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Production of keratinase enzyme from a local isolate of Kocuria rosea using environmental waste

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Abstract

Objective

Keratinase is an enzyme that belongs to the metalloprotease group, along with many other protein-degrading enzymes. Keratinase is a specialized enzyme that acts on the substrate (keratin). It breaks the strong chemical bonds of keratin. This study aimed to produce the enzyme keratinase using local bacterial isolates obtained from soil samples and poultry waste from different areas in Al-Anbar province, using various waste materials such as hooves, horns, and hides.

Materials and methods

Seventeen bacterial isolates were obtained, which demonstrated a high ability to grow on feather agar medium used for isolation, from 20 soil and poultry waste samples. The five most efficient bacterial isolates were selected based on the diameter of the colonies growing on the feather agar medium. Among them, the bacterial isolate designated with the local code A2 was chosen as the most efficient in keratin degradation after culturing the five selected isolates on pure keratin medium. The bacterial isolate was identified based on morphological, cultural, microscopic characteristics, and biochemical tests. The VITEK 2 Compact device was used to confirm the identification.

Results

The results indicated that the isolate was *Kocuria rosea*. The optimal conditions for enzyme production from *Kocuria rosea*, including temperature, pH, inoculum size, carbon source and its concentration, nitrogen source and its concentration, and incubation time were studied. Based on the experiments and their results, the medium prepared using sheep hooves was selected for the growth of *Kocuria rosea* and the production of keratinase, particularly since it is an environmentally available and inexpensive waste in the local environment. The results of the optimal conditions study showed that the best production of keratinase enzyme was at a temperature of 30°C, a pH of 8.0, shaking speed of 150 rpm, with 3g/100mL of sheep hooves in the medium, 5mL/100mL inoculum, using urea as the nitrogen source at a concentration of 0.1g/100mL, and an incubation time of 72 hours. The enzymatic activity reached 0.301 U/mL.

Conclusions

These findings underscore the potential of *Kocuria rosea* in bioindustrial applications, particularly in processes involving keratin waste recycling and sustainable waste management.

Keywords: Bioindustry, Iraq, keratinolytic bacteria, metalloprotease group, Vitek 2 system

Paper Type: Research Paper.

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Introduction

Proteases or protein-degrading enzymes are a group of enzymes that catalyze hydrolysis reactions. These enzymes break down proteins into peptides and amino acids. These enzymes catalyze the breakdown of the peptide bond of proteins at different sites and under different

conditions. Proteases constitute about 60% of the remaining enzymes (Jamir and Seshagirirao 2018). These enzymes are abundant in nature and are produced from different sources. Research and studies have confirmed the presence of these enzymes in plants and animals as well as in various microorganisms. Microorganisms such as bacteria, fungi and yeasts are important sources for the production of protein-degrading enzymes (Razzaq et al. 2019). Proteases can degrade a wide range of substrates, including casein, gelatin, and bovine serum albumin. The average molecular weight of proteases is (18-90) kilodaltons (Gurumallesh et al. 2019). These enzymes vary in their composition and properties depending on the producing organism (animal, plant, microorganism) as well as the growth conditions of these organisms (Liu et al. 2021).

Keratinase is an enzyme that belongs to the metalloprotease group, along with many other protein-degrading enzymes. Keratinase is a specialized enzyme that acts on the substrate (keratin). It breaks the strong chemical bonds of keratin. Apart from traditional proteases, keratinase enzymes have broad specificity for the substrate rich in insoluble keratin. Proteases, in general and keratinase, in particular are of great importance in the field of bioindustry. It is used in textile industries, as well as in the recycling of feathers, leather, textiles, feed, fertilizers, and cosmetics. Comprehensive studies have been conducted on keratinase, describing its sources, production, purification, characterization, examination methods, and applications in economic fields (Kothari et al. 2017).

Keratinases are mostly produced in basic media containing mineral salts bound to keratin. Research by (Emran et al. 2020)indicates that keratinolytic bacteria may utilize processed keratin as their only carbon source. Research has demonstrated that keratinolytic enzyme synthesis does not always require a keratin matrix. Feathers and animal hair, which are both composed of intact keratin, can be used to make the enzyme (Moussa et al. 2021). Keratinases can also be produced using other non-keratin materials that have the property of stimulating the production of keratinase in cells (Gudzenko et al. 2022).

