POULTRY FEATHER DEGRADATION
BY
PLANT GROWTH PROMOTING BACTERIA (PGPB)

A RESEARCH PROJECT

Submitted by

INDRAYANI RAHUL BHANDIGARE

NAZIYA RASHID DESAI

ADITYA DILIP PATIL

UNDER THE GUIDANCE OF

Dr. K.K. Bhise

(Assistant professor)

PG DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(EMPOWERED AUTONOMOUS)

Year 2023-2024

"Dissemination of education for knowledge, science and culture"

-Shikshan Maharshi Dr. Bapuji salunkhe

Shri Swami Vivekanand shikshan sanstha's

VIVEKANAND COLLEGE KOLHAPUR

(EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that **Miss. Indrayani Rahul Bhandigare** studying in M.Sc part 2 Microbiology at Vivekanand College, Kolhapur (Empowered autonomous) has sincerely completed research project work entitled **"POULTRY FEATHER DEGRADATION BY PLANT GROWTH PROMOTING BACTERIA (PGPB)"** during academic year 2023-2024

Dr. K.K Bhise

Project supervisor

Examiner

Dr.G.K Sontakke

Head of the Department

"Dissemination of education for knowledge, science and culture"

-Shikshan Maharshi Dr. Bapuji salunkhe

Shri Swami Vivekanand shikshan sanstha's

VIVEKANAND COLLEGE KOLHAPUR

(EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that **Mr. Naziya Rashid Desai** studying in M.Sc part 2 Microbiology at Vivekanand College, Kolhapur (Empowered autonomous) has sincerely completed research project work entitled **"POULTRY FEATHER DEGRADATION BY PLANT GROWTH PROMOTING BACTERIA (PGPB)"** during academic year 2023-2024

Dr. K.K Bhise

Project supervisor

20/4/24

Examiner

Dr.G.K Sontakke

Head of the Department

"Dissemination of education for knowledge, science and culture"

-Shikshan Maharshi Dr. Bapuji salunkhe

Shri Swami Vivekanand shikshan sanstha's

VIVEKANAND COLLEGE KOLHAPUR

(EMPOWERED AUTONOMOUS)

PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that **Mr. Aditya Dilip Patil** studying in M.Sc part 2 Microbiology at Vivekanand College, Kolhapur (Empowered autonomous) has sincerely completed research project work entitled **"POULTRY FEATHER DEGRADATION BY PLANT GROWTH PROMOTING BACTERIA (PGPB)"** during academic year 2023-2024

Dr. K.K Bhise

Project supervisor

Examiner

Dr.G.K Sontakke

Head of the Department

ACKNOWLEGEMENT

I wish to express my deep sense of appreciation to Prof. Dr. Savita D. Mali, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous) for her valuable support and expert guidance during the course of this study. She has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Dr. G. K. Sontakke, Head of the Department of Microbiology and Dr. R. R. Kumbhar, Principal Vivekanand College, Kolhapur for providing the laboratory facilities in the department to carry out the experimental work.

I express my thanks to my teachers, Dr. K. K. Bhise, Ms. V. V. Misal, Mr. S. D. Gabale, and Ms. S. A. Pise for their valuable suggestions and help during the work.

I convey my gratitude to Mrs. D. S. Shinde (laboratory assistant), Mr. S. K. Maskar and Mr. S. P. Mali (laboratory staff) of the department for their kind help in the laboratory.

I am thankful to the librarian and library staff for providing facilities of computer and reference books. My special thanks and gratitude to my entire classmates who have been constant source of inspiration and help during entire project work. I am highly obliged to authors past and present whose literature has been cited.

Finally, I thank my family members for their blessings and moral and economical support because of which this work has proved satisfactory to me.

Place: Kolhapur

Date: 15/05/2024

Ms. Indrayani Rahul Bhandigare

Ms. Naziya Rashid Desai

Mr. Aditya Dilip patil

INDEX

INTRODUCTION

Introduction-:

The maintenance of high agriculture productivity is a prerequisite to cater the demands of growing population. To increase the productivity of crops, synthetic fertilizers, are being used all over the world. Overuse of these fertilizers leads to their gradual entry into water systems through rain water and causes eutrophication. Recently, several biological approaches for improving crop productivity are gaining strong impetus among agronomists and environmentalists. Among these approaches, the use of plant growth-promoting bacteria. Preparation of bio-fertilizers using chicken feather wastes is attracting the focus of many research scientists. Feather meal is a cheap and easily available source of Nitrogen (15%N) and may serve as a potential bio-fertilizers. The plant-growth promoting activity of protein hydro lysates could also be effectively applied in agriculture. Thus, microbial degradation of feather represents an alternative for development of slow-release nitrogen fertilizers. Microbial conversion of feathers into an value – added products as an animal feed bio-fertilizers used in poultry industries, feathers, being rich source of keratin , proteins, and, amino acids, so that they can get converted into an valuable nitrogen- rich organic fertilizer.

However, increasing poultry production also led to the massive generation of various poultry wastes, which are a great threat to climate change, environmental safety, and, human health. Nowadays, the gradually increase in poultry industries resulted in the generation of chicken feathers as a waste in millions of tons (Bhise et al.2017). Poultry product consumption around the world has been increasing dramatically during the past several decades. The increasing production of the poultry industry has also brought some tricky problems, and a typical one is treatment and management of the massive generation of poultry waste.

Feathers are rich and cheap source of proteins and may function as effective bio-fertilizers. Feathers can be degraded by bacteria and formed lysate having amino acids and peptides that can be used as a bio-stimulant to increase nutrient uptake, growth of plants as well as to tolerant abiotic and biotic stress to increase overall crop yield (Bhise et al.2017) Chicken feathers is one of the most common and cheap by-products which are produced in large quantities. In 2019, there were nearly about 5 million tons of chicken feathers waste were produced world widely. The chicken feather wastes are one of the annoying wastes. Chicken feather consists of keratin (91%), moisture (8%), lipids (1%), in addition to representing 5-7% of the chicken body weight. Keratinous material are unique material they are very abundantly found in various amino acids, especially, sulfur containing amino acids, cysteine, and other amino acids like proline, arginine, glycine, and the essential amino acids like threonine, valine, and leucine. They also contain high minerals content like Nitrogen, Phosphorous, Potassium, Iron, Magnesium, Calcium, Manganese, Zinc, and copper. Although the feather contains large amount of potentially useful proteins and amino acids, they have limited uses as a dietary components in animal feedstuffs owing to their poor digestibility. However, this process requires consumption of large amount of energy and they also destroy certain amino acids, thus yielding products poor digestibility and variable nutrient quality (Boxu, et al. 2009).

The amount of chicken feather generated as a waste from the poultry industry in the last decade has become of technological interest for many researchers regarding both its treatment and application. At a worldwide level, billion of tons of feathers are produced due to the consumption of poultry and , consequently , leading to problems as they are affecting public health and the environment. Biodegradation process of chicken feathers with the greatest international impact used pure strains, which have been isolated from waste of poultry farms. Among the strains with the best degradation efficiency (100% in 1-7days) are *Bacillus spp , Pseudomonas spp, and, Fervidobacterium spp.* At present,microbial consortia or modified

strains are also used to increase the efficiency and profitability of biodegradation. After treatment of poultry waste through different methods, relevant by-products like enzymes, amino acids, etc (Pahua- Ramos ME. et al, 2017). The degraded feathers lysate could generate tryptophan in appreciable amount which is an precursor of plant hormones Indole acetic acid (IAA) (Bhise et al.2017). Feather hydrolysate can also be employed as an nitrogenous fertilizers for plant growth (K.Tamreihao , et al. 2018). The application of hydrolysate increases the water holding capacity , nitrogen content ,carbon content and, mineral content of the soil. It elevates amino acids, chlorophyll content of plant and also the proteins.

Keratin biomass is most complex biological materials, it is responsible of majority of skin, hairs, nails, horns, beaks, claws, tortoiseshells and feathers. Keratin the main structural constituent of feathers contains a lot of valuable amino acids which are potential bioactive compounds as well (Stanly Merin Liya , et al. 2023). The Keratin protein contains of insoluble highly stable structure and it contains hydrophobic bonds and tightly packed in the form of an alpha-helical structure, alpha keratin, beta-pleated sheets, beta keratin into a super coiled polypeptide. 90% of the feather contains beta-keratin by mass (Onifade, et al. 1998). Keratin is an insoluble protein and it is not degradaed by normal proteases such as, tryspsin, papsin and papain. Keratin is introduced as the most abundant polymer in our nature, as that of cellulose and chitin. The process of dissolving and extracting keratin is very complicated as compared to extraction of other polymers like, collagen, chitosan and starch. The applications of keratin includes biocompatibility, low toxicity and biodegradability and it also have an promising biological applications. Some of the applications of keratin are aslo reported in multiple domains such as a bio-plastics, bio- stimulants, animal feed , cosmetics, bio-fertilizers, bio-fibres and also in bio-medical applications, constructing the medicals and drug capsules.

According to the survey by United States (US) Department of Agriculture, the meat produced in the year 2020 was about 100.5 million tons which have the result

producing about more than 4.7 million tons of poultry feathers around the world. According to the United Nations of Food and Agricultural Organization (FAO), there were found to be 24.8 billions of chickens produced annually in 2023 over the world, and there were 37.0 chickens produced in the year 2050.The recent strategies includes physical , chemical and, biological methods. Hence, use of keratin are cost- effective and also have fast extraction hydrolysis methods. So, the hydrolyzation of keratin by physical or chemical treatment requires a high temperature (Beryl, et al. 2021). The degradation of keratin protein by bacteria caused by the solubilisation of amino acids which was confirmed by High Performance Liquid Chromatography (HPLC) (Stanly Merin Liya , et al. 2023). Isolated and characterized feather degraded bacterium shows keratinolytic activities. The use of keratinolytic bacteria having antagonistic and plant growth promoting activities and feather hydrolysate can emerge as an sustainable and alternative tools to promote and improve organic farming, agro eco-sysytem, environmental human health and soil biological activities (K Tamreihao, et al. 2018).

