whereas EDTA brought about slight inhibition. Here also mercapto ethanol enhanced keratinase activity.

Feather degradation under exposed conditions

Visible growth and colonization of the feathers by both isolates started on 3rd day after incubation. However, there was slight difference in the rate of colonization between the two isolates. Colonization was extensive by Ktn-1 on 10th day and part of the feather was already disintegrated (Plate 11). In the case of SG-10 though there was complete colonization of the feathers, degradation was slow. Along with the actinomycete growth on the feathers some fungal growth was also seen. Further observations on 20th and 30th day the feather barbules were almost completely digested by Ktn-1, but SG-10 was little slow. Under exposed and undisturbed conditions on soil surface around 80% of feather was degraded by both the keratinolytic actinomycetes. Along with the actinomycetes soil fungi might also be involved in degradation of the chicken feathers.

Feather composting using keratinolytic actinomycetes

Feather composting experiment showed clearly that feathers can be turned into compost using the right kind of microbes. Both the isolates degraded the feathers in soil environment (Plate 12). The remaining pieces of feathers crumbled into powder when crushed with fingers. In control soil no degradation at all and the feathers were

tough and resisted any crushing effect applied. At the end of the composting period the shafts were still intact and quills remained on the barbs. Microscopic examination showed intact barbules and without any structural damage. In the inoculated soil the barbules were completely degraded due to enzymatic as well as physical disintegration by the actively colonizing keratinolytic actinomycetes. However, the remaining feather pieces/shafts in the compost soils disintegrated into fine powder when crushed by hands. In addition there was reduction of foul odor in inoculated compost than in control. Further it was observed that both composts retained considerable moisture than control. The control displayed poor water holding capacity with a moisture content of 9% at the end of the compositing period. The compost inoculated with Ktn-1 had moisture content of 20% and the compost inoculated with SG-10 had 30% moisture content. Bulk density analysis after pasteurization showed there was increase in bulk density in both compost soils than control.

Next influence of feather compost on green gram seed germination and seedling growth was tested by growing the seeds in the compost in cups (Table 7). In both control and the treatments 100% seed germination was recorded. However, emergence of seedling was slightly delayed in Ktn-1 compost. No such difference was noticed in the seedling growing in SG-10 compost. After ten days the seedlings were removed from the cups and root, shoot lengths were recorded. Plants in SG-10 compost were slightly taller than control and Ktn-1 compost (Plate 13a &b). Some chemical component in the Ktn-1 compost seem to inhibit growth and root and shoot elongation.

Cultural characteristics of the isolate Ktn-1 and SG-10 were examined using a range of media recommended by ISP and other workers. The isolate Ktn-1 grew well

on ISP-1, 3, and 4 and PDA. Moderate growth was observed on ISP- 5, and 7, and NA; poor growth on BA, and no growth on CZ. Aerial mycelium was white in ISP- 1,

Table 7. Effect of feather compost on green gram seed germination and seedling growth

S. No.	Isolate	% Germination	Seedling height (mm)	
			Root	Shoot
1	Control	100	55	124
2	Ktn-1	100	45	116
3	SG-10	100	62	132

2, 3, 4, 5, 6 and 7, and NA and PDA. No aerial mycelium was produced in ISP-2 and 6. Basal mycelium was colorless to creamy yellow. Reverse color was light brown to creamy yellow to colorless. No diffusible pigment or melanin was produced (Table 8a). The isolate SG-10 grew well on ISP-1, 2, 3, and 4 and NA. Moderate growth was observed on ISP- 5, 6, and 7, and PDA; poor growth on CZ, and no growth on BA. Aerial mycelium was white to dirty white in all the media tested. Basal mycelium was light yellow to light brown. Reverse color was light brown to creamy yellow to colorless. No diffusible pigment or melanin was produced (Table 8b).

Table 8a. Colony characteristics of isolate Ktn-1

Medium	Colony characters				
	Growth	Aerial mycelium	Reverse side	Diffusible pigment	Melanin
ISP – 1	++ +	white	cream	-	-
ISP - 2	+ +	bac. like	L.brown	-	-
ISP - 3	+ + +	white	Y.brown	-	-
ISP - 4	+ + +	white	colourless	-	-
ISP - 5	++	white	colourless	-	-
ISP - 6	+ +	bac.like	L.brown	-	-
ISP - 7	+ +	white	white	-	-
CZ	-	-	-	-	-
NA	++	white	cream	-	-
PDA	+ + +	white	cream	-	-
BA	+	white	cream	-	-

+ = poor ; ++= moderate ; +++ = good; Bac.like= bacteria like; L.brown= light brown; Y.brown=yellowish brown

Table 8 b. Colony characteristics of isolate SG-10

Medium	Colony characters				
	Growth	Aerial mycelium	Reverse side	Diffusible pigment	Melanin
ISP – 1	++ +	white	L.yellow	-	-
ISP - 2	++ +	dirty white	L.brown	-	-
ISP - 3	+ + +	white	brown	-	-
ISP - 4	+ + +	dirty white	brown	-	-
ISP - 5	++	white	yellowish brown	-	-
ISP - 6	+ +	white	colourless	-	-
ISP - 7	+ +	white	L.brown	-	-
CZ	+	white	L.brown	-	-
NA	+++	dirty white	L.brown	-	-
PDA	+ +	white	colourless	-	-
BA	++	dirty white	L.brown	-	-

The physiological and biochemical responses of isolate Ktn-1 and SG-10 are listed in Table 9. Isolate Ktn-1was able to grow at temperatures between 20 to 40°C, with an optimum at 35°C; tolerated NaCl up to 8%, maximum growth was recorded at 2% NaCl concentration. Grew at pH ranging between 5-11. However, better growth was recorded at pH 9 and10; no growth was observed at pH 12. It was positive for amylase, protease, urease, lipase, gelatinase, catalase, and phosphatase and negative for cellulase. Neither reduced nitrate nor utilized citrate. No indole was produced. The physiological and biochemical characteristics of the isolate SG-10 are presented in Table 9. Better growth occurred only at 25°C, moderate growth at 30C and poor growth at all other tested temperatures. Tolerated NaCl upto 8%, maximum growth was recorded at 2% NaCl concentration. Grew at pH ranges 5-11. However, better growth was recorded between pH 5 - 9; no growth in pH 12. The isolate was positive for amylase, protease , lipase, gelatinase and catalase; Negative for cellulose, phosphatase and urease. Did not reduce nitrate nor utilized citrate. No indole was produced.

Table 9. Physiological and biochemical properties of isolate Ktn-1

Reaction	Isolate Ktn-01	Isolate SG-10
1. Melanin Reaction		
Medium ISP-1.	Negative	Negative
Medium ISP-7	Negative	Negative
2. Tyrosine Reaction		
Medium ISP-7	Negative	Negative
3. Soluble pigment	Negative	Negative
4. Amylase	positive	Positive
5. Protease	Positive	Positive
6. Urease	Positive	Negative
7. Lipase	Positive	Positive
8. Gelatinase	Positive	Positive
9. Cellulase	Negative	Negative
10. Catalase	Positive	Positive
11. Phosphatase	Positive	Negative
12. Indole production	Negative	Negative
13. Citrate utilization	Negative	Negative
14. Nitrate reduction	Negative	Negative
15. Growth at (°C)		
20°C	Poor growth	Poor growth
25°C	Good growth	Good growth
30°C	Good growth	Moderate growth
35°C	Good growth	Poor growth
40°C	Moderate growth	Poor growth
45°C	Poor growth	Poor growth
16. $NaCl_2$ Tolerance		
0%	Good growth	Good growth
2%	Good growth	Good growth
4%	Good growth	Moderate growth
6%	Good growth	Moderate growth
8%	Moderate growth	Moderate growth
10%	No growth	Poor growth
17. Growth at pH		
5	Moderate growth	Good growth
6	Moderate growth	Good growth
7	Moderate growth	Good growth
8	Moderate growth	Good growth
9	Good growth	Good growth
10	Good growth	Moderate growth
11	Moderate growth	Poor growth
12	No growth	No growth

Utilization of different carbon sources by the isolates Ktn-1 and SG-10 are recorded in Table 10. Isolate Ktn-1 grew well in the medium amended with arabinose, cellobiose, dextrose, fructose, inulin, lactose, maltose, mannose, raffinose, sucrose

Table 10. Utilization of different carbon sources by isolate Ktn-1

Utilization & Growth	Carbon source	
	SG-10	Ktn-01
Good growth	Arabinose, Cellobiose, Dextrose, Galactose , Fructose, Lactose, Maltose, Mannose, Salicin, Trehalose, Xylose	Arabinose, Cellobiose, Dextrose, Fructose, Inulin, Lactose, Maltose, Mannose, Raffinose, Sucrose, Xylose
Moderate	Lactose, Sucrose,	Salicin
Poor growth	Rhamnose	Galactose, Trehalose, Inositol
No growth	Adonitol, Dulcitol, Inulin, Inositol, Melibiose, Raffinose , Sorbitol	Adonitol, Dulcitol, Mannitol, Melibiose, Rhamnose, Sorbitol,

and xylose. Growth was moderate in salicin; poor growth galactose, trehalose and inositol. No growth in adonitol, dulcitol, mannitol, melibiose, rhamnose and sorbitol amended medium. Isolate SG-10 grew well in the medium amended with arabinose, cellobiose, dextrose, fructose, galactose, lactose, maltose, mannose, salicin, sucrose, trehalose and xylose and grew moderately in sucrose, and lactose; poor growth in rhamnose. No growth in adonitol, dulcitol, inulin, inositol, melibiose, raffinose and sorbitol.

Reaction of the two isolates to certain antibiotics is presented in Table 11. The isolate Ktn-1 was resistant to amoxicillin (25mg) and ampicillin (25mg). Highly sensitive reaction was noticed for the remaining antibiotics such as rifamycin (25mg), tetracycline (25mg) chloramphenicol (25mg), erythromycin (25mg), streptomycin (25mg), Clindamycin (25mg) and kanamycin (25mg) and vancomycin (25mg). The

Table 11. Sensitivity of isolates Ktn-1 and SG-10 to antibiotics

Sl. No	Code HiMedia	Name of the antibiotic	Symbol	Mg/ disc	Sensitivity /Resistance Zone (cm)	
					Ktn-1	SG-10
1	SD125	Amoxicillin	A	25	0.0 Rs	0.0 Rs
2	SD007	Ampicillin	AM	25	0.0 Rs	0.0 Rs
3	SD153	Chloramphenicol	C	25	2.4 Hs	2.5 Hs
4	SD164	Clindamycin	CD	10	1.0 Ss	0.0 Rs
5	SD013	Erythromycin	E	15	2.5 Hs	2.0 Ss
6	SD017	Kanamycin	K	30	4.0 Hs	3.5 Hs
7	SD127	Rifamycin	R	30	4.0 Hs	3.0 Hs
8	SD091	Streptomycin	S	25	5.3 Hs	4.3 Hs
9	SD037	Tetracycline	T	30	2.3 Hs	4.2 Hs
10	SD163	Vancomycin	Va	10	3.5 Hs	3.2 Hs

(Rs- Resistant; Hs- Highly sensitive; Ss-Sensitive)

isolate SG-10 was resistant to amoxicillin (25mg), ampicillin (25mg) and clindamycin (10 mg) and highly sensitive chloramphenicol (25mg), rifamycin (25mg), tetracycline (25mg), erythromycin (25mg), streptomycin (25mg), kanamycin (25mg) and vancomycin (25mg).

Examination of the isolate Ktn-1 and SG-10 by scanning electron microscope revealed the presence of unfragmented aerial filaments and smooth spores that were produced in straight chains as well as in coils, a characteristic feature of *Streptomyces* sp. (Plate 14a & b).