Supplements enriched with keratin can boost keratinase production when combined with other carbon or nitrogen sources. Some examples of such carbon sources include sugarcane bagasse, date molasses (Wei et al. 2022), glucose, and additional nitrogen sources like yeast extract, tryptone, urea, peptone, sodium nitrate, and ammonium chloride (Ramnani et al. 2004).

When designing enzymes for mass production in factories, their relative positions to the cell body are an important consideration. Assume for a moment that the enzyme is released into the fermentation medium from outside the cell. Since cell breaking is not required, it is the preferable method for commercial enzyme synthesis in that circumstance. Thus, the cost of enzyme production is reduced, and the target enzymes can be easily isolated. On the contrary, if the enzyme is intracellular and not secreted into the fermentation medium, cell breakage is required,

and enzyme isolation and purification are difficult. Moreover, livestock production in general and domestic chicken production in particular plays a vital socio-economic role for people living in low-income countries of Africa and Asia (Mohammadifar et al. 2014; Moazeni et al. 2016a; Mohamadinejad et al. 2024). Domestic chickens are widely distributed avian species around the world, due to their short generation interval and adaptability in a wide range of agro ecologies (Mohammadifar and Mohammadabadi, 2018; Moazeni et al. 2016b; Khabiri et al. 2022). The domestic chickens provide high quality protein and income for the poor rural households and are the most widely kept livestock species in the world (Mohammadabadi et al. 2010; Mohammadifar and Mohammadabadi, 2018). This is due to the presence of the valuable traits of chicken like disease resistance, adaptation to harsh environments and ability to utilize poor quality feeds (Shahdadnejad et al. 2016; Khabiri et al. 2023). Poultry manure is very suitable for many agricultural products such as vegetables, garden and field crop due to its high nitrogen content. However, it should be noted that each type of agricultural product and each type of soil has specific needs in terms of fertilization. For this reason, it is better to examine the needs of the agricultural product and soil before use and determine the amount of fertilizer according to the type of product, the amount of nitrogen in the soil and other factors. We must also properly manage the amount of chicken manure added to the soil to prevent soil and groundwater contamination. Thus, this study aimed to produce the enzyme keratinase using local bacterial isolates obtained from soil samples and poultry waste from different areas in Al-Anbar province, using various waste materials such as hooves, horns, and hides.

Materials and methods

Isolation of keratinase production bacteria: 20 soil samples were collected from different areas in Anbar Governorate. They included soils contaminated with poultry waste and soils planted with various field crops in order to isolate keratinase-producing bacteria from the soil. (1 g) of each soil sample was taken after cleaning it from impurities and conducting a series of decimal dilutions on it in distilled water. The pouring plate method was used, as (1 mL) was taken from the fourth dilution, 4-10 and the fifth dilution, 5-10. Each of them was placed in sterile Petri dishes. Then, a sterile feather agar medium consisting of 10 g of chicken feather powder, NaCl (0.5 g), KH2PO4 (0.7 g), K2HPO4 (1.4 g), MgSO4.7H2O (0.1 g), MgCl2.6H2O (0.48 g), and agar (15 g), in 1000 mL of distilled water, was added to the plates containing the dilutions. The plates were moved clockwise and counterclockwise to ensure a homogeneous distribution of the sample. They were incubated at 30 °C for 72 h. Growth and density was observed on these plates. These isolates were re-purified by sub-culturing them with several transfers on a feather agar

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medium to obtain pure single colonies. Then, the purified isolates were screened on feather agar medium by culturing them in a circle with a diameter of (1 cm) in the center of the plate. It was incubated at 30°C for 48 hours to select the most efficient isolates in enzyme production. The keratinase production trait was then detected by measuring the diameter of the colony or the transparent area around the growing colony in the middle of the dish.

Identification of bacterial isolates: Morphological features, such as colony form, boundaries, height, color, and texture, were used to identify the most productive bacterial isolates. After that, Gram's staining of the bacterial cells allowed for their microscopic examination in accordance with (Holt et al. 1994). Biochemical tests were also performed on these isolates for their identification (MacFaddin, 2000). Then, "the diagnosis was confirmed using the Vitek 2 compact system provided by the French company BioMerieux".