This PGPB (Plant growth promoting bacteria) influence the growth & development of plants by various mechanisms which includes atmospheric nitrogen fixation, phosphate solubilization, etc. PGPB are the diverse group of micro- organisms living in plant rhizospheres and have been widely studied for increasing agricultural productivity. PGPB promotes the plant directly by producing indole acetic acid (IAA), a plant hormone playing a role in division of cells and elongation (Bhise et al.2017). PGPB are free living bacteria which also forms a symbiotic relationships with plants. It also releases certain metabolites which directly stimulates the growth. . PGPB are some of the beneficial organisms because they are able to supply essential plant nutrients to gain soil enrichment and to sustainable environmental health.

To overcome the hazardous effects of chemical fertilizers, Plant Growth Promoting Bacteria (PGPB) could serve as an alternative to chemical fertilizers, sustaining and

uplifting agricultural sectors for crop production. Due to the wide usage of chemical fertilizers, enormous threat to microbial population present in soil, human health via consumption and water ecosystem diseases are emerging extensively. Boundless use of synthetic fertilizers is cost- effective, but at same time it also creates an environmental issues. They colonize the plant rhizospheres and also support the growth of the plant directly as well as indirectly therefore it also promotes the plant stress management. It is believed that by using PGPB, the synthetic fertilizers to increase the crop productivity will reduce in larger extent. It aslo have certain mechanisms for promoting the crop productivity by interacting with plants via nitrogen fixation, solubility and Mineralization of phosphate, secretion of siderophores for enhanced iron absorption, phyto-hormones production and, secondary metabolites production.

A diverse group of bacteria, fungi and actinomycetes are capable of degrading feather keratin. The most prevalent and easy to handle micro-organisms among there is *Bacillus spp.* Feather waste was converted as a bio- fertilizer by treating feather with *Chryseobacterium spp.*, to enhance the release of nitrogen in soil. The micro- organisms that are able to deteriorate keratin present in feathers achieving applications providing cost-effective, environmentally benign and nutritious produced to the poultry as well as applied industries. Another study reflected the efficiency of Bacillus amyloliquefaciens 6B, isolated from the dumped feathers soil sample that had the capability to deteriorate native feathers within 24hrs (Goldy Primo Beryl., et al . 2021). There are variety of bacteria, actinomycetes and fungi which are known to produce keratinolytic enzymes. Among the bacteria , Gram-positive bacteria which includes, *Bacillus spp.,* Actinomycetes which includes *Streptomyces spp.* are the most common ones.The strains which have shown best degradation are *Bacillus pumilis A1, Bacillus cereus Wu2, Bacillus megaterium F7-1, Kocuria rosea, Pseudomonas stutzeri, Fervidobcterium islandicum AW-1, Alcaligens spp., Stenotrophomonas maltophilia*

and , Actinomadura keratinilyca Cpt29, they have accomplished total chicken feather degradation in a period of about 1.5 to 2.5 years (Brandelli ,2008).

REVIEW OF LITERATURE

Review of literature-:

Worldwide, the poultry meat processing industry generated large quantity of feathers by-products that amount to 40 $x10^9$ kg annually. The feathers are considered wastes although small amounts are often proceed into valuable products such as feather meal & fertilizers. The remaining waste is disposed of by incineration or by burial in controlled landfills. In proper disposal of these biological waste contributes to environmental damage and transmission of diseases. Economic pressures, environmental pressures, increasing interested in using renewable and sustainable raw materials, and the need to decrease reliance on non-renewable petroleum resources behind the industry to find better ways of dealing with waste feathers. A closer look at the structure & composition of feathers shows that the whole part of chicken feathers (Rachis & Barb) can be used as a source of a pure structural protein called keratin which can be exploited for conversion into a number of high value by-products. Additionally, several technologies can be used to convert other biological components of feathers into high value added by- products. Thus, conversion of the waste into valuable products can make feathers an attractive raw materials for the production of by- products in this review, possible applications of chicken feathers in a variety of technologies and products are discussed. Thus, using waste feathers as a valuable resource can help the poultry industry to disposed of the waste feathers in an environmentally sustainable manner that also generates extra income for the industry. Their valorization can result in their sustainable conversion into high value materials and products on the proviso of existence of development of cost effective technologies for converting these waste into useful products (Tamrat Tesfaye, et al. 2017)

The amount of chicken feathers generated as waste from the poultry industry in the last decade has become of technological interest for many researchers regarding both its treatment and applications. At the worldwide level, billions of tons of feathers are produced due to the consumption of poultry and, consequently, leading to problems as they are affective public health & environment. Biodegradation process of chicken feathers with the greatest international impact used pure strains, which have been isolated from wastes of poultry farms. Among the strains with the best degradation efficiency (100%) in 1-7days (ME Pahua- Ramos, et al.2017). The increase in consumption of chicken results in generation of large amounts of waste that need to be disposed of properly. Chicken feathers constitute about 5-10% of the weight of the chicken and thus, they comprise a significant protein of the poultry waste. Disposal of waste chicken feathers is problematic in that they do not readily should shortage of landfill space, and they are contaminated with microbial biomass that makes them hazardous waste. Feathers contain around 91% keratin protein and thus, potentially feathers can be beneficiated into high value compounds or products comprised of keratin proteins or keratinous fibres. Thus, valorization of feathers could be viable option for sustainable disposal of the waste (Tamrat Tesfaye , et al. 2018)

The characterization of the chicken feathers is an essential step to identifying possible avenues for valorisation of these waste biomass. While chemical, physical and, morphological properties of chicken feathers and related potential valorisation routes have described by the authors, identification of their mechanical , thermal & electrical properties have not been reported and these information is necessary to have a complete & comprehensive characterization of waste chicken feathers. Hence, in this research, the mechanical, thermal & electrical properties of feathers were determined & evaluated to as certain suitability of the feathers for production of high value materials. The feathers and fractions their of were characterized by TGA/DSC (Thermo gravitimetric analysis / Digital signature certificate),

instron (material & structural testing) dynamic mechanical analyzer, and a two probe measurement of resistivity instrument. Under heated condition, the TGA of chicken feathers confirmed the occurrence of three zones of weight loss. The TGC/DSC results relevated a glass transition temperature around 67 degree Celsius and a melting temperature 230 degree Celsius in the crystalline phase. The tendency of chicken feathers barbs at maximum load was 16.93cN-tex. The results from electrical properties indicated that chicken feathers fraction have low conductivity. Overall, the results indicates that chicken feathers have potential to be used in a variety of application such as electrical insulator materials, yarn production for use in textiles, nonwoven fabric production, filler for winter clothing geotextile and construction materials (Tamart Tesfaye, et al.2018).

Feathers are the major by- product of poultry industry and considered as waste. Feathers (composed of protein keratin) are metabolized by a number of micro-organisms as a source of carbon and nitrogen. Feathers are keratin just-like wool, but the surface area is much larger because the diameter of the fibres is smaller. As a result, the fibre can absorb more moisture than wool or cellulose fibres. The crystal structure of feathers fibres also makes them naturally stable & durable. Chicken feathers have a hollow structure. The average length of chicken feather is 135mm, with an aspect ratio varying from 400-2200. The feathers diameter is in the range of 5-6 micrometer, which is the smallest among all natural fibres available. The small diameter and high aspect ratio mean fibres weight has a higher surface area which makes them appropriate for lightweight applications. The chemical composition of chicken feather show 82-91% protein (keratin), 1% lipid, crude fibres (2-2.5%) and 8-12% water. The specific application of chicken feathers fibres includes- Chicken feathers fibres reinforced polymer composite & chicken feather fibres reinforced composite with epoxy & reinforcement in cement bonded composite & also additive and media density feathery broad (MDF) composite & also it is used in durable and low cost building materials for tropical climate(Adil A. Khan ,2023).

Feathers are the major waste by-products of chicken poultry and it is gradually increasing day by day. Specifically around 8.5 billion tons of chicken feathers are produced annually worldwide as a waste from the production unit of chicken meat and the Indians contribution alone is about 350 million tons. Accumulation of such huge volume of feathers which is a recalcitrant in nature, leads to environmental pollution and feather protein wastage. The feathers is rich protein source because it contains over 90% keratins. The keratin protein consists of insoluble, highly stable structure that is cross linked by bisulphide, hydrogen and, hydrophobic bonds and tightly packed in the form of alpha helix (alpha- keratin) and Beta- sheets (Beta- Keratin) into a supercoiled polypeptide. 90% of the feathers contains beta-

keratin by mass cross linking of protein chains by cysteine rich confers high mechanical stability and resistant to hydrolysis degradation by pepsin, trypsin and papain. Traditional feathers degradation process such as alkali hydrolysis and steam pressure cooking not only destroys an amino acids but also consume large amount of energy. Therefore, biodegradation feathers by microbial keratinase may provide a viable alternative. In enzymatic catalysis, the disulphide bond of keratin is reduced by the disulphide reductase followed by the action of proteolytic keratinase which simultaneously degrade the keratin into oligo and monomeric products. Keratin is produced by some micro- organisms like bacteria such as *Bacillus spp.* , fungi such as , *Aspergillus, Rhizomucors.* Some species of *Dermatophyte*s and and some species of *Actinomycetes* such as *Streptomyces bactum.* The keratinase obtained from micro-organisms have several applications in feed, fertilizer detergent, leather and pharmaceutical industries during last few decades many researchers have focused upon improvement in agronomic utilization of organic waste (Tanmay paul, eta al. 2012).