Finally attempt was made to identify the two isolates based on 16S rRNA gene sequence homology analysis. The sequences have been submitted to Gene Bank (accession number awaited). The nucleotide sequence and the phylogenetic tree are presented below:

16s r RNA Sequence of Ktn - 1

Forward sequence:

TATCCTATACATACACAGGGCGGACGACGGGGTAGCCGGCCTGAGAGGGCGACC

GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG

AATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACG

GCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG

CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGC

AAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCG

GGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAG

AGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATC

AGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGA

GCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA

CGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCA

TTAAGTTCCCCGCGTGGGGGAATTACGGGA

Reverse sequence:

AATTGGTTTCCGGCCAAACCAACCTTAGGTTTCCCAACGTTTTACGGCGTGGGAA

CTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTA

ATGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCA

CCGCTACACCAGGAATTCCGATCTCCCCTACCACACTCTAGCCTGCCCGTATCGG

ATGCAGACCCGGGGTTAAGCCCCGGGCTTTCACACCCGACGTGACAAGCCGCCTA

CGAGCTCTTTACGCCCAATAATTCCGGACAACGCTTGCGCCCTACGTATTACCGC

GGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTTTCGCTT

CTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTC

GCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGA

GTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCGGCTACC

CGTCGTCGCCTTGGTGAGCCGTTACCTCACCAACAAGCTGTAAGGCCGCGA.....

16s r RNA Sequence of SG-10

SG-10F (Forward sequence)

ATGATAATTGCCACAAAAGGCGAAGACAGGGTAGCCGGCCTGAGAGGGCGACCG

GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA

ATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGG

CCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGC

AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCA

AGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGG

TTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGA

GTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCA

GGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAG

CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC

GGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCAT

TAAGTGCCCAGCGTGGGGAAGTAACGGAACGTGGGAATGTTGCCCACACCTAGT

GCCCACCGTTTACGGCGTGAACCACCAGGGTATCTAATCCTGTTCGCTCCCCACG

CTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGATCCGCCTCGCCACGGGTGTTCC

TCCGGATATCTGCGCATTTCACCGCTCACCAGGAATTCCGATTTCCCTACCGAACT

CTACCCGCCCGTATCGGCGGCAGACCCGGGGTTTAGCCCGGGCTTCACACGACAT

GACAGCCGCCACAGCTCTATACGCCAGCATTCAGACACCCTGCGGCCTACGTAAT

TACGCGCCGCTGGCACGTAATTGACAGCGCTTCTTCTGCACGTACGGTGACTGTG

CTTCTTGCCTGCTGAAGGAGAGTGTTATACCCGAAAGGCGTCGTCCTCAGGGGGC

GTGCTAGCAGCAGGCTTTCCCCATGTGGAAATATGCCACTGCTGCCTCGATAGAG

TCTGAGCTGGTCTCACTTCAGTGTGGCGATGCCCACTCAAGCGCTACCAGCATTC

ATGGATAGGCCCATAGCTTACCACGTTTTAGTCGGGAGTA

SG-10R (Reverse sequence)

TGGGCCCGGCATATAAGAGTTTTGGGGGCCGGCCACGTTGGATGTTGCCAACACC
TAAGTGCCCACCGTTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCC
CCACGCTTTCGCTCCTCAGCGTCAGTATCGGCCCAGAGATCCGCCTTCGCCACCG
GTGTTCCTCCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGATCTCCCC
TACCGAACTCTAGCCTGCCCGTATCGACTGCAGACCCGGGGTTAAGCCCCGGGCT
TTCACAACCGACGTGACAAGCCGCCTACGAGCTCTTTACGCCCAATAATTCCGGA
CAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCT
TCTTCTGCAGGTACCGTCACTTTCGCTTCTTCCCTGCTGAAAGAGGTTTACAACCC
GAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTG
GCCGGTCGCCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTGAGCCACTACCTC
ACCAACAAGCTGTAAGGCCGCGACACGTGGGAATGTTGCCCAGCCTAGTGCCCA
CCGTTTTACGGCGTGGACTACCAGGGTATCTGATCCTGTTCGCTCCCCACGCTTTC
GCTCCTCAGCGTCAGTATCGGCCCAGAGATCCGCCTTTGCCACCGGTGTTCCTCCT
GATATCTGCACTTTCACCGCTCACCAGGAATTCGATCTCCCTACCGACTCTACCTG
CCGTATTGGTGCAGAACCGGGGTTAAGCCCAGGCTTTACAACGACATGTACAGCC
CCTAGAGCTTTTAGCCAATAATTCGGACAACGCTGGGCCCTACGAATAAGCGGCT
GCGGCAGTAGGTAAGCGGGCTTTTTCTGCTGGTGCCGTCCATTTCGTTTTTTCCTG
CGGAAGAAGGTTTTATAACCCGAAGGCCGTCATCCTCCAGCGGGTGCTGCAGGA
GGGTTTCCCCCATTGAGGCAATTAGATTG

The 16S rRNA gene sequence was compared with those deposited in the public data bases. Phylogenetic analysis was performed using software package MEGA (Molecular Evolutionary Genetic Analysis), version 4, after multiple alignment of data using CLUSTRAL X and clustering was determined using the neighbor-joining method. Fig. 9 shows the phylogenetic placement of isolate Ktn-1

Fig. 9. Comparative analysis of the 16S rRNA partial gene sequence from Ktn-1 and representative strains from GenBank. The significance of each branch is indicated by a bootstrap value calculated from 1000 subsets.

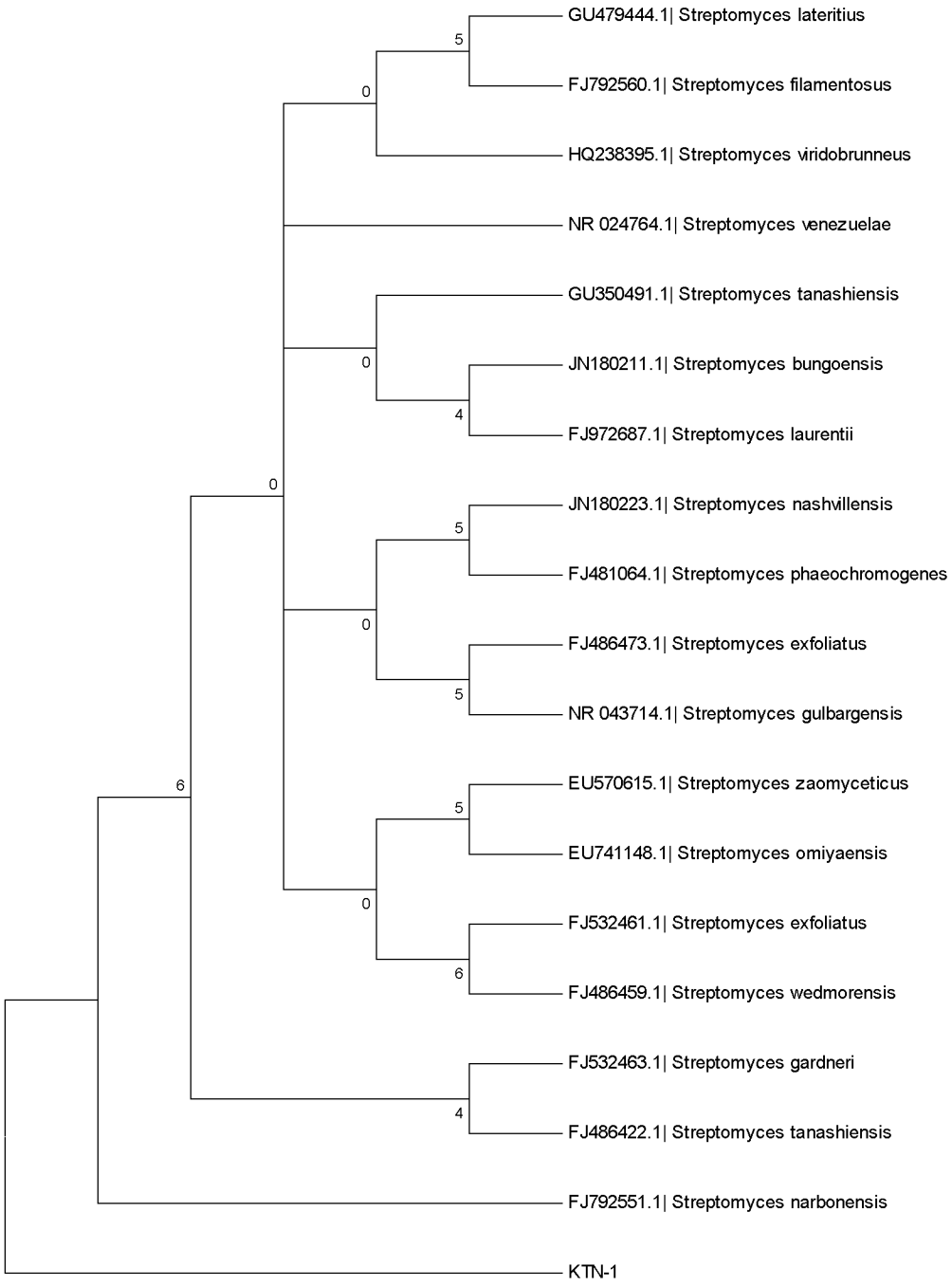
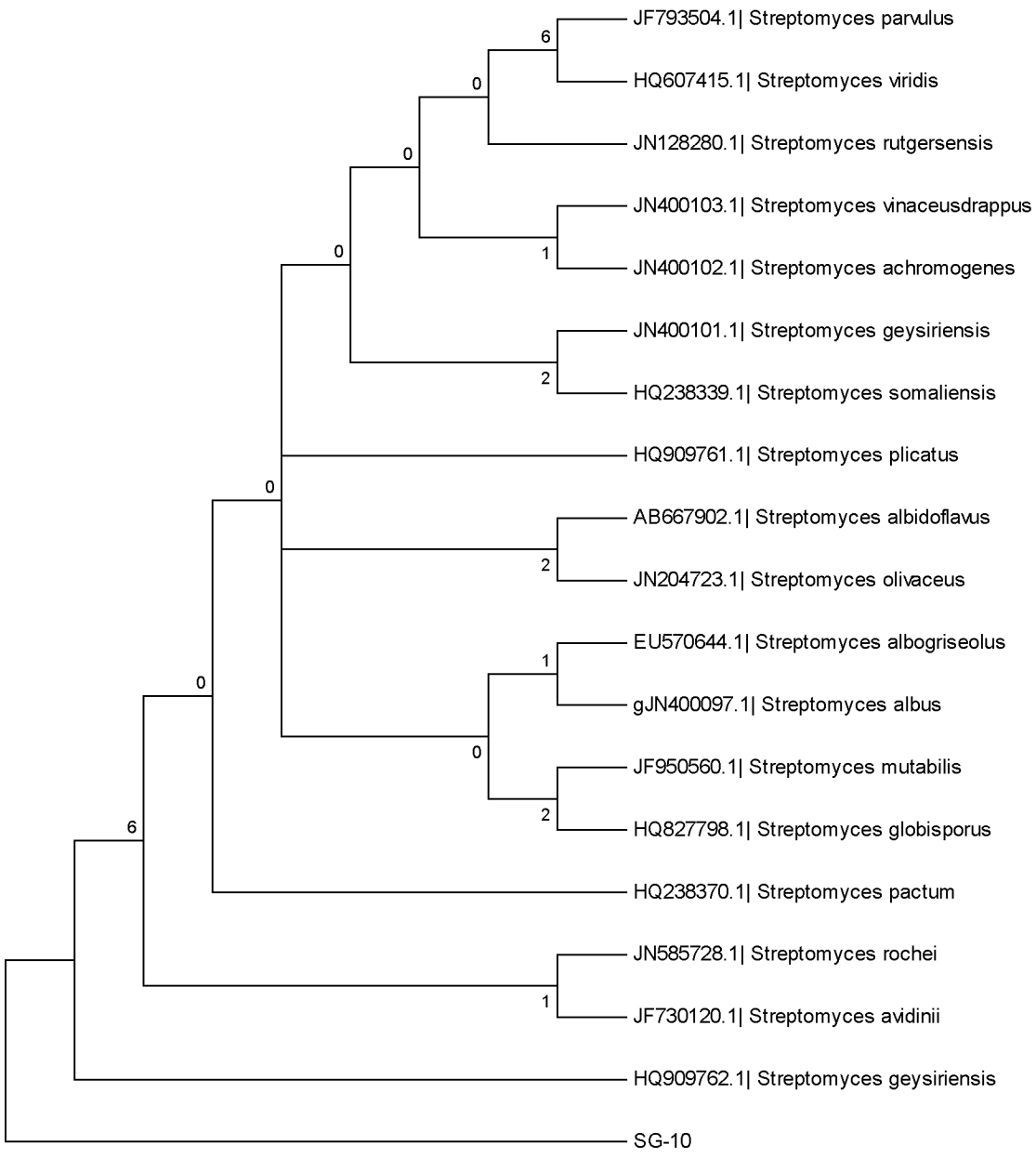


Fig. 10. Comparative analysis of the 16S rRNA partial gene sequence from SG-10 and representative strains from GenBank.



within the genus *Streptomyces*. Based on the sequence similarity and placement within the phylogenetic tree indicate the closest relatives of the isolate Ktn-1 are S*treptomyces narbonensis*, followed by *Streptomyces tanashiensis*. Therefore this isolate Ktn-1 is designated as *Streptomyces* sp. Ktn-1. Similarly the 16S rRNA gene

sequence was compared with closest in the data base and phylogenetic analysis was made as mentioned above. Fig.10 shows the phylogenetic position of the isolate SG-10. The search shows that there was no exact match for the isolate SG-10. However, it seems to be closely related to *Streptomyces geysiriensis*. Therefore the isolate SG-10 is designated as *Streptomyces* sp. SG-10 (Fig.10).