Selection of the best local carbon source for keratinase enzyme production: The optimal local carbon source for the synthesis of the keratinase enzyme was determined by comparing three different local carbon sources: horn powder, hoof powder and hides. Horn agar medium, hoof agar medium and hide agar medium were prepared by adding 2 g of horn powder, 2 g of hoof powder and 2 g of hide powder (each separately) to NaCl (0.05 g), KH2PO4 (0.07 g), K2HPO4 (0.14 g), MgSo4.7H2O (0.01 g) and 1.5 g of agar in 100 mL of distilled water. Then, the media were sterilized by autoclaving. Petri dishes were used to pour the cooled medium, which was then permitted to harden. Next, three copies of each source were used to plant the chosen bacterial isolate in the middle of the dish, creating a circle with a diameter of 1 cm. A 72-hour incubation period was carried out at 30°C. The diameters of the bacterial colonies on each of the aforementioned mediums were used to choose the optimal residue as a carbon source for the manufacture of the keratinase enzyme.

Keratinase assay: Keratinase activity assay According to (Cai et al. 2008), the keratinolysis technique was used to measure enzyme activity using 1% chicken feather powder in 0.05 M Tris-HCl solution (pH 8.0). The reaction mixtures included 1 mL of substrate and crude enzyme solutions. The mixture was incubated in a water bath at 50 °C for 20 min. Add 2.0 mL of 0.4 M trichloroacetic acid to stop the process. Without adding chicken feather powder, the enzyme solution was incubated with 2.0 mL of TCA to make the control solution. For 30 min, the solution was centrifuged at 3825 rpm. A spectrophotometer assessed absorbance at 280 nm, with one unit (U/mL) of enzyme activity defined as a 0.01 absorbance increase.

pH: The growth medium pH was adjusted to 6, 7, 8, 9, 10 to find the best pH for keratinase enzyme synthesis in liquid media.

Inoculum size: In order to examine the impact of the increased inoculum size on the production of the keratinase enzyme, the liquid medium was inoculated with several sizes of the chosen bacterial isolate. (1, 2, 3, 4, 5) mL/100 mL media was the inoculum size that was utilized.

Temperature: Different temperatures were tested (25, 30, 35, 40, 45) C "to determine the optimum temperature for the production of keratinase enzyme in the liquid production medium".

Carbon source concentration: After screening the waste materials using a solid medium, it was found that hoofs produced the highest concentration of keratinase enzyme, making them an ideal candidate for use as a local carbon source in enzyme synthesis. Other waste materials investigated included skin and horns. It is abundantly available as environmental waste and is inexpensive to obtain from an economic point of view. It was added in an amount of (1, 2, 3, 4, and 5) g/100 mL of medium to determine the optimal concentration for the production of keratinase enzyme.

Type of nitrogen source: Organic sources of nitrogen, such as peptone and urea, and inorganic sources, such as sodium nitrate and ammonium sulfate, were tested to find the optimal nitrogen source for keratinase enzyme synthesis.

Concentration of the nitrogen source: Urea was selected as the best nitrogen source based on the previous step for the production of keratinase enzyme, as it was added to the liquid medium in different weights (0.1, 0.2, 0.3, 0.4, and 0.5) g/100 mL of medium to determine the optimal concentration for the production of keratinase enzyme.

Period of incubation: To find the optimal incubation period for keratinase enzyme production, the chosen isolate was tested on a liquid medium with hooves as a carbon source. The incubation periods were 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. Temperature, pH, and concentration of carbon source as well as the type and concentration of nitrogen source were also varied.

Results and discussion

Isolation of bacteria producing keratinase enzyme: Table 1 displays the first isolation results, which revealed that out of 30 bacterial isolates obtained from 20 soil samples sent to the lab, 17 were able to grow successfully on feather agar medium. The growth density of these isolates on feather agar medium, a source of carbon and energy, determined their capacity to manufacture the keratinase enzyme. The results of the secondary screening showed that obtaining five local isolates was efficient in analyzing the residue. These isolates carried the local codes (A2, E1, F1, G1, and H1), and the diameters of the colonies of these isolates were (3.5, 3, 3, 3, and 3.5) cm, respectively.

Table 1. Growth diameter rate of bacterial isolates on isolation medium (cm)

No.	Isolate symbol	Average diameter of growth zone (cm)
1	A1	2.5
2	A2	3.5
3	B1	2.5
4	C1	2
5	D1	1.5
6	E1	3
7	E2	2.5
8	F1	3
9	F2	2.5
10	G1	3
11	H1	3.5
12	Z1	2.5
13	J1	2
14	K1	2.5
15	U1	2.5
16	U2	2
17	X1	1.5

Testing the efficiency of the selected isolates by re-culturing them on keratin medium: the most efficient isolate in keratin analysis was selected by re-culturing the five isolates selected in the previous paragraph on keratin agar medium by making a circle with a diameter of 1 cm in the centre of the dish. The dishes were incubated for 72 hours at 30°C. The isolates appeared with different diameters, as shown in Table 2. The isolate bearing the local symbol A2 was selected from among the five selected isolates (Figure 1) based on the growth rate of its diameter (Deba et al. 2023).