Degradation of feathers results in production of amino acids and peptides, which can be employed as precursor of plant growth promoting metabolites such as Indole acetic acid (IAA), ammonia and, HCN. The microbial degradation of feathers represents an alternative for development of slowly released nitrogen fertilizer. The degraded product of chicken feathers could generate appropriate amount of tryptophan which can serve as the key source of IAA synthesis which is a major plant growth regular. The isolate also showed a poultry feather degrading activity which is the main waste material of poultry industry and opulent source of protein, amino acids, Nitrogen, phosphorous, calcium, potassium, magnesium, zinc and copper. The management of poultry waste disposal and utilization of feathers degraded products as a biostimulant for better growth of plants. When feathers degrading *Bacillus licheniformes* is grown in culture, it secretes a keratinase that hydrolyses the beta keratin matrix of a feathers, thereby releasing oligopeptides that dissolved into the

medium surrounding the feathers and feather degrading *Bacilli*. These peptides absorb like passed through a sample of medium from which feather fragments, melanin granules, and, bacteria have been removed by centrifugation sample of medium in which white non- melanic feathers are degrading absorb more like than samples of medium in which black, melanic feathers are degrading which indicates that more oligo peptides are dissolved in medium surrounding white feathers than the medium surrounding black feathers. The differential absorption of light supports that *B.licheniformes* degrades white feathers more rapidly than black feathers (Gerald Goldstein , et al. 2004).A feather degrading culture was enriched with isolates from a poultry waste that digestors and adapted to grow with feathers as its primary source of carbon, sulphur and energy subsequently a feathers hydrolytic endospores forming, motile, rod- shaped bacterium was isolated from the feather degrading culture. The organism was gram stained variable and catalase positive and demonstrate facultative growth at thermophillic temperatures. The optimum rate of growth in nutrient broth occurred at 45-50 degree Celsius and at pH7.5 electronmicroscopy of the isolate showed internal crystals.The micro- organism was identified as *Bacillus licheniformes* (Ci M Williams, et al. 1990).

The Mechanism of extracellular keratin degradation by keratinolytic bacteria was proposed by Yamamura et al. The crude enzyme from the keratinolytic strain Stenotrophomonas sp. showed two peaks in ion exchange chromatography. The enzyme fraction of the first peak showed protease activity only, while the enzyme fraction in the second peak showed disulfide bond-reducing activity. None of the enzyme fractions independently showed keratinolytic activity. However, the keratinolytic activity was regained after the two enzyme fractions were mixed together. Mixing of the two enzyme fractions increased the keratinolytic activity to more than 50-fold, while the proteinase acitivity increased only to more than 2-fold in absence of disulfide reductase. The proposed mechanism demonstrated that the

two enzymes acted cooperatively resulting in higher keratinolytic activity for effective keratin degradation.

Keratin is a crystalline protein occurring in vertebrates in two forms, the α helix and the β sheet, stabilized by sulfur cross-links. Keratin is found in skin, horn, hair, claws and nails, feather, and hoof, often forming hierarchical structures. The stiffness of a keratin composite at a "normal" water content is, water has a platicizing effect by interfering with intermolecular hydrogen bonding. Structures such as hoof use both the stiffness and toughness of keratin. In hair, the α helices are bound, probably by hydrogen bonds, in a heterogeneous, poorly characterized, proteinaceous matrix. Under excessive tensile strain

in the α helices in hair transform into the β sheet form, leading to a pronounced two-phase stress–strain relationship and large hysteresis.

Keratins belong to the superfamily of intermediate filament (IF)-forming proteins. They are heterogeneous in size (40–72 kDa) and charge, and they are insoluble. The original keratin nomenclature, devised in 1982 by Roland Moll, Werner Franke, and their colleagues, was based upon protein separation by both charge and size via two-dimensional electrophoresis. A revised nomenclature was produced in 2006 to accommodate a large number of novel keratins (uncovered mainly through whole genome sequencing efforts) while complying with the human and mouse genome nomenclature committees. Type I keratins tend to be smaller (40–64 kDa) and acidic compared to the larger (52–68 kDa) and basic–neutral type II keratins ,Homology at both the protein or DNA sequence levels, as well as gene substructure (number and position of introns), readily confirms the partitioning of keratin genes and proteins into type I and II IF sequences.

Soil is replete with microscopic life forms including bacteria, fungi, actinomycetes, protozoa, and algae. Of these different microorganisms, bacteria are by far the most common (i.e., 95%). It has been known for some time that the soil hosts a large number

of bacteria (often around 10^8 to 10^9 cells per gram of soil) and that the number of culturable bacterial cells in soil is generally only about 1% of the total number of cells present . However, in environmentally stressed soils the number of culturable bacteria may be as low as 10^4 cells per gram of soil . Both the number and the type of bacteria that are found in different soils are influenced by the soil conditions including temperature, moisture, and the presence of salt and other chemicals as well as by the number and types of plants found in those soils . In addition, bacteria are generally not evenly distributed in soil. That is, the concentration of bacteria that is found around the roots of plants (i.e., in the rhizosphere) is typically much greater than in the rest of the soil. This is because of the presence of nutrients including sugars, amino acids, organic acids, and other small molecules from plant root exudates that may account for up to a third of the carbon that is fixed by a plant. (Bernard R Glick , 2012).

Regardless of the number of bacteria in a particular soil sample, the bacteria may affect plants in one of three ways. The interaction between soil bacteria and plants may be (from the perspective of the plant) beneficial, harmful, or neutral . However, the effect that a particular bacterium has on a plant may change as the conditions change. For example, a bacterium that facilitates plant growth by providing either fixed nitrogen or phosphorus, compounds that are often present in only limited amounts in many soils, is unlikely to provide any benefit to plants when significant amounts of chemical fertilizer is added to the soil. In addition, it is possible for a particular bacterium to affect different plants disparately. Thus, for example, an IAA overproducing mutant of the bacterium *Pseudomonas fluorescens* a stimulated root development in blackcurrant cuttings while inhibiting the development of roots in cherry cuttings. This observation may be interpreted as indicating that the blackcurrant cuttings contained a suboptimal level of IAA that was enhanced by the presence of the bacterium. On the other hand, with the cherry cuttings the IAA level was optimal prior to the addition of the bacterium and the additional IAA provided by the bacterium became inhibitory.

Notwithstanding, it is usually a straightforward matter to decide whether a bacterium either promotes or inhibits plant growth (Bernard R Glick , 2012).

The bacteria that can promote plant growth, that is, PGPB, include those that are free-living, those that form specific symbiotic relationships with plants (e.g., *Rhizobia* spp.), bacterial endophytes that can colonize some or a portion of a plant's interior tissues, and cyanobacteria (formerly called blue-green algae). Notwithstanding the differences between these bacteria, they all utilize the same mechanisms. PGPB may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria (Bernard R Glick , 2012).

Historically, *Rhizobia* spp. were studied extensively, from physiological, biochemical, and molecular biological perspectives, before much interest was shown in trying to understand or utilize other PGPB to facilitate plant growth. Thus, these early studies became a conceptual starting point for mechanistic studies of PGPB. However, since unlike *Rhizobia* spp., most PGPB fix no or only a limited amount of nitrogen, studies to better understand some of the mechanisms used by PGPB have addressed a wide range of different mechanisms (Bernard R Glick , 2012).

The plant analysis showed improvement in root & shoot length, fresh & dry weight, chlorophyll, proteins, amino acids, phenolics, flavonoids content and decreased level of proline. In addition, sodium uptake was decreased and potassium uptake was increased. Therefore, application of novel bioformulation could increase the yield of crop by ameliorating growth of plants.

OBJECTIVES

Objectives-:

1) Collection of poultry dumped soil.

2) Isolation of poultry feather degradation organism.

3) Characterization of poultry feather degrading organism.

4) Poultry feather degradation study.

5) Effect of poultry feather degraded lysate on seed germination.

6) Effect of poultry feather degraded lysate on plate growth.

MATERIALS AND METHODOLOGY

Materials and Methodology -:

Collection of soil sample –:

The soil sample is collected from poultry feathers dumping site in Sangli district and Kolhapur district. The six representative soil samples [(kookood khath – Bavada, Kolhapur), Poultry farm (sangli), Budhgoan (Sangli), Bavada (Kolhapur), Kavalapur (Sangli), PVPIT college ground (Sangli)] were collected in sterile polythene bag after thorough mixing and transported to the laboratory (Siddharthan Nagarajan, et al.2017).

Isolation of bacteria from the soil sample -:

One gram of soil sample was mixed with 100ml sterile distilled water and serially diluted from 10^{-1} to 10^{-8} The diluted sample was plated on Skim milk agar plates (Gelatin agar plates, Nutrient agar plates) for detecting the proteolytic bacteria showing protein utilization zone around the bacterial colonies. The plates were incubated at 37 degree Celsius for 24hrs. After incubation plates were observed for clear zone around the colony (Siddharthan Nagarajan, et al.2017).

Gram staining and motility -:

The gram staining of the organism was performed by standard procedure. Firstly, we prepared the smear of bacterial culture then, stained with crystal violet stain for about 30sec which is the primary stain. Rinse in d/w for 2 secs the, Grams Iodine which acts as an mordant for 1min. Again, rinse it with d/w. Wash it with 95% ethanol for 10-30secs which act as an decolorizing agent. Again, rinse it with d/w. Then, add Safranin stain

for 30-60secs which acts as counter stain and finally, rinse the slide with d/w. Observe the slide under oil immersion lens (OIL) in microscope.