6. DISCUSSION

Worldwide 24 billion chickens are killed annually and around 8.5 billion tons of poultry feather are produced. According to a recent report India's contribution alone is 350 million tons. The poultry feathers are dumped, used for landfilling, incinerated or buried, which involves problems in storage, handling, emission control and ash disposal. Discarded feathers also cause various human ailments including chlorosis, mycoplasmosis and fowl cholera (Williams et al., 1991).

Poultry feathers contain up to 90% by mass pure keratin protein (Onifade et al., 1991). Keratin is a recalcitrant fibrous protein with extensive disulphide and bond and cross-linkages. The keratin fiber is insoluble tightly packed in α helix (α keratin) and β-sheets (β-keratin) into super coiled polypeptide chains (Parry and North, 1998). Keratin-containing materials (feather, hair, wool, etc.) are abundant in nature but have limited uses in practice as they are resistant to degradation by the common proteolytic enzymes. Keratinous wastes represent a source of valuable proteins and aminoacids and could find application as fodder additive for animals or source of nitrogen for plants. For this purpose, degradation of the rigid keratin structure is necessary. Biodegradation by microorganisms possessing keratinolytic activity represents an alternative attractive method for improving the nutritional value of keratin wastes, as

it offers cheap and mild reaction conditions for the production of valuable products. There have been some reports on microorganism capable of degrading keratinous wastes.

In the present investigation actinomycetes, isolated from soil samples collected from feather decomposing site and a sacred grove soil, were directly screened for feather degradation in whole feather medium under submerged conditions. Out of the 25 isolates screened from feather dumping site, four isolates (Ktn-1, Ktn-8, Ktn-10 and Ktn-16) could completely degrade the chicken feather and four more showed partial degradation (Isolates Ktn-3, Ktn-6, Ktn-9 and Ktn-20). Similarly out of 220 isolates tested from sacred grove soil 67 isolates degraded feather; 27 isolates brought about complete disintegration of feathers and the remaining 40 isolates effected partial degradation. Keratinolytic activity of microbes isolated from keratin containing wastes from poultry and leather industries has been reported (Williams et al., 1990; Onifade et al., 1998; Bockle et al., 1995; Bressollier et al., 1999). However, keratinolytic activity of sacred grove actinomycetes has not been reported earlier. Occurrence of keratinolytic or feather degrading actinomycetes in sacred grove is interesting though they not directly exposed to chicken feathers. Hoever, they may be involved in removing and recycling the3 molted feathers of birds living and visiting the sacred grove.

Despite the recalcitrance, keratin waste can be efficiently degraded by a variety of bacteria, actinomycetes (Bockle et al., 1995; Al-Zarban et al., 2002) dermatophytes (Kunert,1973) and saprophytic fungi (Kim, 2003) which secrete keratinolytic proteases. Most of our isolates can be considered as opportunistic species which do ntot need keratin to survive in soil. Even if not directly used, hydrolysis of high molecular weight compounds is an essential first step in the degradation of

organic matter in nature (Corttrell and Kirchman,2000). However, one might expect that in soil feathers are degraded by a consortium of bacteria and fungi, which act in synergy or compete for keratin (Onifade et al., 1998). This study revealed that the diversity of feather degrading actinomycetes is higher than previously described. It also suggests that improved isolation or culture-independent techniques are needed to explore this group and its ecological role.

These isolates were able to colonize and digest feather as the sole carbon and nitrogen. They were also able to secrete extracellular enzymes that digest feather keratin, indicating effective keratinolytic activity under mesophilic conditions. Feather utilization was initially assessed by appearance of turbidity and foaming of the culture medium of the active isolates. Whereas, the ability of the strain to grow in media containing chicken feathers as substrate indicate that it can obtain its carbon and nitrogen directly from this substrate. This is in line with previous findings (Hossain et al., 2007; Fakhfakh et al., 2010). Feather degradation by actinomycetes like *Streptomyces pactum* (Bockle et al., 1995), *Streptomyces* sp. (Letourneau et al., 1998), *Streptomyces thermoviolaceus* (Chitte et al. 1995), *Streptomyces albidoflavus* (Bressollier et al., 1999), *Streptomyces graminofaciens* (Szabo et al., 2000) *Actinomadura keratinolytica* (Puhl et al., 2009), *Nocardiopsis halotolrans, Saccharomonospora halophile* (Al-Zarban et al., 2002), *Amicolatopsis keratinephila* (AL-Musallam et., 2003), *Microbispora aerata* (Gushterovaa et al., 2005) has been reported.

These strains though could utilize feather as the sole carbon and nitrogen but addition of yeast extract in low concentration is helpful for promoting growth of the actinomycetes, especially immediately after inoculation. This additive is presumably necessary for the inoculated actinomycete before the induction of the degrading

enzymes. The growth in the feather medium started intensively after a lag period of 2-4 days. Another four isolates (Ktn-3, Ktn-6, Ktn-9 and Ktn-20) brought near complete or partial disintegration of the feather. These isolates completely disintegrated barbules and major portion of the rachis. The remaining isolates colonized the feathers and showed little amount of growth but no lysis occurred. Visible growth of these isolates probably due to utilization of the soluble nutrients released during autoclaving of the feather. The hydrolysis most likely occur through the proteolytic enzymes produced by the strains, which degrades the β-keratin and other proteins found in feathers producing usable carbon, sulfur and energy for growth and maintenance of the actinobacteria (Hansen et al., 1993: Burtt and Ichida, 1999b). In all cultures, some common characteristics were observed. Insoluble non-degraded feathers gradually disappeared with time, presumably due to keratin hydrolysis. The pH of the culture filtrate also increased towards alkalinity with time. The pH value was more in the culture filtrate which degraded the feather faster than the slow degraders (data not shown). This is characteristic of strong keratinolytic microorganisms. It is suggested that the basis of keratinolysis is the high level of deamination, which renders the medium alkaline (Yu *et al.*, 1968). Both Ktn-1 and SG-10 could grow on chicken feather and degrade it, releasing sulphydryl-containing compounds detected as cysteine (Table 5), keratinase (Fig. 1,3,5 & 7) and total proteins (Fig. 2,4,6 & 8). In fact, the evidence of keratinolysis lies on the ability of the actinomycetes to release soluble sulphur-containing amino acids and polypeptides into medium in significant quantities (Weary *et al.*, 1965). These amino acids may enter the protein pool of the actinobacteria, thereby enhancing its growth and simultaneous keratinase production (Kaul and Sumbali, 1999). Although deamination

and alkalization of the medium surely play a role in keratinolysis, they cannot alone cannot cause substrate denaturation (Kunert, 1995).

Microbial keatinases are inducible enzymes and is substrate specific (Cheng et al., 1995; Malviya et al., 1992). Various keratinous materials like chicken feathers, feather meal, wool, bovine hair, human foot skin have been used as inducer of keratinase (Kumar et al., 2008, De Toni et al., 2002; Ignatova et al., 1999). For instance the gene *KerA*, which encodes keratinase in *B. licheniformis*, is expressed specifically for feather hydrolysis (Lin et al., 1995). Therefore the presence of feather keratin as sole carbon and nitrogen source in the culture medium may result in preferential expression of the keratinolytic protease. The keratinolytic isolates Ktn-1 and SG-10 produce inducible protease which exhibits extraordinary activity against insoluble native feathers. The major difference between keratin and other proteins lies in the presence of a higher level of disulfide bonds which are responsible for the mechanical stability of keratin. Reports on feather degradation suggest that the reduction of disulfide bonds by disulfide reductase (Yamamura et al., 2002) or the production of sulfitase and thiosulphate to be involved in keratin degradation.

The crude enzyme (cell free broth) of both Ktn-1 and SG-10 also completely dissolved the chicken feather. But it took more time than degradation in the presence of the keratinolytic organisms. This might be due to the absence of the mechanical component ie. substrate colonization and mechanical invasion into the feather by the organism. Keratinolytic actinomycetes attack feather by both mechanical and enzymatic action like keratinolytic fungi. Mechanically colonization of the feather result in surface erosion and further invasion into the keratinized structure weakens the feather. This is often followed by enzyme action. This is supported by the observations of Goeth et al. (2008) who documented that an enzymatic reduction of

disulfide groups in feather keratin by disulfide reductase that actually assisted the feather degradation by keratinolytic protease. Moreover, disulfide reductase activity in extracellular medium was found to be less compared to intracellular level. The colonization of actinobacteria on feather provides continuous supply of disulfide reductase that ultimately helps keratinolytic protease to complete the further digestion (Bockle and Muller, 1997).

The two isolates Ktn-1 from feather dumping site and SG-10 from sacred grove soil with strong keratinolytic activity were selected for further study. Effect of culture condition of feather lysis exerted profound influence. Under shake culture condition feather lysis was faster 7-8 days than in still condition 13-15 days. Feathers disintegration started 3 days after incubation with Ktn-1 and SG-10. Macroscopic digestion of feathers residues increased considerably upon prolonged incubation. This resulted in change in color of the medium from colorless to yellowish fermentation broth. Whole degradation of chicken feathers was achieved in 7-8 days (Plate 6). Protein estimation in the culture broth also suggest that Ktn-1 SG-10 are capable of digesting and releasing proteins from chicken fathers accordingly formation of a peptide rich fermentation broth. Measurement of pH also showed more alkaline pH in shake culture (8.6) than in still culture (8.2). The two selected keratinolytic actinomycetes effectively degraded the chicken feathers into a protein and aminoacids rich broth. Keratin sources such as feather, horn, nails and hair, are abundantly available in nature as wastes. Worldwide, commercial poultry processing generate an excess of million tons of feathers per year (Shih, 1993; Schrooyen et al., 2001), which are currently converted to feather meal through steam pressure and chemical treatments. However, these methods destroy amino acids and require significant energy input (Papadopoulos et al., 1986). Alternatively, keratin can be converted to

useful biomass, protein concentrate or amino acids using proteases derived from keratinolytic micro-organisms (Onifade et al., 1998). The nutritional upgrade of feather meal and the use of microbial feather lysate in feed trials showed that the treatment with keratinase might significantly increase amino acid digestibility of feather keratin (Williams et al., 1991; Odetallah et al., 2003). Keratinases may have other important uses in biotechnological processes. The use of keratinolytic enzymes as an alternative to de-hairing pelts and skins has been investigated (Raju et al., 1996; Riffel et al., 2003a). The potential for commercial use of enzymes in leather industry is considerable because of their properties as highly efficient and selective catalysts, avoiding the problem caused by sulfide in tanneries (Wiegant et al., 1999). In addition, keratin wastes have the potential for conversion to products with high added value such as slow-release nitrogen fertilizers, cosmetics and biodegradable films (Choi and Nelson 1996; Schrooyen et al., 2001).