Diagnosis: Tables 3 and 4 show the results of the biochemical, microscopic, and cultural tests conducted on the chosen bacterial isolate with the local code (A2), which was based on (Holt et al. 1994). The VITEK2 Compact device's ability to diagnose the isolation verified this.

Selection of the best carbon source for keratinase enzyme production: Figure 2 shows the findings of the experiment demonstrating that the best isolate for manufacturing the keratinase enzyme, Kocuria rosea, varied in its capacity to utilize the carbon sources represented by horns,

hooves, and skin. The optimal carbon source for enzyme synthesis was determined to be hoof, which resulted in an average bacterial colony diameter of 3.5 cm.

Table 2. Average growth diameters of bacterial isolates on keratin medium (cm)

No.	bacterial isolation symbol	Average diameter of growth zone (cm)
1	**A2**	**3**
2	**E1**	**2.5**
3	**F1**	**2**
4	**G1**	**2**
5	**H1**	**2**

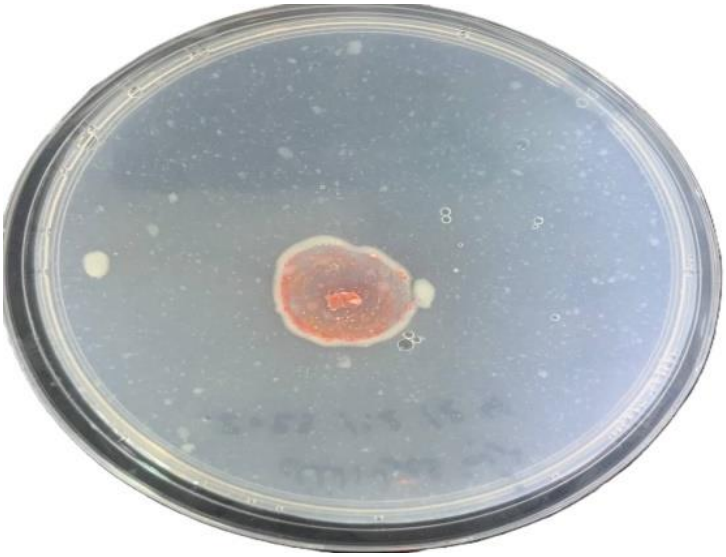

Figure 1. Bacterial isolate with local code A2 on keratin medium with a lysis diameter of 3.5 cm

Table 3. Cultural and microscopic specifications of the bacterial isolate with local code A2

Nature of colonies on solid medium						Bacteria shape	Cell aggregation	Gram dye
Shape	Color	Texture	Edge	Transparency	Height	Spherical	double, groups	positive
circle	pink	mucous	soft	dark	Convex			

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Table 4. Results of biochemical tests for the bacterial isolate with local code A2

No.	Type of test	Result
1	Catalase	+
2	Oxidase	-
3	Indole	+
4	Methyl red	-
5	Voges-Proskauer	-
6	Motility	-
7	Urease	-
8	Starch hydrolysis	-
9	Gelatin hydrolysis	+

(+) positive for the test (-) negative for the test

This result is attributed to the high content of keratin in the hooves. In addition, the chemical composition of cattle hooves is characterized by containing a large amount of keratin in the form of alpha-helical with a mixture of beta sheets and about 90% crude protein (Falaye et al. 2020).

Figure 2. The efficiency of selected bacterial isolates in exploiting local carbon resources

Optimizing the optimal conditions for the growth of the selected bacterial isolate producing the keratinase enzyme-pH: Figure 3 shows that of all the pH values tested, the enzyme productivity utilizing Kocuria rosea bacteria at pH 8 produced the best yield. At pH 9, the enzyme concentration was 0.101 U/mL, after which it reached 0.110 U/mL. At other pH values, however, enzyme production decreased. One of the variables that determine enzyme synthesis is the pH. It does this by influencing how the microbes involved in production affect the solubility and readiness of the nutrients. This causes the microbes to produce the enzyme since

it will show up in their growth. There is a correlation between the production medium's pH and the efficiency of enzyme activities and nutrient and material transfer across cell membranes. The microbe that produces it has its metabolism impacted. To get the most out of the enzyme manufacturing, the pH needs to be right for the microbe doing the creating (Moradi et al. 2020).