The motility of the organisms was performed by standard procedure. Firstly, take a clean slide with small depression in the middle. Put a drop of culture with the help of inoculating loop above, the culture put a coverslip and fix the slide using wax. Now, put the slide upside down to bring the culture into hanging position. Observe the motility slide under 45x lens in microscope.

Screening of Keratinolytic bacteria-:

Morphologically distinct bacterial colonies were selected and sub cultured till pure cultures were obtained. The purified cultures were preserved as slants. Bacterial strains were screened for feather degradation in chicken feather medium (CFM).

Minimal media / Chicken feather medium (CFM) composition-

NaH2PO4	**0.5g**
KH2PO4	**0.7g**
FeCl3	**0.016g**
CaCl3	**0.1g**
MgCl2	**0.01g**
NaCl	**0.5g**
K2HPO4	**1.4g**
MgSO4	**0.1g**
Chicken feathers	**1g**
pH	**7**

Hence, these inoculated culture medium was incubated at 37 degree Celsius.

One loopful of bacterial culture was inoculated into the chicken feather medium (CFM) and was kept in shaker at 150rpm for 4 days. Extend of feather degradation was studied and feather weight loss was observed. After incubation under optimum conditions, the potent organisms were selected. The most promising strains was selected for further studies (Siddharthan Nagarajan, et al.2017).

Optimization of Bioprocess parameters-:

For choosing the most appropriate medium conditions for maximum protease production, parameters such as , pH (6,7,8,9,10), Temperature (25,37,40,45 degree Celsius), 0.5% carbon source each (Ribose, Glucose, Arabinose, Lactose, Xylose, Maltose, Dextrose, Galactose, Sucrose, Mannitol) and IMVic test was also performed which includes (Indole test, Methyl red test, Vogus prauskaur test, Citrate utilization test).

After every 24hrs of incubation, the amount of soluble protein and amino acids was quantified.

Protein Estimation-:

The estimation of soluble protein was done by 'Biuret method'. Firstly, we he pipetted out 0.1, 0.2, 0.4, 0.6, 0.8 and, 1ml of working standard protein (casein) solution into the series of labelled test tubes. Then, we pipetted 1ml of given sample into the another test tube. Making up the volume to 1ml in all the test tubes and the tube of 1ml distilled water was labelled as blank. Now, we added 3ml of Biuret reagent to all the tubes including the tube labelled as blank & unknown. Mix the contents of the tube by vortexing the tubes warm at 37 degree Celsius for 10mins. Now, we cool the content to room temperature and recorded the absorbance at 540nm against blank. Then, we plotted the std. graph and calculated the concentration of the proteins in the given sample (Gustaw Piotrowski, 1857).

Table 1- Estimation of protein content

Vol. of std. BSA(ml)	**Vol. of d/w (ml)**	**Concn. of protein(mg)**	**Vol. of Biuret reagent**	
0.0	1.0	0.0	3	Incubated
0.2	0.8	1	3	at
0.4	0.6	2	3	37 degree
0.6	0.4	3	3	celsius
0.8	0.2	4	3	for
1.0	0.0	5	3	24hrs
Blank	0.0	To be estimated	3	

Amino acid estimation-:

The estimation of amino acid content was done by 'Ninhydrin method'. Firstly, we he pipetted out 0.1, 0.2, 0.4, 0.6, 0.8 and, 1ml of working standard amino acid (Alanine) solution into the series of labelled test tubes. Then, we pipetted 1ml of given sample into the another test tube. Making up the volume to 1ml in all the test tubes and the tube of 1ml distilled water was labelled as blank. Now, we added 1ml of Ninhydrin reagent to all the tubes including the tube labelled as blank & unknown. Mix the contents of the tube by vortexing the tubes. Then, place all the tubes in boiling water bath for about 15mins, cool the tubes in cold water and add 5ml of diluent solvent (ethanol/ Glycerol) to each tube and mix it well. Record the absorbance at 540nm against blank. Then, we plotted the std. graph and calculated the concentration of the amino acids in the given sample (Siegfried ruhemann, 1910).

Table 2- Estimation of amino acid content

Vol. of std. amino acid soln (ml)	Vol. of d/w (ml)	Concn. of amino acid (ug)	Vol. of Ninhydrin reagent		Vol. of solvent (ml)
0.0	1.0	0.0	1	Incubate	5
0.2	0.8	20	1	in boiling	5
0.4	0.6	40	1	Water bath	5
0.6	0.4	60	1	for 15mins	5
0.8	0.2	80	1		5
1.0	0.0	100	1		5
Blank	0.0	To be estimated	1		5

Feather degradation study-:

- **Inoculum preparation-**

  To put forth feather degradation studies, inoculum was prepared. 100ml of physically optimized Minimal media production broth was sterilized at 121 degree Celsius for 20 mins. The sterilized broth, a loopful of protease producing bacterial culture was inoculated under sterilized conditions. Broth culture was incubated for 72hrs on rotary shaker (150rpm) at 37 degree Celsius and this served as an inoculum (Goldy primo beryl, et al.2021).

- **Degradation of feather sample in liquid medium-**

  The effects of temperature, pH, substrate concentration and incubation time on feather degradation was studied. The factors studied included initial pH of medium (7.0-9.5) adjusted by 1M HCL or 1M NaOH , incubation temperature (20-35 degree Celsius), feather concentration (1-12%) and incubation time (12-84hrs).

Incubation temperature profoundly influenced feather degradation. Incubation temperature of 37 degree Celsius resulted in maximum feather weight loss (80%). The feather degradation was drastically reduced at 37 degree celsius. Initial pH of the medium also affected the feather degradation and soluble peptide production. Maximum feather degradation (85%) and soluble peptide was observed. Optimum incubation time for feather degradation was observed at 72hrs. Even at 10% (W/V) feather concentration, 74% feather weight loss was achieved. Maximum soluble peptide production was observed. Feathers concentration was further increased in feather concentration retarded feather weight loss and soluble peptide production.

Chemical composition of the feather samples was determined using standard method. Biodegradation of keratin rich material (chicken feathers) was analyzed. The feathers are considered as a sole source of carbon and nitrogen (Goldy primo beryl, et al.2021).

In Vitro plant growth studies-:

Seed germination study-:

The seeds of *Vigna radiata* were surface sterilized with 75% ethanol for 10mins and where washed several times with distilled water. 5 petri plates were taken, in each petri plate 50 seeds of *Vigna radiata* were taken and labelled the plates as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and Control. In the plates of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. 0.5 ml of lysate was inoculated and, in the controlled plate d/w was poured to compare between the plates and hence, the plates with lysate (Nutrient rich medium) showed effective seed germination than the control plate

Pot analysis-:

The ability of studied organism along with chicken feather lysate to promote growth of *Vigna radiata* was evaluated by pot trials. 5 plant pots were taken, in each pot 5 seeds of *Vigna radiata* were planted and the pots were labelled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and Control. In the pots of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} .5 ml of lysate was inoculated and, in the controlled pot water was poured to compare between the pots and hence, the pots containing lysate (Nutrient rich medium) showed effective growth than the controlled one.

Plant analysis-:

Plant growth studies was performed according to modified method presented by for in vitro pot assay, seeds of *Vigna radiata* crops were adopted. Pots were filled with corresponding controlled soils (10-15%). Per pot (15seeds) were shown and the pots were watered regularly. The germinated seeds were allowed to grow for 10-15days, after which plant

growth parameters where evaluated and recorded. After 10-15days, matured plants were uprooted from the pots and their growth parameters where measured and tabulated. Parameters checked where germination efficiency of seeds, shoot length , root length, fresh weight & dry weight.

RESULTS AND DISCUSSIONS

Results-:

Collection of soil sample-:

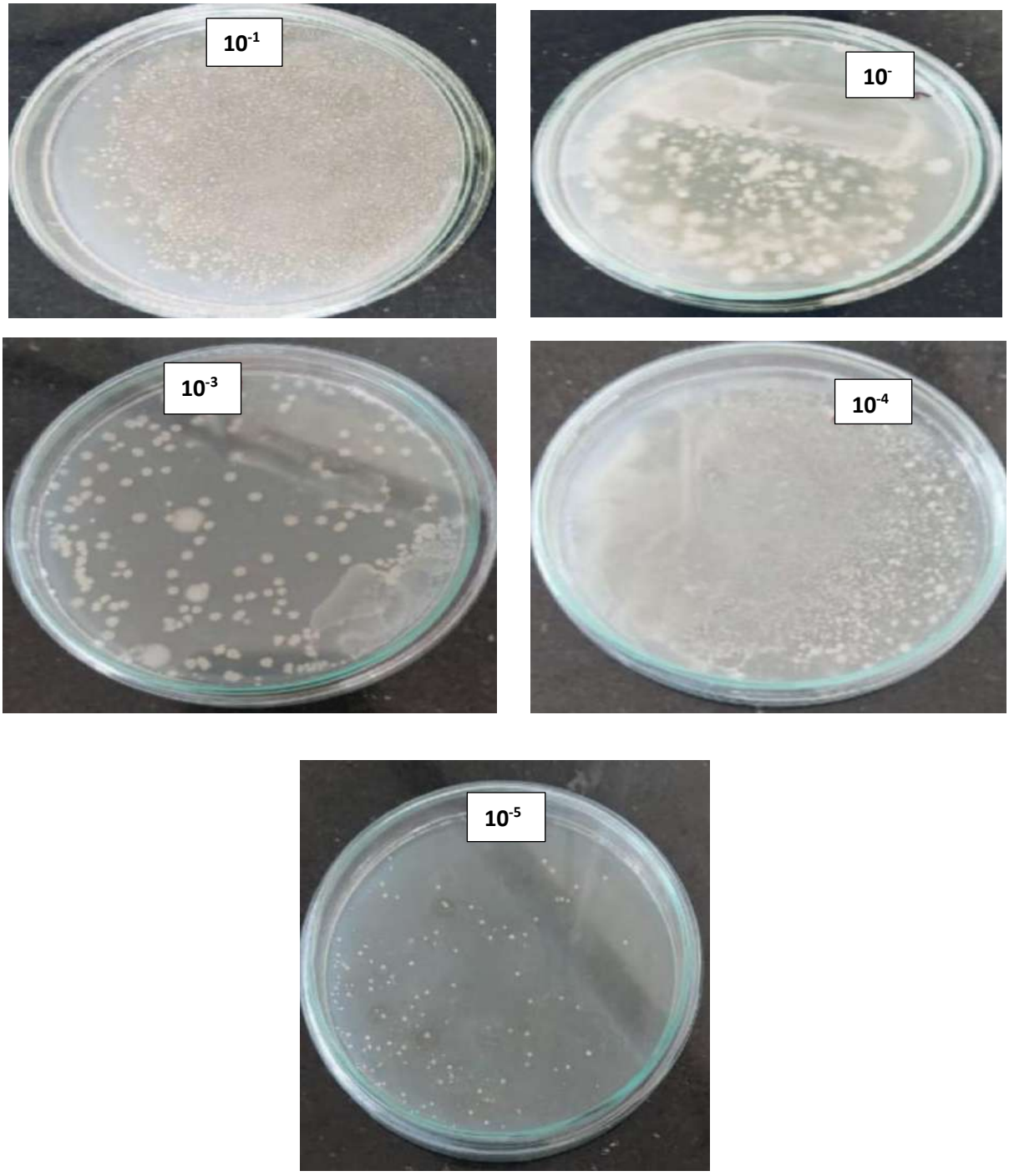
Firstly, we have collected the soil sample from poultry feathers dumping site which is located in Sangli district and Kolhapur district. The six representative soil samples [(kookood khath – Bavada, Kolhapur), Poultry farm (sangli), Budhgoan (Sangli), Bavada (Kolhapur), Kavalapur (Sangli) , PVPIT college ground (Sangli)] we have collected it in the polythene bag, after thorough mixing and transported to the laboratory.

Fig 1- Collection of soil sample

Isolation of bacteria from soil sample-

Collected soil samples were used to isolate organisms. The isolates were selected on the basis of clear zone around their growth. We have isolated around 75 bacteria from the soil sample. The isolated colonies were selected and purified. The culture of same colony was maintained on sterile nutrient agar slants. The cultures were stored at low temperature.

Fig 2- Isolation of bacteria



Colony characters-:

The bacterial colony were isolated from number of soil samples. The colony characters of this organisms where noted down. The colony characters are as in Table no.1

Table 1- Colony Characteristics

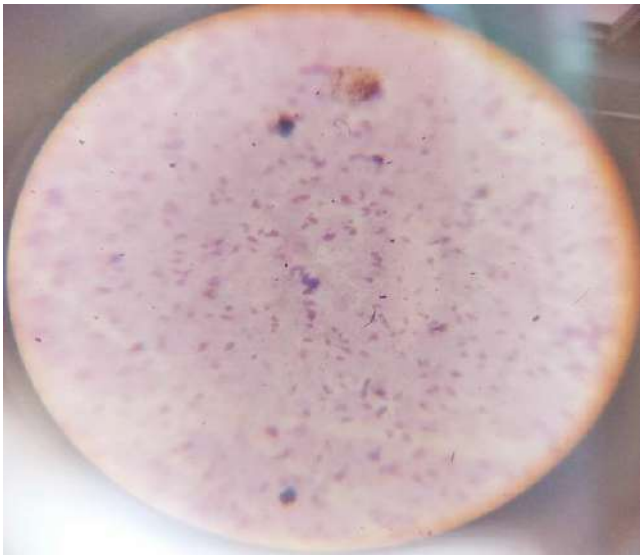
	Size	Shape	Color	Margin	Opacity	Elevation	Surface	Consistency
10^{-1}	1mm	circular	whitish	Entire	opaque	convex	smooth	moist
10^{-2}	2mm	irregular	whitish	wavy	transparent	flat	smooth	sticky
10^{-3}	1.5mm	circular	yellowish	Entire	opaque	convex	smooth	moist
10^{-4}	2 mm	tiny	orange	Entire	opaque	convex	smooth	mucoid
10^{-5}	1.7mm	circular	yellowish	Entire	translucent	flat	smooth	moist
10^{-6}	1.5mm	irregular	whitish	Entire	opaque	convex	smooth	sticky

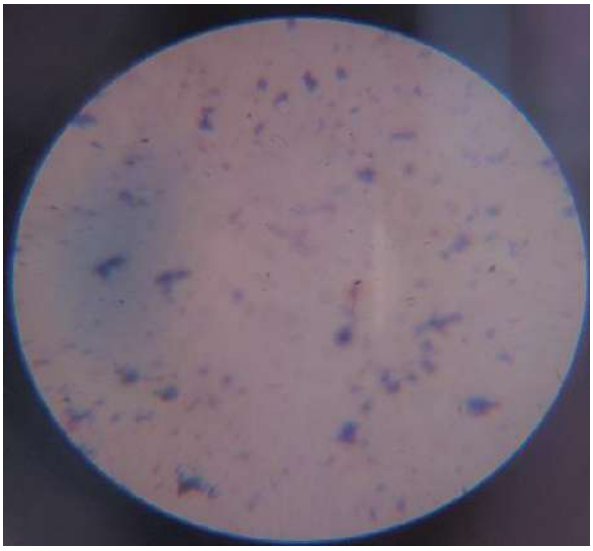
Gram staining-:

The isolated colonies were maintained on sterile nutrient agar slants under low temperature.

For initial characterization suspension of growth was prepared and gram staining was also performed.

Fig 3- Gram staining of selected isolates





Motility-:

The isolated colonies were maintained on sterile nutrient agar slants under low temperature. For initial characterization suspension of growth was prepared and Motility was also performed.

The motility of the organisms were noted down in fig 5.

Table No. 2 - Motility of selected isolates

Dilutions	Motility
10^{-1}	Non- motile
10^{-2}	Non-motile
10^{-3}	Non-motile
10^{-4}	Non-motile
10^{-5}	Non-motile
10^{-6}	Non-motile

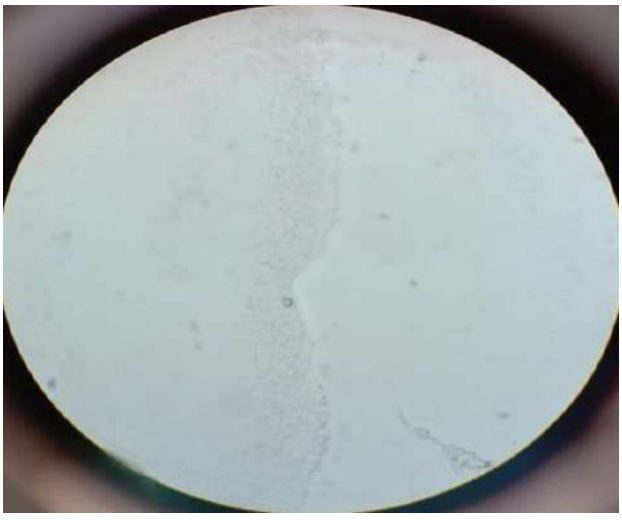


Fig 4-Motility

Biochemical results -:

The biochemical tests of various sugars were also performed. The type of sugars includes, Ribose, Glucose, Arabinose, Lactose, Xylose, Maltose, Dextrose, Galactose, Sucrose and Mannitol and, the IMVic test was also performed which includes Indole test, Methyl red test, Vogus prauskauer test and Citrate utilization test. The results of biochemical test is shown in Table No.3

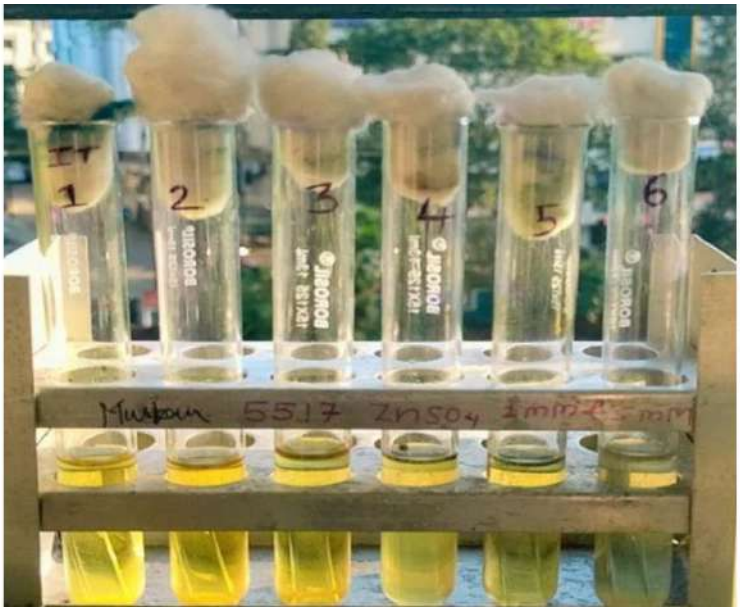
Among these tests, Maltose, Xylose, Methyl red, Vogus prauskauer and Indole test shows positive test for the lysate effectiveness. After, incubation at 37°C for 24hrs.