Studies on effect of temperature and pH on feather degradation showed that the two parameters did influence the rapidity with which the feathers undergo disintegration. Feather degradation by SG-10 and Ktn-1 was accomplished by the extra cellular production of keratinolytic protease. Temperature has been found to be having profound effect on the extent of feather degradation and actinobacterial growth. Degradation of feather in the media and the keratinase activity maximize at temperature ranging from 30°C to 35°C (Fig 3). Both the extent of feather degradation and enzyme activity declines sharply at high temperature which suggests the mesophilic nature of two keratinophilic actinomycetes Ktn-1 and SG-10. Mesophilic organisms can have important implications towards the industrial use because they do not require high energy inputs unlike thermophilic keratin degrading microorganism (Chitte et al., 1999).

The optimum pH for maximizing the feather degradation and enzyme activity was determined by growing the two isolates in pH ranging from 5 to 11. At acidic and neutral pH both the enzyme activity and the extent of feather degradation were low but at pH higher than that of 7.5, improvement in the values of these two parameters has been seen. A maximum of 180 U/ml of enzyme activity with about 95-100% degradation of feather was observed at pH value of 9 &10 (Plate 9). The enzyme produced by Ktn-1 and SG-10 can be classified as alkaline protease because maximum activity was achieved at the alkaline pH (Altalo and Gashe, 1993).

However, the parameters for keratinase production are species specific and thus vary with respect to the organism (Sangali and Brandelli, 2000; Thys et al., 2004; Yamamura et al., 2002; Friedrich and Antranikian, 1996; Riffel et al., 2003). It has been observed that alkaline pH supports keratinase production and feather degradation in most microorganisms. The optimum pH for SG-10 and Ktn-1 was found to be 9 and 10 respectively. Alkaline pH possibly favors keratin degradation because higher pH modifies cysteine residues to lathionine, making it accessible for keratinase action (Riffel et al., 2003).

Characterization of the enzyme of Ktn-1 showed the temperature optimum and pH optimum at pH 10 and temperature 35°C and that of SG-10 had optimum temperature and pH of 25C and 9 respectively. The keratinases from the two isolates are likely to be a serine protease since it was mostly inactivated by PMSF, partially by EDTA and their pH range were in the alkaline range 9-10. Most of the keratinases are classified as serine type proteases. The keratinases produced by *B. licheniformis, B. subtilis, Microsporum canis* are serine proteases, and their genes shared sequence homology with subtilisins, which are typical serine-type proteases (Lin et al., 1995; Laghloul, 1998; Deschamps et al., 2003). Among actinomycetes, several keratinases

described for *Streptomyces* spp. are also classified as serine proteases (Bockle et al., 1995; Bressollier et al., 1999). Metalloproteases are seldom found associated with keratinolytic activity as reported for *Lysobacter* sp. (Allpress et al., 2002*);* *Chrysobacterium* sp. (Riffell et al., 2003b) and *Mycrobacterium* sp. (Thys and Brandelli, 2006). Several reports have shown serine proteases to be slightly affected by metalloprotease inhibitors (Manachini et al. 1988; Bockle et al. 1995; Singh et al 2001). The keratinases produced by actinomycetes are often serine proteases such as the enzymes produced by *Streptomyces pactum* (Bockle et al., 1995) and *S. albidoflavus* (Bressollier et al., 1999). The reducing agent mercaptoethanol enhanced the enzyme activity of both the isolates. The use of reducing agents to enhance keratin degradation by keratinases has been described (Bockle et al., 1995; Thys and Brandelli, 2006). The reducing agents like glutathione, mercaptoethanol, cysteine, and sodium sulfite stimulated the hydrolysis of keratin by keratinase of *Bacillus* sp. JB99 (Pushpalata and Naik, 2010). The keratinase of *Bacillus subtilis* KS-1 also showed increased keratin degradation in the presence of reductants (Suh and Lee, 2001). Vignardet et al. (2001) observed that increased quantity of proteins was released when thioglycolated nails or hooves were treated with keratinase. The keratin degradation by keratinases in vitro, therefore, accompanied by simultaneous reduction in the disulfide bond or cysteine bonds (Pushpalata and Naik, 2010).

The isolate presented optimum growth at mesophilic temperatures, as expected of its environmental origin. Other previously described keratinolytic bacteria generally gave optimum growth and feather degradation activity at higher temperatures (Williams et al., 1990: Nam et al. 2002). The isolate Ktn-1 caused a significant increase in the pH of the medium during cultivation on raw feathers and was able to complete feather degradation, indicating its strong keratinolytic character.

Organisms with higher keratinolytic activity alkalinize the media to a greater extent than those exhibiting lower keratinolytic activity (Kaul and Sumbali, 1997). This tendency to alkalinize the medium results from the production of ammonia by means of the deamination of peptides and aminoacids originating from keratin degradation. The resulting increase of pH is typical of microorganisms growing on protein substrates. The precise mechanism underlying keratinolysis has yet to be elucidated. It has been proposed that the first step in keratin degradation involves deamination, which creates an alkaline environment needed for substrate swelling, sulphitolysis, and proteolytic attack (Kunert, 2000).

Analysis of the feather lysate of Ktn-1 and SG-10 showed presence of 20 different aminoacids. The feather hydrolysate contained good amount of essential aminoacids (phenylalanine, valine, leucine and isoleucine) and rare aminoacids (serine, cysteine and proline) along with the other aminoacids more importantly the sulphur containing aminoacids like cysteine and methionine. The most distinctive character of keratin is its high cysteine content. The disulphide linkage between the amino acids renders the keratin molecule more resistant to enzyme digestion. The two isolates showed fairly good amounts of cysteine in the culture medium. Maximum cysteine was released in the basal medium supplied with 1.0 g of chicken feather as a keratin source by SG-*10* (4.9mg/ml) followed by Ktn-1 (3mg/ml). Kunert (1973) reported the release of cysteine by *Trichophyton rubrun* and *Microsporum gypseum* growing on wool and hair, respectively. Hasija *et al.* (1990) isolated thirty keratinophilic fungi for their ability to degrade human scalp hair and found that most of them could release cysteine. Malviya *et al.* (1992) have reported that similar values of protein release after prolonged incubation. In the investigation of keratinolytic activity measured in keratinase units (KU), the two actinomycete isolates secreted

considerable amounts of keratinase (Fig. 3B), which is known to be responsible for hydrolysis of feather keratin.

The two keratinolytic actinomycetes selected in this study were tested for feather degradation in soil over a period of 30 days. The two isolates showed great variation in their ability to degrade the feathers in soil environment, where they are ultimately intended to work. Of the two isolates SG-10 brought about maximum degradation when compared to Ktn-1. Ktn-1 could not perform better in soil but degraded feathers completely in submerged conditions (Plate 10). Often microbes with activity in in vitro conditions may not always work in vivo. On the other hand the isolate SG-10 intensely degraded feathers under exposed, submerged and in soil conditions and emerged as a potential keratinolytic organism suitable for industrial applications as well as for feather composting purpose. The inoculation of the indigenous actinomycetes can be valued as a contributing factor in the success of a decreased composting time when compared to usually used composting cycles of 3-6 months (Baldwin and Greenfield, 2000; Pettett and Kurtboke, 2004). Moreover, degradation of the feathers did not occur in the control soil without the actinomycete inoculum. Accordingly the composting process and feather degradation observed in this study clearly indicates that the introduction of indigenous actinomycetes could multiply in the soil environment and speed up the process of feather disintegration. Thus composting using actinomycetes resulted in rapid removal of the recalcitrant feather content resulting in acceptable quality end product. Reduction of malodors was another significant achievement of composting using keratinolytic actinomycetes. Reduction of odor nuisances reduces the risks associated with air quality problems, flies, vermin and scavenging animals, protection of surface and ground water.

Further the usefulness of the feather compost in terms of manure or fertilizer was tested by sowing green gram seeds in cups. The compost produced by the isolate Ktn-1 had some inhibitory effect on seed germination, seedling emergence and seedling growth. Whereas the compost produced by SG-10 had no harmful effect on seed germination and enhanced seedling growth. Consequently seedlings in Ktn-1 compost were slightly shorter than the seedlings in SG-10 compost. Feathers being rich in protein their microbial degradation in soil would release peptides and aminoacids along with other products which may stimulate soil microbial activity there by facilitating the assimilation of nutrients by plants (Paluszak and Olszewska, 2000) and better growth. Addition of feather lysate to soil has been shown to increase urease and protease enzyme activity. Proteases and ureases in soil play a significant role in nitrogen mineralization, an important process in regulating the amount of plant available nitrogen for plant growth (Ros et al., 2003). On the other hand inhibitory effect of the Ktn-1 compost may be due to production of phytotoxic organic metabolites (O'Brien et al., 2002).

Finally this keratinolytic isolate was identified based on colony morphology, growth characteristics, and several biochemical tests. The isolate produced a creamy white colony with creamy yellow base, produced no diffusible pigment or melanin. Positive for amylase, catalase, lipase protease, gelatinase, neither reduced nitrate nor utilized citrate. Tolerated salt concentrations up to 8% with better growth between 0-6%, grew better at pH 9 and 10, and temperatures 25-35C°. Scanning electron microscopic examination of the cultures showed unfragmented mycelium and straight and coiled chains of smooth spores. These characteristics confirmed that the isolate is a member of the genus *Streptomyces*. Phenotypic characterization of any organism is the first step towards its identification and taxonomic classification. However, many

members of from the same genus share overlapping phenotypic characters and therefore, they are difficult to distinguish only on the basis of phenotypic and biochemical traits. Over the years, determination of the phylogenetic relationship or identification of bacteria based on 16S rDNA sequencing (ribotyping) has been widely accepted to confirm the identity of the microorganism.

Therefore, next 16S rRNA gene sequence was compared with those deposited in the public data bases. Phylogenetic analysis was performed using software package MEGA (Molecular Evolutionary Genetic Analysis), version 4, after multiple alignment of data using CLUSTRAL X and clustering was determined using the neighbor-joining method. Fig. shows the phylogenetic placement of isolate Ktn-1 within the genus *Streptomyces*. Based on the sequence similarity and placement within the phylogenetic tree indicate the closest relatives of the isolate Ktn-1 are *Streptomyces narbonensis*, followed by *Streptomyces tanashiensis*. On the basis of phylogenetic analysis the isolates Ktn-1 and SG-10 are classified as members of the genus *Streptomyces*. However, in spite of the phenotypic, biochemical data and rDNA sequence analysis both the isolates could not be identified to species level. The two isolates were thus designated as *Streptomyces* sp. strain Ktn-1 and *Streptomyces* sp. strain SG-10.

To conclude the results obtained in this study clearly demonstrate that feathers can be decomposed in vitro and in vivo by specialized group of microbes. Such microbes can be isolated and employed to digest the recalcitrant feathers into a nutrient rich feather meal which can be used as feed supplement. Alternately the nitrogen rich feather lysate can be processed to separate rare and essential aminoacids in pure form for cosmetic and pharmaceutical application or the nitrogen rich, concentrated feather meal can also be used for organic farming as semi-slow release

nitrogen fertilizer. Thirdly such keratinolytic microbes can be used to compost feathers to produce low-cost organic manure for restoration of soil fertility. Thus bioconversion of the recalcitrant chicken feathers by suitable keratinolytic microbes will predictably benefit the poultry industry, man and the environment.

7. SUMMARY

In the present investigation an attempt was made to convert raw chicken feathers into nutrient rich feather meal / nitrogen rich organic manure using keratinolyic actinomycetes.