This result is almost consistent with what was reached by Barman et al. (2017), who were able to produce the keratinase enzyme from Arthrobacter sp. bacteria at pH (7.5). Also, Hashem et al. (2018) were able to obtain the optimal production of the keratinase enzyme from Bacillus licheniformis bacteria at pH 8.5.

This result is completely consistent with what was reached by Mazotto et al. (2022), who obtained the highest production of the keratinase enzyme from Bacillus subtilis bacteria at pH 8.

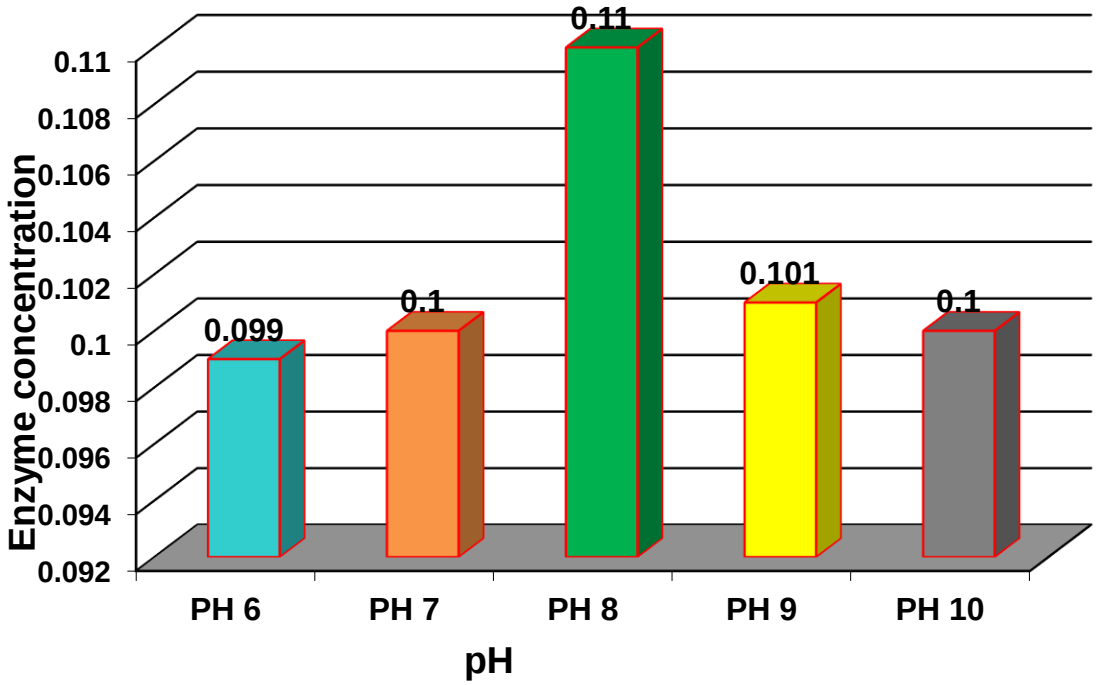

Figure 3. Effect of pH on the production of keratinase enzyme (L.S.D0.0035)**

Inoculum Size: Figure 4 displays the data showing that the optimal inoculum size for producing the keratinase enzyme from *Kocuria rosea* bacteria was 5 mL/100 mL of media. After 72 hours of incubation, the enzyme concentration was 0.224 U/mL. After that point, every further change in inoculum size resulted in a decline in production rate. The inoculum size that resulted in the lowest enzyme production was 1 mL/100 mL medium, which reached 0.189 U/mL. Because the inoculum concentration is insufficient to supply a biomass of the bacterial isolate, enzyme synthesis is minimal at low inoculum sizes. Therefore, it has a detrimental effect on enzyme synthesis. On the other hand, the high density of cells in the culture increases viscosity and

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competition for nutrients. This also reduces the possibility of cells reaching the stage of high production. Thus, their ability to produce the enzyme (Abdel-Fattah et al. 2018). This result is consistent with what was reached by Demir et al. (2015), who were able to obtain the best production of keratinase enzyme from Streptomyces sp. bacteria when using an inoculum size of (5) mL/100 mL medium.