Table No 3-: Biochemical results

	Ribose	Glucose	Arabinose	Lactose	Xylose	Maltose	Dextrose	Galactose	Sucrose	Mannitol
10^{-1}	-	-	-	-	+	+	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	+	-	+	-	-
10^{-4}	-	-	-	-	-	-	-	-	-	-
10^{-5}	-	-	-	-	-	-	-	-	-	-
10^{-6}	-	-	-	-	-	-	-	-	-	-

	Indole test	Methyl red test	Vogus prauskaur test	Citrate utilization test
10^{-1}	+	+	+	+
10^{-2}	+	+	+	+
10^{-3}	+	+	+	+
10^{-4}	+	+	+	+
10^{-5}	+	+	+	+
10^{-6}	+	+	+	+

Results for Vogus Prauskaur test



Results for Indole test

Results for Methyl red test

Results for Maltose test

Results for Citrate Utilization test

Results for Xylose test

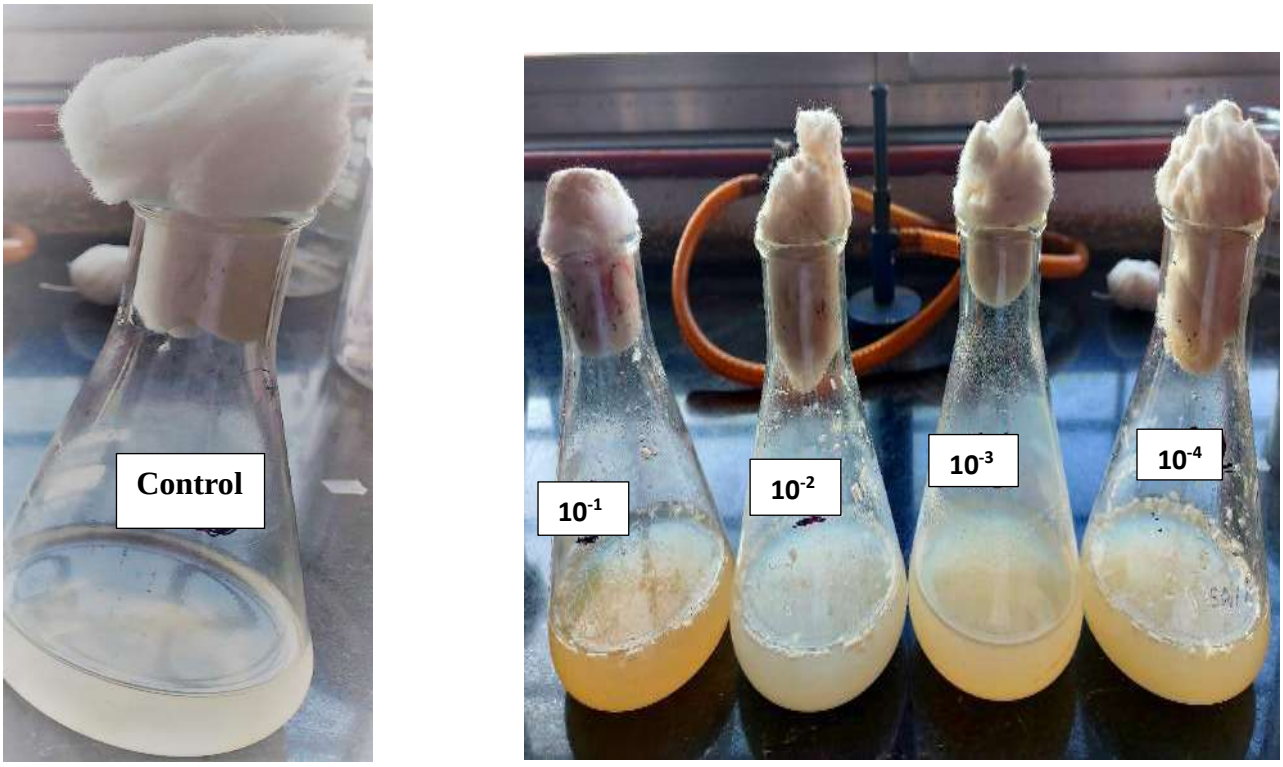
Degradation of chicken feathers-:

The bacterial strain were selected for feather degradation process in chicken feather medium (Minimal media). Lastly, these inoculated culture medium is incubated at 37 degree Celsius for 24hrs.

The chicken feather medium (CFM) was kept into the incubator shaker.. Then, finally it was observed that the feather was degraded and there was also a feather loss. The most promising strains was selected for further studies.

On the basis of effect of temperature, pH, substrate concentration and incubation time on which the further feather degradation study is done.

Fig 8- Degradation of chicken feathers



Estimation of Protein content-:

After 24hrs of incubation, the estimation of protein content was performed. The protein was estimated by a method called as *Biuret method.*

Std. Protein (Casein) solution	O.D. (530nm)
10^{-1}	0.10
10^{-2}	0.15
10^{-3}	0.20
10^{-4}	0.25
10^{-5}	0.28
Blank	0.00

Test	O.D. (530nm)
10^{-1}	0.13
10^{-2}	0.14
10^{-3}	0.17
10^{-4}	0.13

Fig 7-: Estimation of protein content

Estimation of amino acid content-:

The estimation of amino acid was also performed. The amino acid was estimated by a method called as *Ninhydrin method.*

Std. amino acid (Phenyl alanine) solution	O.D. (620nm)
10^{-1}	0.20
10^{-2}	0.23
10^{-3}	0.22
10^{-4}	0.26
10^{-5}	0.30
Blank	0.00

Test	O.D. (620nm)
10^{-1}	0.20
10^{-2}	0.21
10^{-3}	0.24
10^{-4}	0.25

In vitro plant study-:

Germination of seeds-

The ability of the selected organism along with the poultry feather lysate to promote the plant growth of *Vigna radiata* was performed. Finally, germination of seeds was performed.

Control

In the control plate, the water was poured there was no plant growth due to less amount of nutrient availability.

10^{-1}

In 10^{-1} dilution plate, 0.5ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

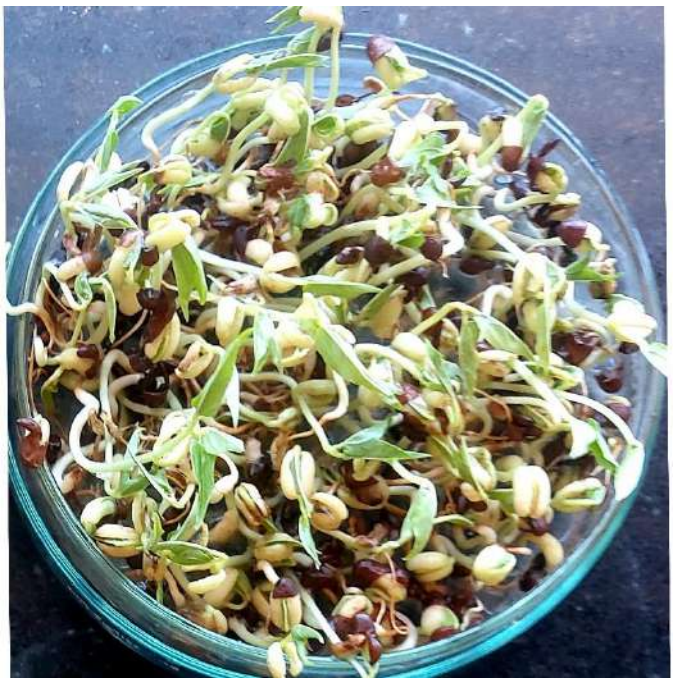
10^{-2}

In 10^{-2} dilution plate, 0.5ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

10^{-3}

In 10^{-3} dilution plate, 0.5ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days. This dilution showed most maximum plant growth.

10^{-4}



In 10^{-4} dilution plate, 0.5ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

10^{-5}

In 10^{-5} dilution plate, 0.5ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

Effect of inoculum on plant growth-:

Control

In the control plant pot, the water was poured there was no plant growth observed due to less amount of nutrient availability.

10^{-1}

In 10^{-1} dilution plant pot, 10ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

10^{-2}

In 10^{-2} dilution plant pot, 10ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

10^{-3}

In 10^{-3} dilution plant pot, 10ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days. This dilution showed most maximum plant growth.

10^{-4}

In 10^{-4} dilution plant pot, 10ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

10^{-5}

In 10^{-5} dilution plant pot, 10ml of lysate (nutrient rich) was poured which showed effective plant growth in 10-15 days.

Effect of inoculum on Length-:

In 10^{-1}, the root length is 6.4cm and shoot length is 16cm. In $10^{-2,}$ the root length is 5.2cm and shoot length is 15.2cm. In $10^{-3,}$ the root length is 8.8cm and shoot length is 15.8cm, which shows maximum effect on root length and shoot length of the plant. In $10^{-4,}$ the root length is 7.3 cm and shoot length is 14.3cm. In $10^{-5,}$ the root length is 6.6 cm and shoot length is 14.8cm. In $10^{-6,}$ the root length is 4.8 cm and shoot length is 14.3cm.

	Root length	**Shoot length**
10^{-1}	6.4cm	16cm
10^{-2}	5.2cm	15.2cm
10^{-3}	8.8cm	15.8cm
10^{-4}	7.3cm	14.3cm
10^{-5}	6.6cm	14.8cm
10^{-6}	4.8cm	14.3cm

Effect of inoculum on Weight-:

In 10^{-1}, the fresh weight is 0.32g and dry weight is 0.07g. In 10^{-2}, the fresh weight is 0.33g and dry weight is 0.14g. In 10^{-3}, the fresh weight is 0.53g and dry weight is 0.16g, which shows maximum effect on fresh weight and dry weight of the plant. In 10^{-4}, the fresh weight is 0.33g and dry weight is 0.09g. In 10^{-5}, the fresh weight is 0.42g and dry weight is 0.14g. In 10^{-6}, the fresh weight is 0.33g and dry weight is 0.08g.