Soil actinomycetes isolated from feather decomposing site and sacred grove were screened for feather degradation. Four out of 25 isolates from feather dumping site and 27 out of 220 isolates from sacred grove completely degraded chicken feather in whole feather mineral medium at pH 7.2 and 28-30□C after 14-25 days incubation. Two isolates ie. Ktn-1(from feather dumping site) and SG-10 (from sacred grove) with strong keratinolytic activity were selected and the optimum conditions to maximize keratinolytic activity was determined.

Ktn-1 degraded feathers completely at pH 10 and at 35□C, in 8 days whereas SG-10 lysed feathers within 7 days at pH 9 and at 25□C in shake culture (180 rpm). In both case the enzyme was found to be serine protease. Analysis of the feather lysate showed presence of 20 amino acids including essential and rare amino acids. Both isolates could actively colonize the feather under aerobic conditions and could lyse the same within 30 days. Feather composting experiments with the two

keratinolytic isolates showed that SG-10 is more active than Ktn-1 under soil conditions. The feather compost by SG-10 supported better growth of green gram seedlings than the Ktn-1 compost.

Finally, the two isolates were identified based on colony morphology, growth characteristics and biochemical characteristics and 16S rRNA gene sequence similarity analysis. Both the isolates Ktn-1 and SG-10 were identified as member of the Genus *Streptomyces*. However, they could not be identified to species level. Hence they are designated as *Streptomyces* sp. strain Ktn-1 and *Streptomyces* sp. strain SG-10.

To conclude the results obtained in this study clearly demonstrate that feathers can be decomposed in vitro and in vivo by specialized group of microbes. Such microbes can be isolated and employed to digest the waste feathers into a high-protein feather meal which can be used as feed supplement. Alternately the nitrogen rich feather lysate can be processed to isolate rare and essential amino acids in pure form for cosmetic and pharmaceutical application or the lysate can also be used as a slow release nitrogen fertilizer. Thirdly such keratinolytic microbes can be used to compost feathers to produce low-cost organic manure for restoration of soil fertility. Thus bioconversion of feather wastes and dead chickens could be a safe method of recycling these organic materials. This microbial process not only solve economic and environmental problems but at the same time generating value added bio-products with potential industrial and organic farming application. These encouraging results in feather wastes management should be continued and the isolated actinomycetes may also be evaluated in the treatment of other kind of wastes.

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Dr. T. Ganesan

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Fig . 5. Effect of pH on Keratinolytic activity of isolate SG-10

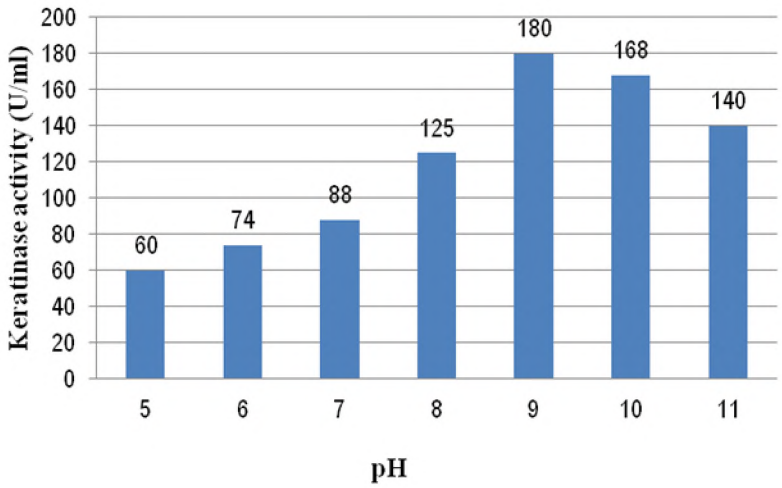
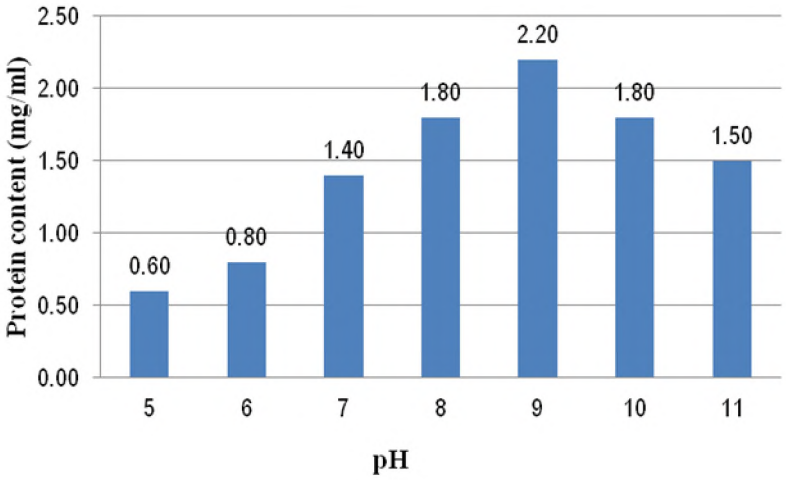


Fig . 6. Effect of pH on Feather degradation by SG-10 and protein accumulation



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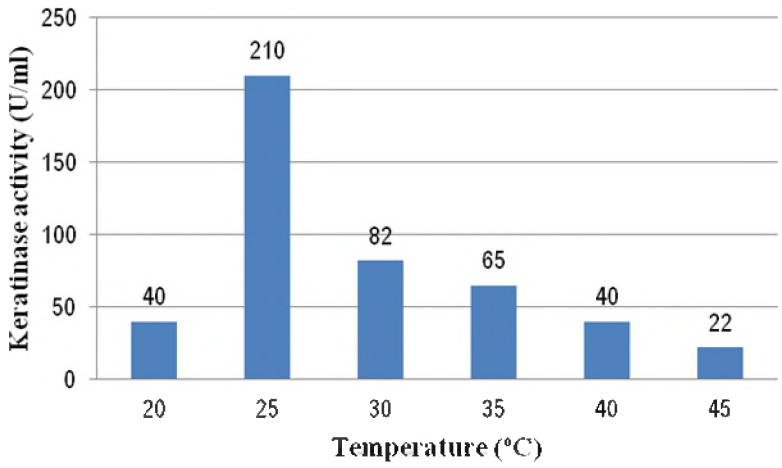

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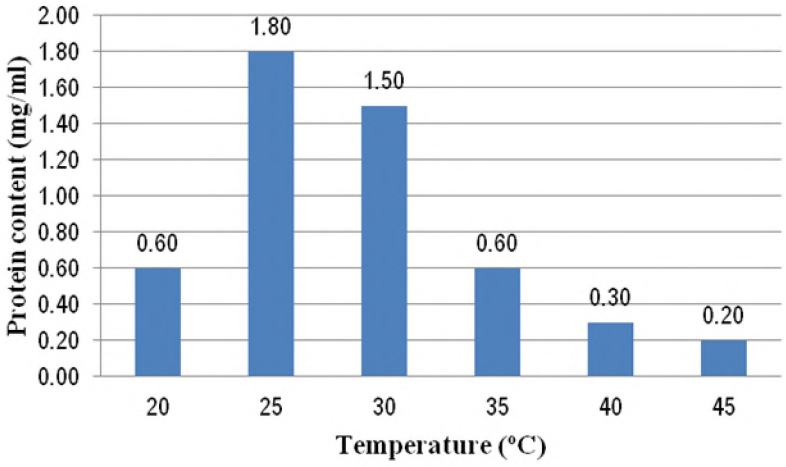
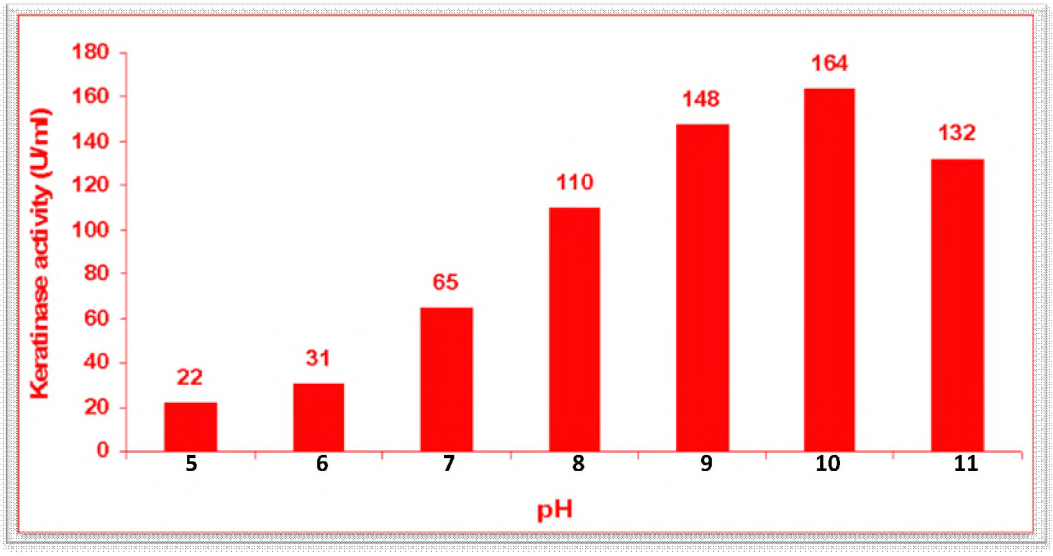