This result is also consistent with what was reached by Barman et al. (2017), who were able to produce keratinase enzyme from Arthrobacter sp. bacteria at an inoculum size of 5 mL/100 mL medium. While Abdul Gafar et al. (2020) were able to obtain keratinase enzyme production from Bacillus sp. bacteria at an inoculum size of 5 mL/100 mL medium.

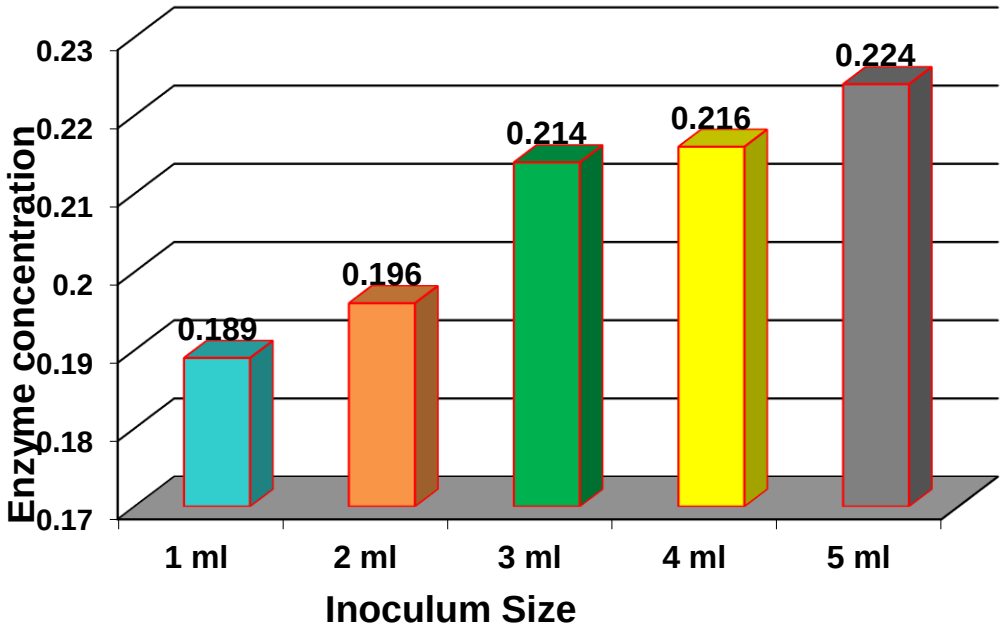

Figure 4. Effect of vaccine size on keratinase enzyme production

Temperature: Figure 5 shows that the optimal temperature for the keratinase enzyme synthesis by the *Kocuria rosea* bacteria was 30°C. A concentration of 0.247 U/mL of enzyme was achieved. Enzyme production was shown to be lowest at 45°C. A concentration of 0.228 U/mL of enzyme was achieved. The development of the microbe and the enzymatic activity are both affected by the incubation temperature. The temperature range within which an enzyme is created varies with the microorganism responsible for its synthesis, the media in which it is produced, and the sources of the enzyme. The reactions catalyzed by enzymes increase in speed with increasing temperature to a certain limit. After that, they begin to decrease due to the dissociation of hydrogen bonds and other forces that maintain the tertiary form of the enzyme. Then, the nature of the enzyme is denatured, and thus its effectiveness is lost (Whitaker 2018).

These results are completely consistent with what was reached by Mazotto et al. (2022). They obtained the best production of keratinase enzyme from Bacillus subtilis bacteria at a temperature of 30°C.

These results also agree with what was reached by Gafar et al. (2020). They were able to produce the enzyme from Bacillus sp. bacteria at a temperature of 31°C. While Jayalakshmi et al. (2011) indicated that the highest production of keratinase enzyme from Streptomyces Sp. Bacteria was at a temperature of 28°C.