	Fresh weight	**Dry weight**
10^{-1}	0.32g	0.07g
10^{-2}	0.33g	0.14g
10^{-3}	0.53g	0.16g
10^{-4}	0.33g	0.09g
10^{-5}	0.42g	0.14g
10^{-6}	0.33g	0.08g

Finally, pot analysis was done. Hence, root length, shoot length, dry weight and fresh weight was analyzed.

Discussions-:

In the present study, the soil sample is collected from poultry feathers dumping site in Sangli district and Kolhapur district. The soil were collected in sterile polythene bag after thorough mixing and transported to the laboratory. One gram of soil sample was mixed with sterile distilled water and serially diluted. The diluted sample was plated on Skim milk agar plates for detecting the proteolytic bacteria showing protein utilization zone around the bacterial colonies. The plates were incubated at 37 degree Celsius for 24hrs. After incubation plates were observed for clear zone around the colony. As well as in the previous study,firstly the keratinolytic activity of the isolated bacterial strain was determined in the milk agar medium hence, these isolates showed efficient keratinolytic acitivity (ashjan khalel,et al. 2020).

The identification of selected isolate was characterized based on there Gram staining property, motility, biochemical tests for the keratinolytic bacterial isolate respectively. The isolated strain was Gram positive and this strain showed negative result on motility. The indole test, methyl red test, vogus prauskaur test and, citrate utilization test is positive for the bacterial isolates. The sugar included which showed positive result is Xylose and Maltose. In the previous study, the identification of selected isolate was characterized based on their morphological, cultural, physiological and biochemical properties for the keratinolytic bacterial isolate respectively. The isolated strain was Gram positive and this strain showed positive result on motility, catalase, gelatin, starch, casein and, urease activity. The isolated keratinolytic bacteria from poultry waste dumping sites soil sample. The *Bacillus* strain are reported to have keratinolytic activity in many studies in past few decades (Siddharthan Nagarajan, et al. 2017).

Initially, strain was inoculated in minimal media and incubated for 24hrs at 150rpm and 32°C in flask containing 100 ml minimal medium broth supplemented with 1g chicken feathers. After few days, feather degradation or feather loss is observed whereas, initially the strain was inoculated in peptone water and incubated for 24hrs at 150rpm and 32°C in flask containing 25ml minimal medium supplemented with 1% chicken feathers, 5% inoculum were transferred. The flask were incubated for 24hrs at 150rpm at 32°C.

For Plant growth study, the seeds of *Vigna radiata* were surface sterilized with 75% ethanol for 10mins and where washed several times with distilled water. 5 petri plates were taken, in each petri plate 50 seeds of *Vigna radiata* were taken and labelled the plates as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and Control. In the plates of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. 0.5 ml of lysate was inoculated and, in the controlled plate d/w was poured to compare between the plates and hence, the plates with lysate (Nutrient rich medium) showed effective seed germination than the controlled one. The maximum seed germination was observed in 10^{-3} dilution plate which exhibited high plant growth parameters. Whereas, in the previous study after the 7days of seed germination it was observed that the plant growth in T2 treatments exhibited high plant growth parameters when compared to other treatments.

CONCLUSION

Conclusion-:

Poultry feathers are widely discarded as waste worldwide and are considered as environmental pollutant and a reservoir of pathogenic bacteria. Therefore, developing sustainable and environmentally friendly methods for managing feather waste is one of the important environmental protection requirements. Feathers feel breakdown within just a few months if mixed with a good carbon source. This feathers pollute both the soil and the water and create a heaven for dangerous micro- organisms, so, this feather waste pause a measure threat to the ecosystem and turn into a source of environmental pollution. Poultry feathers are considered a growing and annoying waste and in many countries, they are disposed of as environmental waste, which constitutes a burden on landfills in addition to showing their decomposition. Poultry feather degradation property could be efficiently utilized in feather waste management. This study is useful in rapid removal of the un-management waste of feather content with the release of valuable by-products.

The proper treatment of poultry feathers waste (PFW) might be an environmental friendly solid waste management tool and a good source of nitrogen rich organic fertilizers. Traditionally, feather waste had been disposed of through burning and dumping, leading to severe environmental damage, increased pollution, landscape degradation, and contamination of soil and groundwater. The soil sample is collected from poultry feathers dumping site in Sangli district and Kolhapur district. The soil samples were collected in sterile polythene bag after thorough mixing and transported to the laboratory. The isolation of bacterial strains from the soil sample is done. The colony characters was noted, Gram staining and motility was performed. The purified culture was preserved as slants at low temperature.

The feather degradation ability of isolates were tested by using minimal media containing poultry feather as only source of nutrient in the media. During feather

degradation study, the protein and amino acid contents of degraded lysate were determined after incubation period.

The ability of feather degraded lysate support plant growth was analysed. Initially seed germination study was performed the results of which indicated improvement in germination of mung beans. The effect of same inoculum on plant growth was tested. The *Vigna radiata* inoculated with feather degraded lysate along with the organism promoted the growth of plant significantly compared to control plant. The feather degraded lysate also helps for enrichment of soil with useful nutrients. Hence the use of multifarious bacteria with feather degraded lysate and holds a great potential to be used as biofertilizer to improve agricultural productivity.

BIBLIOGRAPHY

Bibliography -:

1) Bhise, K. K., Bhagwat, P. K., & Dandge, P. B. (2017). Synergistic effect of Chryseobacterium gleum sp. SUK with ACC deaminase activity in alleviation of salt stress and plant growth promotion in Triticum aestivum L. *3 Biotech*, *7*, 1-13.

2) Beryl, G. P., Thazeem, B., Umesh, M., Senthilkumar, K., Kumar, M. N., & Preethi, K. (2021). Bioconversion of feather composts using proteolytic Bacillus mycoides for their possible application as biofertilizer in agriculture. *Waste and Biomass Valorization*, *12*(12), 6795-6809.

3) Tamreihao, K., Mukherjee, S., Khunjamayum, R., Devi, L. J., Asem, R. S., & Ningthoujam, D. S. (2019). Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. *Journal of basic microbiology*, *59*(1), 4-13.

4) Pahua-Ramos, M. E., Hernández-Melchor, D. J., Camacho-Pérez, B., & Quezada-Cruz, M. (2017). Degradation of chicken feathers: a review. *Biotechnol. Ind. J*, *13*, 658-666.

5) Liya, S. M., & Umesh, M. (2023). Bioconversion of chicken feather waste into feather hydrolysate by multifaceted keratinolytic Bacillus tropicus LS27 and new insights into its antioxidant and plant growth-promoting properties. *Biomass Conversion and Biorefinery*, 1-11.

6) Liya, S. M., Umesh, M., Nag, A., Chinnathambi, A., Alharbi, S. A., Jhanani, G. K., ... & Brindhadevi, K. (2023). Optimized production of keratinolytic proteases from Bacillus tropicus LS27 and its application as a sustainable alternative for dehairing, destaining and metal recovery. *Environmental research*, *221*, 115283.

7) Onifade, A. A., Al-Sane, N. A., Al-Musallam, A. A., & Al-Zarban, S. (1998). A review: potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Bioresource technology*, *66*(1), 1-11.

8) Ningthoujam, D. S., Tamreihao, K., Mukherjee, S., Khunjamayum, R., Devi, L. J., & Asem, R. S. (2018). Keratinaceous wastes and their valorization through keratinolytic microorganisms. In *Keratin*. IntechOpen.

9) Brandelli, A., Sala, L., & Kalil, S. J. (2015). Microbial enzymes for bioconversion of poultry waste into added-value products. *Food Research International*, *73*, 3-12.

10) Brandelli, A., Daroit, D. J., & Riffel, A. (2010). Biochemical features of microbial keratinases and their production and applications. *Applied microbiology and biotechnology*, *85*, 1735-1750.

11) Tesfaye, T., Sithole, B., Ramjugernath, D., & Chunilall, V. (2017). Valorisation of chicken feathers: Characterisation of physical properties and morphological structure. *Journal of Cleaner Production*, *149*, 349-365.

12) Tesfaye, T., Sithole, B., Ramjugernath, D., & Mokhothu, T. (2018). Valorisation of chicken feathers: Characterisation of thermal, mechanical and electrical properties. *Sustainable Chemistry and Pharmacy*, *9*, 27-34.

13) Khan, A. A., Parikh, H., & Qureshi, M. R. N. (2022). A review on chicken feather fiber (CFF) and its application in composites. *Journal of Natural Fibers*, *19*(16), 12565-12585.

14) Paul, T., Das, A., Mandal, A., Halder, S. K., DasMohapatra, P. K., Pati, B. R., & Mondal, K. C. (2014). Valorization of chicken feather waste for concomitant production of keratinase, oligopeptides and essential amino acids under submerged fermentation by Paenibacillus woosongensis TKB2. *Waste and Biomass Valorization*, *5*, 575-584.

15) Paul, T., Das, A., Mandal, A., Halder, S. K., DasMohapatra, P. K., Pati, B. R., & Mondal, K. C. (2014). Production and purification of keratinase using chicken feather bioconversion by a newly isolated Aspergillus fumigatus TKF1: detection of valuable metabolites. *Biomass conversion and Biorefinery*, *4*, 137-148.