Fig. 1. Effect of pH on Keratinolytic activity of isolate Ktn-1



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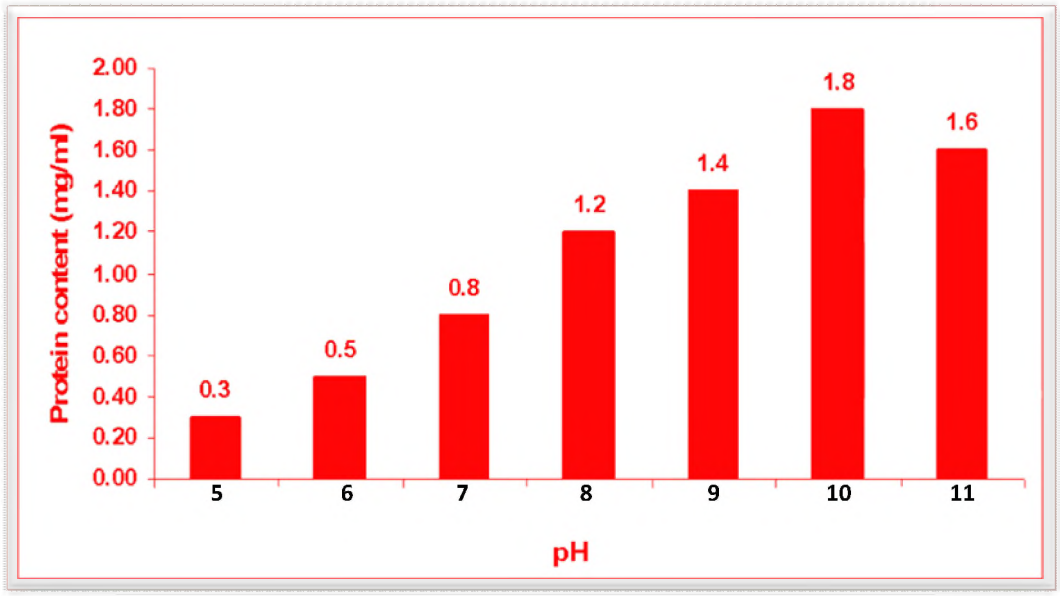
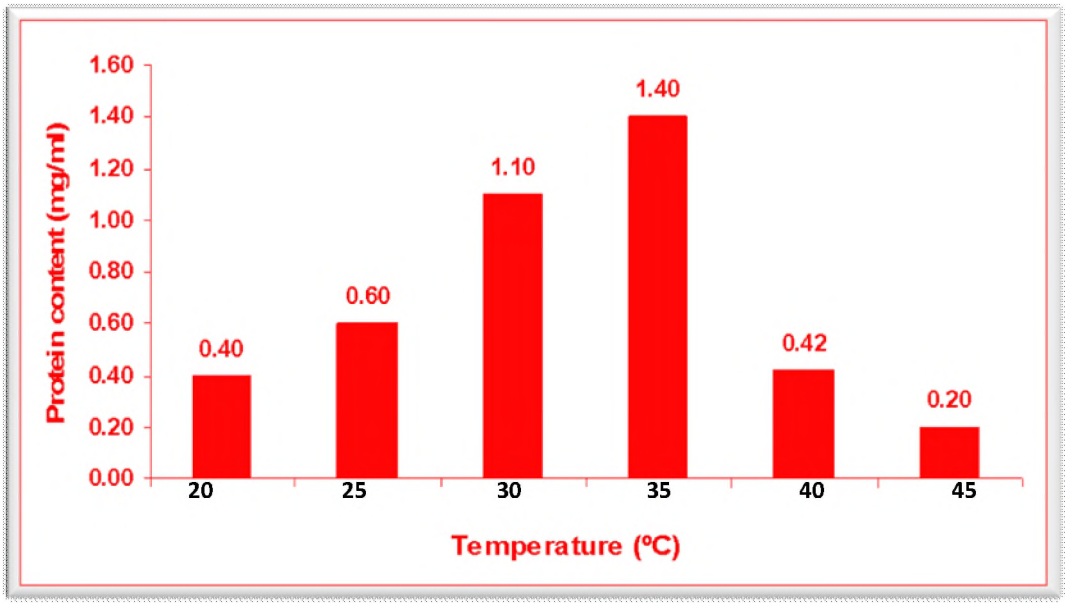
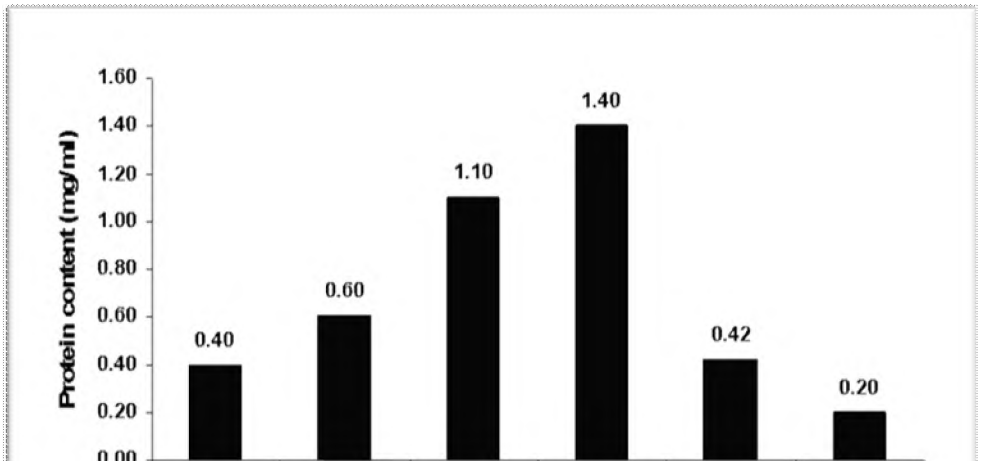


Fig . 3. Effect of temperature on Keratinolytic activity of Ktn-1

20 25 30 35 40 45

Fig. 4.Effect of temperature on feather degradation by Ktn-1 and protein accumulation.





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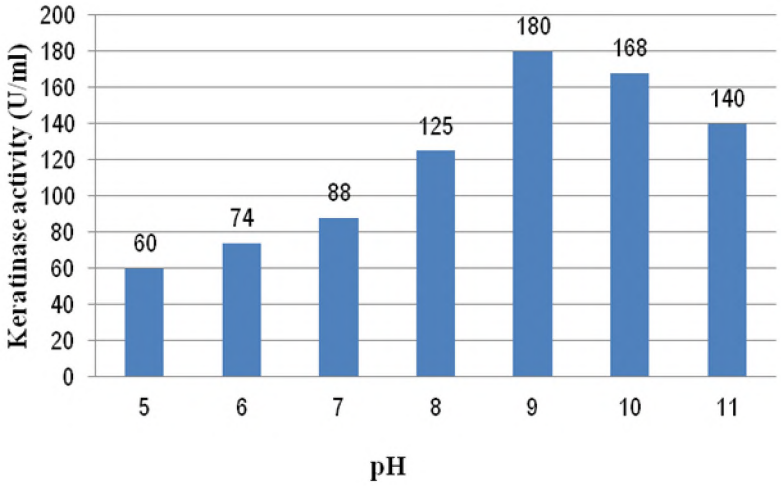

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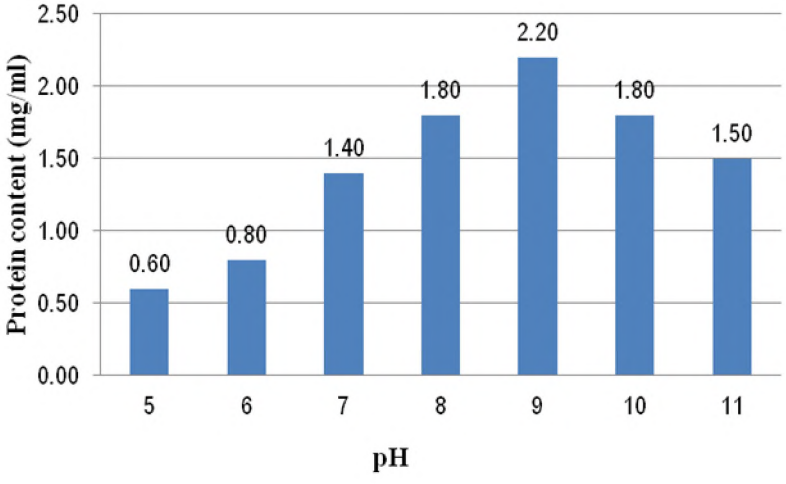
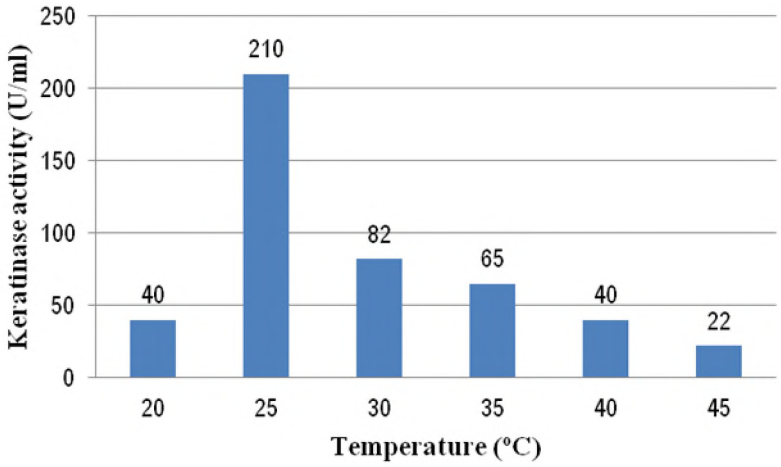


Fig . 7. Effect of temperature on Keratinolytic activity of SG-10



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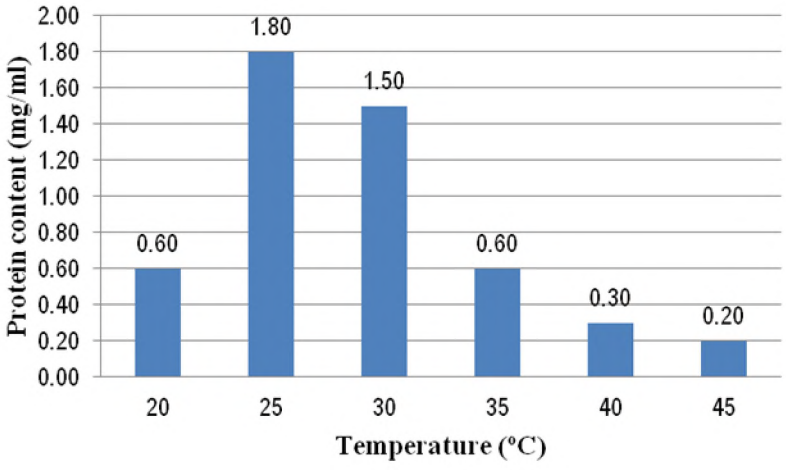