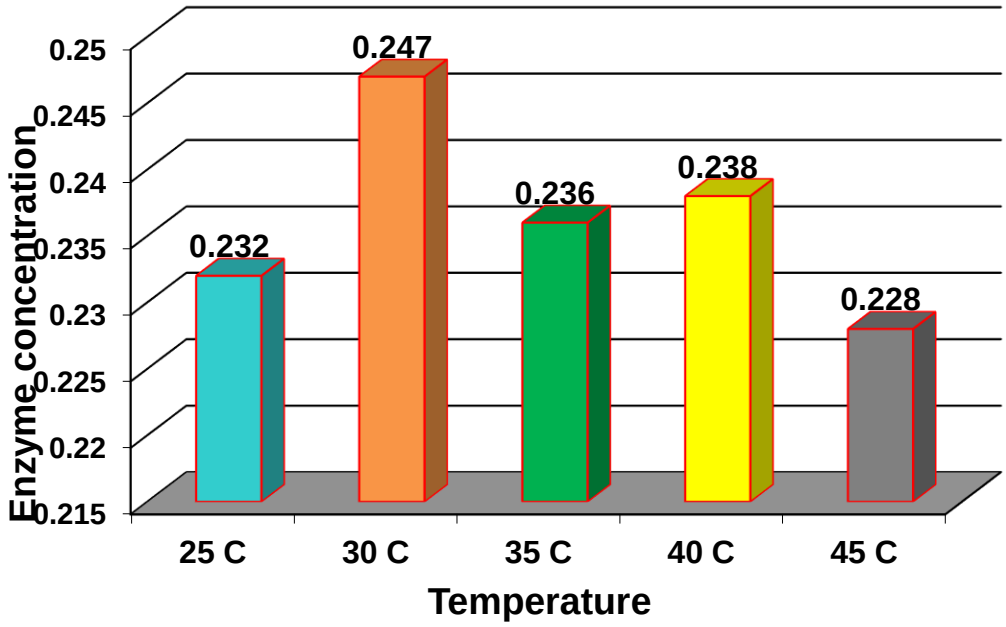

Figure 5. Effect of temperature on the production of keratinase enzyme

Carbon Source: Figure 6 shows that the optimal carbon source for enzyme synthesis was determined to be 3 g/100 mL of hoof waste media, as indicated by the optimum generation of keratinase enzyme. At a pH of 8, a vaccination volume of 5 mL, and a temperature of 30°C, the enzyme concentration reached 0.248 U/mL. However, other ratios resulted in a reduction in enzyme concentration. When the enzyme concentration was 5 g/100 mL of trash, it was at its lowest. This is because the level of compatibility between the microbe density and the carbon supply ratio plays a significant role in the manufacturing process. The producing microbe needs these nutrients and energy sources; therefore, this is how it gets them. When hooves are added in large quantities, they prevent microbes from making enzymes. This is because the enzyme's active sites are filled with amino acids and proteins, leaving an excess of basic material without enough active sites (Izadi et al. 2020). These results are almost consistent with what was reached by Moonnee et al. (2021), as they were able to produce the keratinase enzyme from Pseudomonas

aeruginosa bacteria at a residue concentration of 2 g/100 mL of medium. These results differ from what was reached by Govarthanan et al. (2015). He indicated that the best production of the keratinase enzyme from Bacillus sp. bacteria was at a residue concentration of 6 g/100 mL of medium.