16) Goldstein, G., Flory, K. R., Browne, B. A., Majid, S., Ichida, J. M., & Burtt Jr, E. H. (2004). Bacterial degradation of black and white feathers. *The Auk*, *121*(3), 656-659.

17) Williams, C. M., Richter, C. S., MacKenzie Jr, J. M., & Shih, J. C. (1990). Isolation, identification, and characterization of a feather-degrading bacterium. *Applied and environmental microbiology*, *56*(6), 1509-1515.

18) Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, *2012*.

19) Nagarajan, S., Eswaran, P., Masilamani, R. P., & Natarajan, H. (2018). Chicken feather compost to promote the plant growth activity by using keratinolytic bacteria. *Waste and biomass valorization*, *9*, 531-538.

20) Jeong, J. H., Jeon, Y. D., Lee, O. M., Kim, J. D., Lee, N. R., Park, G. T., & Son, H. J. (2010). Characterization of a multifunctional feather-degrading Bacillus subtilis isolated from forest soil. *Biodegradation*, *21*, 1029-1040.

21) Sun, Z., Li, X., Liu, K., Chi, X., & Liu, L. (2021). Optimization for production of a plant growth promoting agent from the degradation of chicken feather using keratinase producing novel isolate Bacillus pumilus JYL. *Waste and Biomass Valorization*, *12*, 1943-1954.

22) Jeong, J. H., Park, K. H., Oh, D. J., Hwang, D. Y., Kim, H. S., Lee, C. Y., & Son, H. J. (2010). Keratinolytic enzyme-mediated biodegradation of recalcitrant feather by a newly isolated Xanthomonas sp. P5. *Polymer degradation and stability*, *95*(10), 1969-1977.

23) Kumari, M., & Kumar, J. (2020). Chicken feather waste degradation by Alternaria tenuissima and its application on plant growth. *Journal of Applied and Natural Science*, *12*(3), 411-414.

24) Jeong, J. H., Lee, O. M., Jeon, Y. D., Kim, J. D., Lee, N. R., Lee, C. Y., & Son, H. J. (2010). Production of keratinolytic enzyme by a newly isolated feather-degrading Stenotrophomonas maltophilia that produces plant growth-promoting activity. *Process biochemistry*, *45*(10), 1738-1745.

25) Jeong, J. H., Park, K. H., Oh, D. J., Hwang, D. Y., Kim, H. S., Lee, C. Y., & Son, H. J. (2010). Keratinolytic enzyme-mediated biodegradation of recalcitrant feather by a newly isolated Xanthomonas sp. P5. *Polymer degradation and stability*, *95*(10), 1969-1977.

26) Jagadeesan, Y., Meenakshisundaram, S., Pichaimuthu, S., & Balaiah, A. (2023). A scientific version of understanding "Why did the chickens cross the road"?–A guided journey through Bacillus spp. towards sustainable agriculture, circular economy and biofortification. *Environmental Research*, 117907.

27) Jagadeesan, Y., Meenakshisundaram, S., Raja, K., & Balaiah, A. (2023). Sustainable and efficient-recycling approach of chicken feather waste into liquid protein hydrolysate with biostimulant efficacy on plant, soil fertility and soil microbial consortium: A perspective to promote the circular economy. *Process Safety and Environmental Protection*, *170*, 573-583.

28) Kumari, M., & Kumar, J. (2020). Chicken feather waste degradation by Alternaria tenuissima and its application on plant growth. *Journal of Applied and Natural Science*, *12*(3), 411-414.

29) Bhari, R., Kaur, M., & Sarup Singh, R. (2021). Chicken feather waste hydrolysate as a superior biofertilizer in agroindustry. *Current Microbiology*, *78*(6), 2212-2230.

30) Ahmadi, L., Baharlouei, J., Khavazi, K., & DAVOUDI, M. H. (2024). Decomposed chicken feather: A biostimulant to lettuce (Lactuca sativa) growth. *The Indian Journal of Agricultural Sciences*, *94*(3), 246-250.

31) Wang, L., Cheng, G., Ren, Y., Dai, Z., Zhao, Z. S., Liu, F., ... & Tang, B. (2015). Degradation of intact chicken feathers by Thermoactinomyces sp. CDF and characterization of its keratinolytic protease. *Applied microbiology and biotechnology*, *99*, 3949-3959.

32) Peng, Z., Mao, X., Zhang, J., Du, G., & Chen, J. (2019). Effective biodegradation of chicken feather waste by co-cultivation of keratinase producing strains. *Microbial cell factories*, *18*, 1-11.

33) Peng, Z., Zhang, J., Du, G., & Chen, J. (2019). Keratin waste recycling based on microbial degradation: mechanisms and prospects. *ACS sustainable chemistry & engineering*, *7*(11), 9727-9736.

34) El-Naghy, M. A., El-Ktatny, M. S., Fadl-Allah, E. M., & Nazeer, W. W. (1998). Degradation of chicken feathers by Chrysosporium georgiae. *Mycopathologia*, *143*, 77-84.

35) El-Gindy, Y. M., Sabir, S. A., Zahran, S. M., Ahmed, M. H., Reuben, R. C., & Salem, A. Z. (2023). Effect of dietary onion (Allium cepa L.) powder as an antioxidant on semen quality, blood biochemicals, and reproductive parameters, as well as immunological variables of rabbit bucks under severe heat stress. *Tropical Animal Health and Production*, *55*(6), 380.

36) Kim, J. M., Lim, W. J., & Suh, H. J. (2001). Feather-degrading Bacillus species from poultry waste. *Process Biochemistry*, *37*(3), 287-291.

37) Kumar, E. V., Srijana, M., Chaitanya, K., Reddy, Y., & Reddy, G. (2011). Biodegradation of poultry feathers by a novel bacterial isolate Bacillus altitudinis GVC11.

38) Suntornsuk, W., & Suntornsuk, L. (2003). Feather degradation by Bacillus sp. FK 46 in submerged cultivation. *Bioresource technology*, *86*(3), 239-243.

39) Chaturvedi, V., Agrawal, K., & Verma, P. (2021). Chicken feathers: A treasure cove of useful metabolites and value-added products. *Environmental Sustainability*, *4*, 231-243.

40) Wang, T., Liang, C., Sun, Y., Gao, W., Luo, X., Gao, Q., ... & Yuan, H. (2019). Strategical isolation of efficient chicken feather–degrading bacterial strains from tea plantation soil sample. *International Microbiology*, *22*, 227-237.

41) Yadav, S., & Khosla, B. (2021). Biodegradation of poultry feather waste by keratinase producing Bacillus cereus strain isolated from poultry farms waste disposal site. *Case Studies in Chemical and Environmental Engineering*, *4*, 100114.

42) Kumar, J. I. T. E. N. D. R. A., Kumar, P. A. N. K. A. J., & Kushwaha, R. K. S. (2020). Recycling of chicken feather protein into compost by Chrysosporium indicum JK14 and their effect on the growth promotion of Zea mays. *Plant Cell Biotechnology and Molecular Biology*, *21*(37&38), 75-80.

43) Kumari, M., & Kumar, J. (2020). Chicken feather waste degradation by Alternaria tenuissima and its application on plant growth. *Journal of Applied and Natural Science*, *12*(3), 411-414.

44) Kumar, J., & Mahal, S. (2021). Isolation of Chrysosporium indicum from poultry soil for keratinase enzyme, its purification and partial characterization. *Journal of Applied and Natural Science*, *13*(2), 744-751.

45) Kumar, J., & Yadav, R. (2020). Keratinolysis of chicken feather and human hair by nondermatophytic keratinophilic fungi isolated from soil. *Journal of Applied and Natural Science*, *12*(4), 568-574.

46) Sahoo, S., Dash, S., Rath, B., Mondal, K. C., & Mandal, A. (2023). Commercial initiation of feather hydrolysate as supreme fertilizer: A smart bio-cleaning strategy of poultry waste. *Waste and Biomass Valorization*, *14*(7), 2151-2166.

47) Ly, T. T. N., Toán, Q. T., & Thiên, T. V. (2015). A study of IAA producing ability of chicken feather biodegradation bacteria and application of feather lysate as plant growth promoting agent. *Tạp chí Khoa học và Công nghệ-Đại học Đà Nẵng*, 89-92.

48) Bhari, R., Kaur, M., & Sarup Singh, R. (2021). Chicken feather waste hydrolysate as a superior biofertilizer in agroindustry. *Current Microbiology*, *78*(6), 2212-2230.

49) Mann, G. (1906). *Chemistry of the proteids*. Macmillan and Company, limited.

50) Hansen, D. B., & Joullié, M. M. (2005). The development of novel ninhydrin analogues. *Chemical Society Reviews*, *34*(5), 408-417.\

51) Chang, W. T., Hsieh, C. H., Hsieh, H. S., & Chen, C. (2009). Conversion of crude chitosan to an anti-fungal protease by Bacillus cereus. *World Journal of Microbiology and Biotechnology*, *25*, 375-382.

52) Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles,

mechanisms of action, and future prospects. *Applied and environmental microbiology*, *71*(9), 4951-4959.

53) Muleta, D., Assefa, F., Börjesson, E., & Granhall, U. (2013). Phosphate-solubilising rhizobacteria associated with Coffea arabica L. in natural coffee forests of southwestern Ethiopia. *Journal of the Saudi Society of Agricultural Sciences*, *12*(1), 73-84.

54) Joardar, J. C., & Rahman, M. M. (2018). Poultry feather waste management and effects on plant growth. *International Journal of Recycling of Organic Waste in Agriculture*, *7*, 183-188.

55) Masih, H., & Singh, S. (2014). Degradation of keratinous waste products by keratinolytic bacteria isolated from soil. *International journal of engineering and computer science*, *3*(08).