Plate: 1. a. A view of poultry farm

b. Feather waste from poultry processing industry

Plate: 2. Dumping of feather wastes on road sides - I

Plate: 2. Dumping of feather wastes on road sides - II

Plate: 2. Dumping of feather wastes on road sides - IV

Plate: 2. Dumping of feather wastes on road sides - V

Plate: 3. Structure of a typical feather

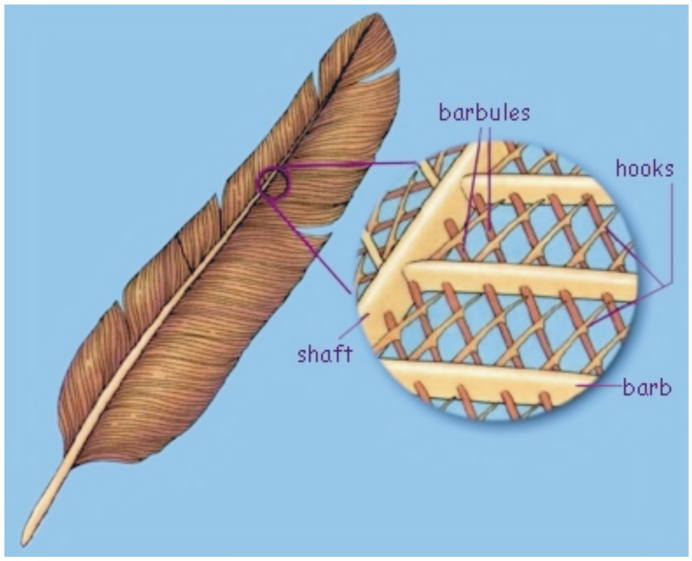


Plate: 4. Actinomycete colonies on isolation plates

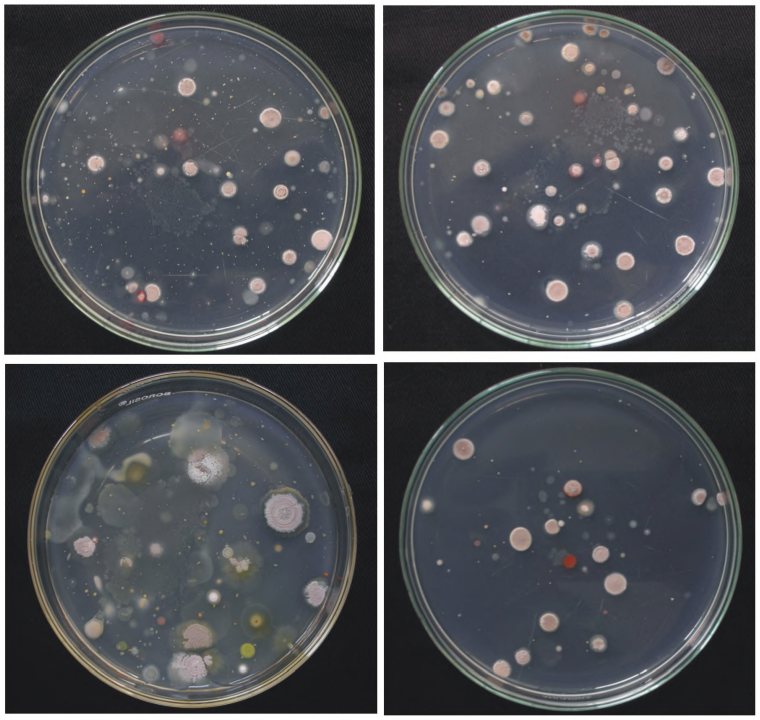
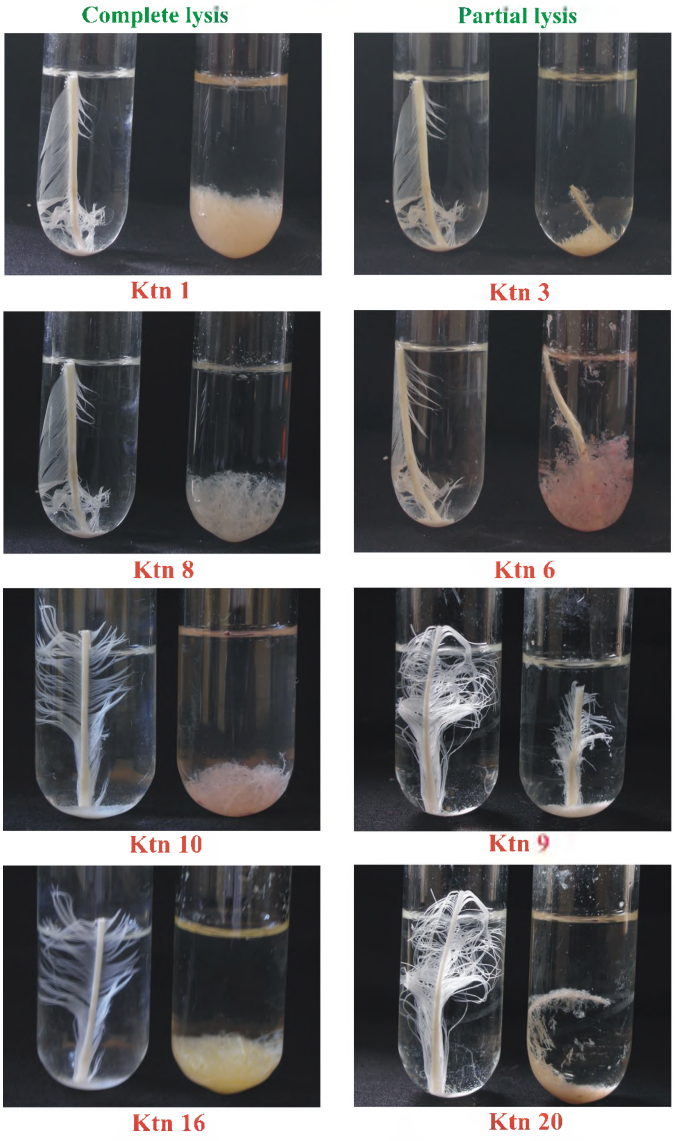


Plate : 5

a. Pure cultures obtained from feather dumping site

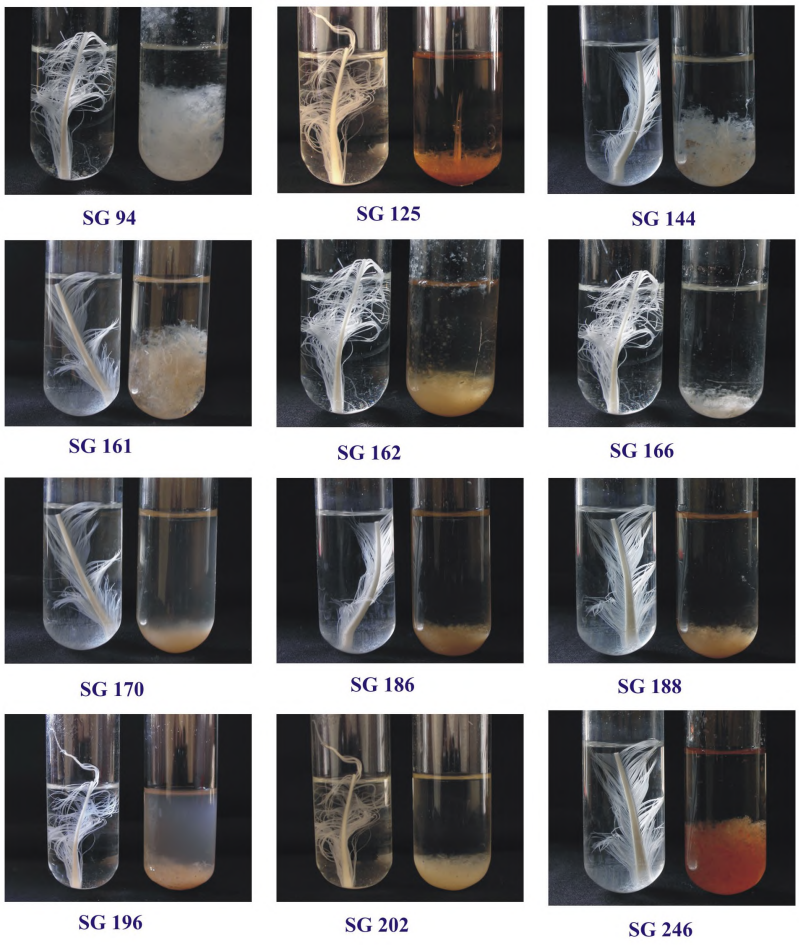
b. Pure cultures obtained from sacred grove soil

Plate: 6. Keratinolytic activity of actinomycetes from feather dump site



In each panel tubes on left is control, on right inoculated with actinomycetes

Plate : 7a. Complete degradation of feather by sacred grove actinomycetes.



SG 94 SG 125 SG 144

SG 161 SG 162 SG 166

SG 170 SG 186 SG 188

SG 196 SG 202 SG 246

Plate : 7a. Complete degradation of feather by sacred grove actinomycetes.

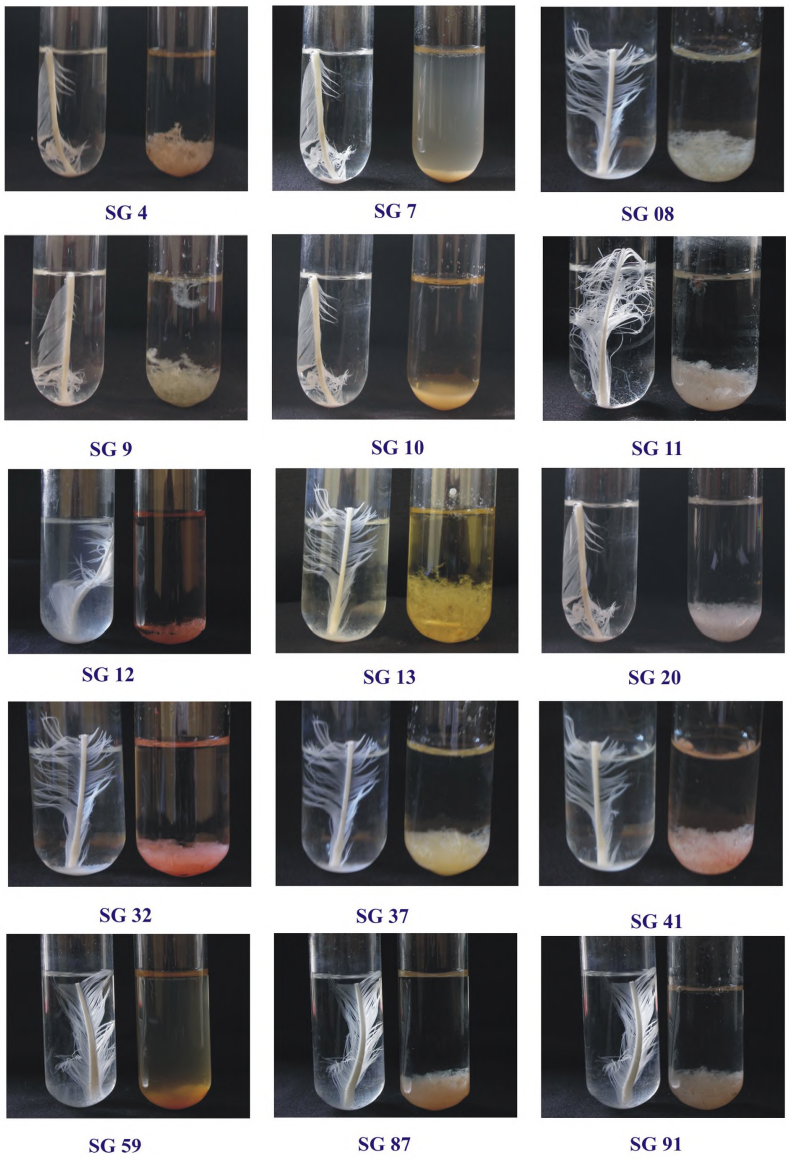
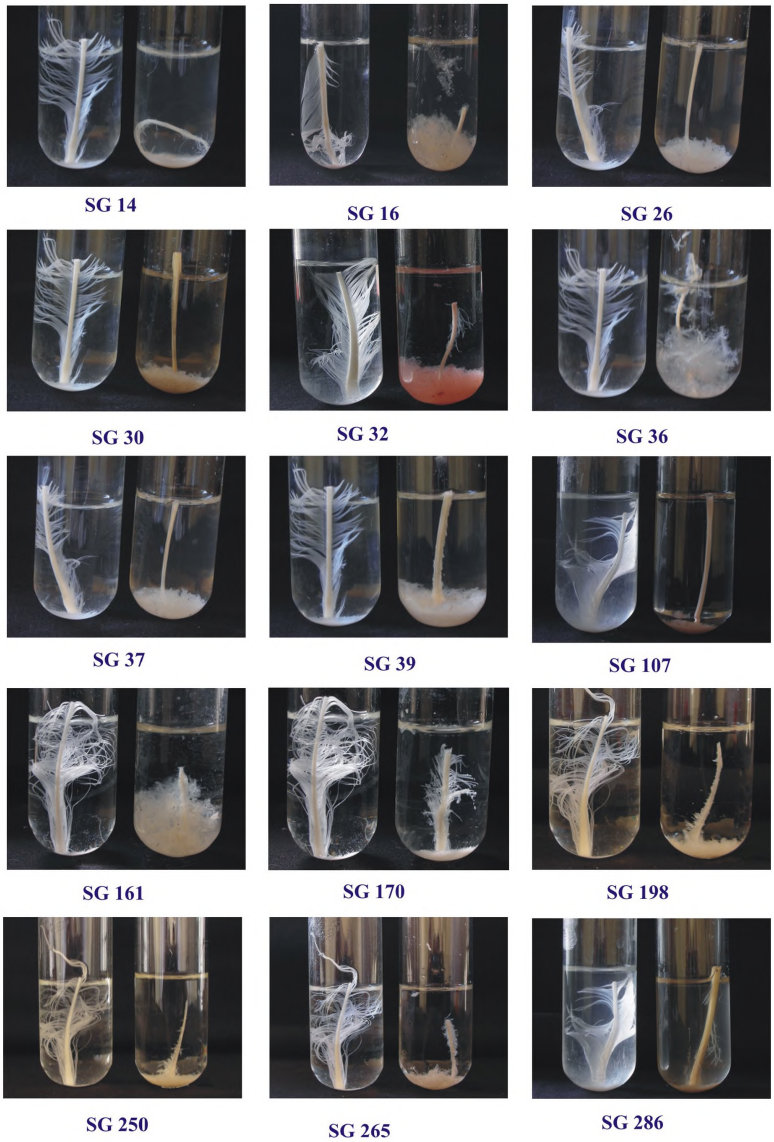


Plate : 7b
Partial degradation of feather by sacred grove actinomycetes.



SG 14 SG 16 SG 26

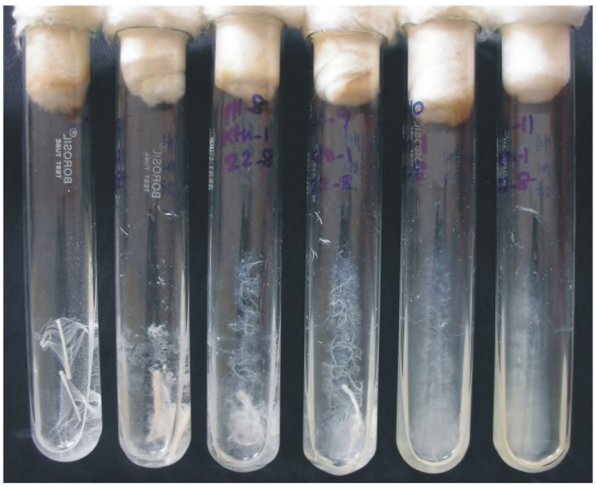
SG 30 SG 32 SG 36

SG 37 SG 39 SG 107

SG 161 SG 170 SG 198

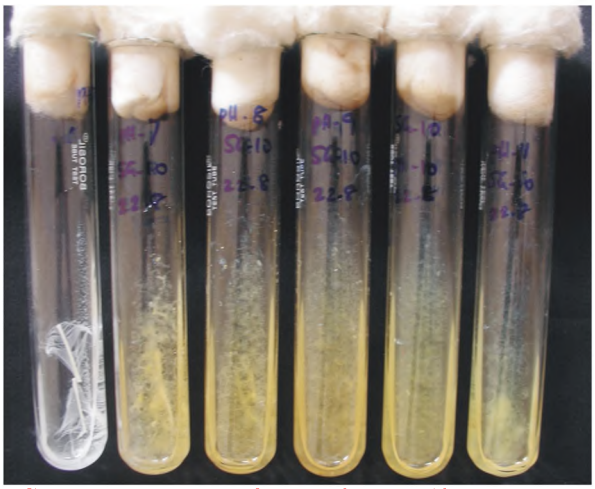
SG 250 SG 265 SG 286

Plate: 9a. Feather lysis by Ktn-1 in defferent pH.



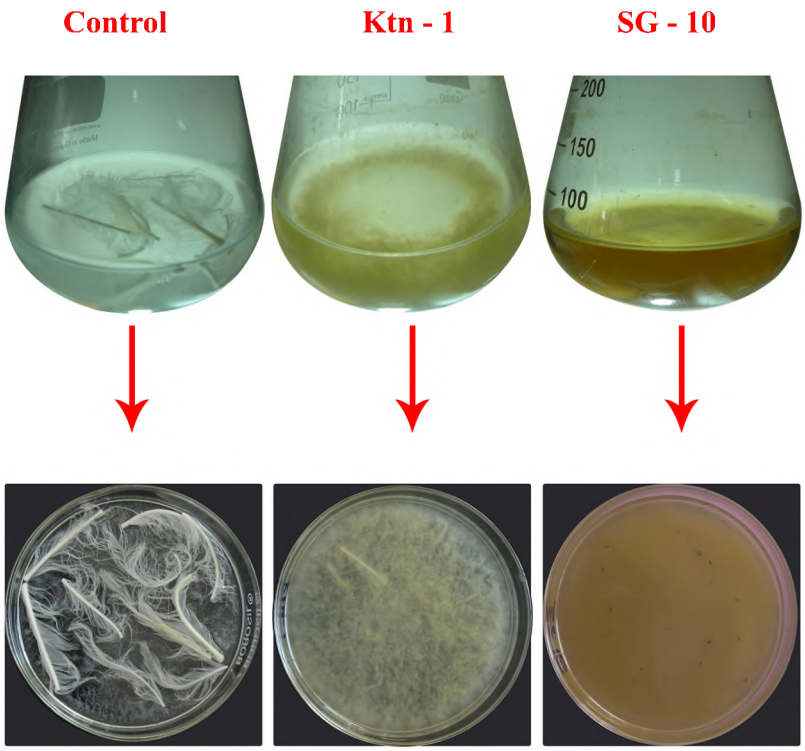
Control pH7 pH8 pH9 pH10 pH11

9b. Feather lysis by SG-10 in defferent pH.



Control pH7 pH8 pH9 pH10 pH11

Plate: 10. Feather degradation by keratinolytic actinomycete isolates under submerged condition.



Ten days after incubation the contents in the flasks were transferred to petriplates for photography

Plate: 11. Feather degradation by keratinolytic actinomycete isolates under exposed condition.

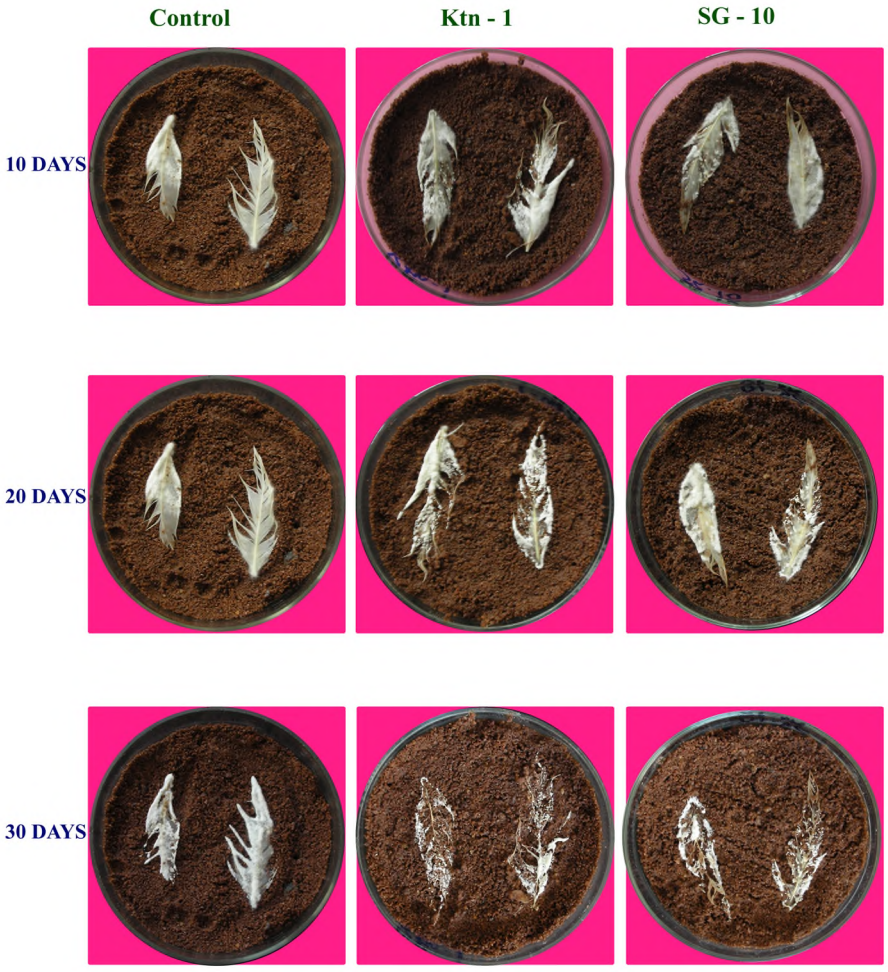


Plate: 12. Feather composting in soil by keratinolytic actinomycetes

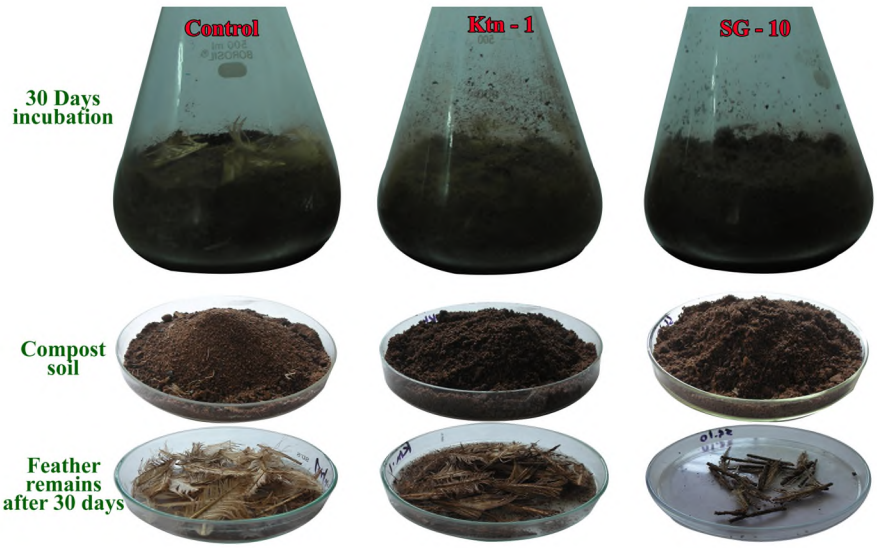
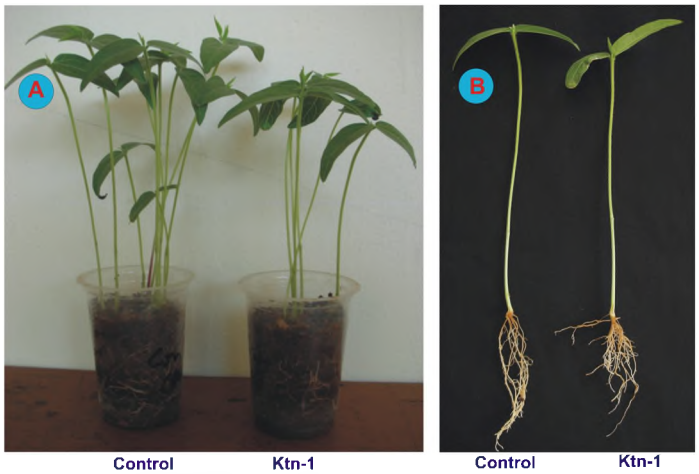


Plate : 13a. Plant growth in feather compost soil



A. Ten days old green gram plants grown in feather compost soil (Isolate Ktn-1). B. Ktn compost did not support plant growth.

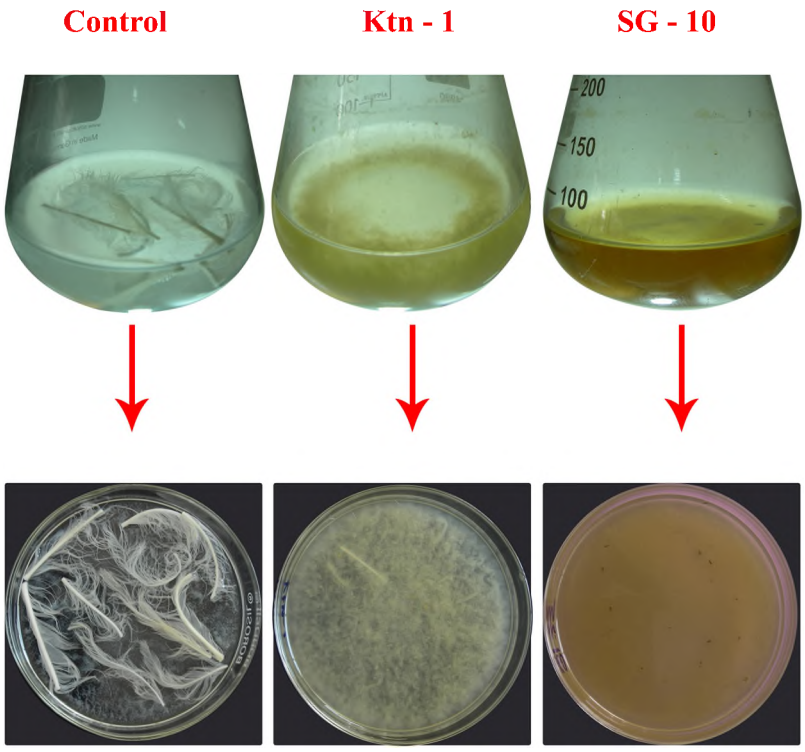
Plate : 13b. Plant growth in feather compost soil

A. Ten days old green gram plants grown in feather compost soil (Isolate SG-10)

B. Note better growth in feather compost soil than in control soil.

Plate: 2. Dumping of feather wastes on road sides - III

Plate: 10. Feather degradation by keratinolytic actinomycete isolates under submerged condition.



Ten days after incubation the contents in the flasks were transferred to petriplates for photography