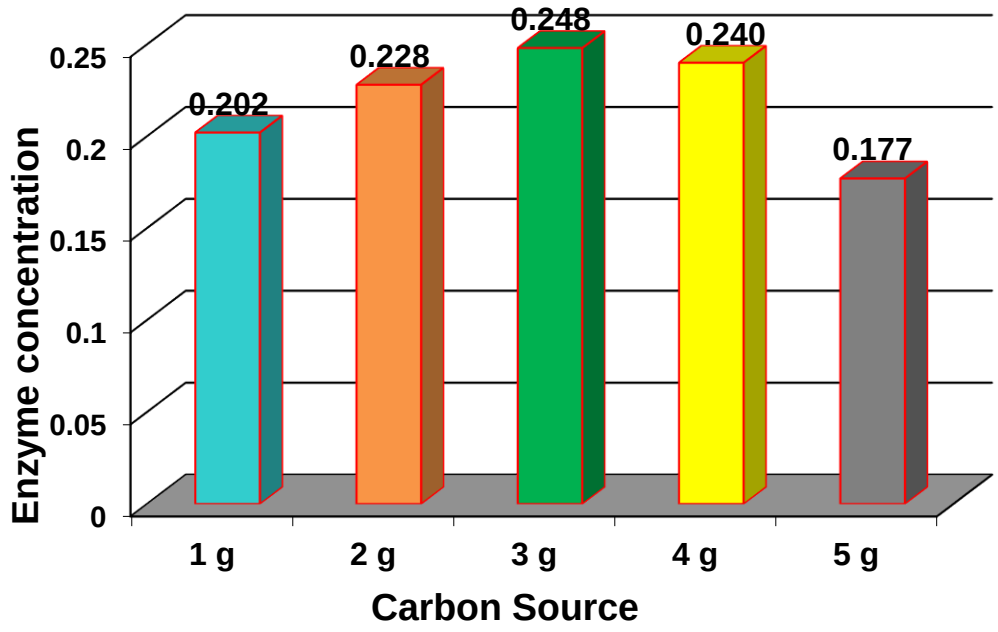

Figure 6. Effect of carbon source concentration on keratinase enzyme production

Nitrogen source: Figure 7 shows that when given urea as a nitrogen source, the keratinase enzyme was most effectively produced by the Kocuria rosea bacterium (0.301U/mL). At the same time, other nitrogen sources resulted in a reduction in enzyme concentration. Ammonium sulfate caused an even more dramatic drop in enzyme output, and the final enzyme concentration was 0.231 U/mL. When sodium nitrate was used as a nitrogen source, the enzyme productivity was at its lowest point at 0.216U/mL. Nitrogen sources greatly affect the growth of microorganisms. The optimum concentrations enhance the content of biomass, protein, fat and carbohydrates. In contrast, high concentrations of nitrogen can reduce growth rates in submerged farms (AnanadhiPadmanabhan et al. 2010). Nitrogen sources greatly affect the structure of the microbial community, metabolic functions, and pollutant removal efficiency in submerged farms. Urea and nitrate show clear effects on biodegradation and nutrient transformation (Li et al. 2024). These results differ from those of da Gioppo et al. (2009), who were able to obtain the highest production of keratinase enzyme from Myrothecium verrucaria bacteria when using peptone as the best nitrogen source. These results also differ from those of Sivakumar et al. (2012), who were able to

produce keratinase enzyme from Bacillus thuringiensis TS2 bacteria using peptone as the best nitrogen source.

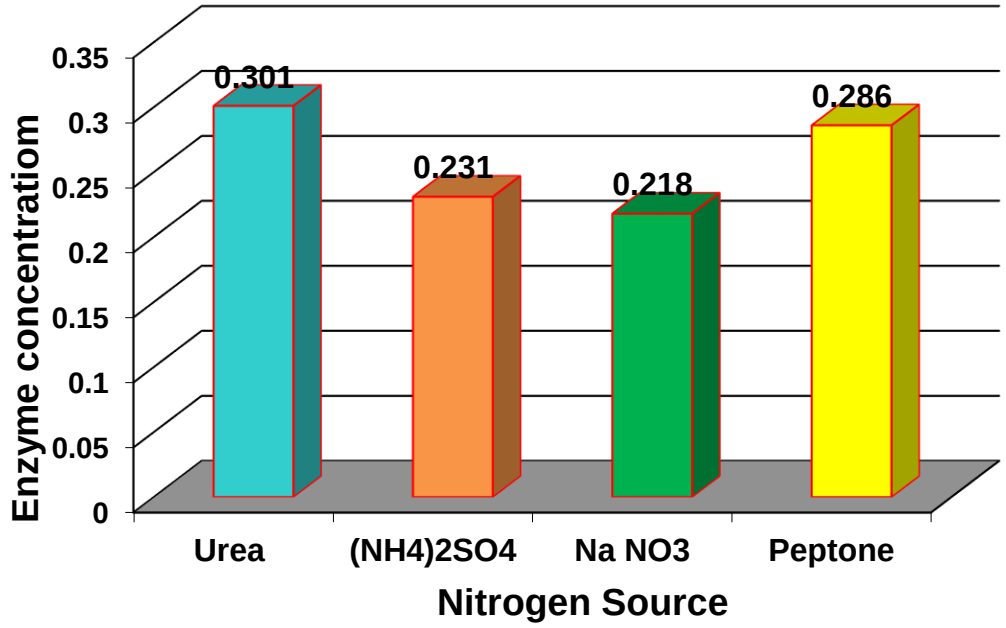

Figure 7. Effect of the type of nitrogen source on the production of keratinase enzyme Nitrogen source concentration

Figure 8 shows that the keratinase enzyme was most abundantly produced by the Kocuria rosea bacterium. Using a concentration of 0.1g urea/100 mL medium, the enzyme concentration was obtained at 0.301U/mL. As the concentration of urea rose, the efficiency of the enzymes fell. With an addition rate of 0.5g/100 mL of medium, the enzyme concentration was 0.045 U/mL. Bacterial growth and keratinase synthesis are very sensitive to nitrogen supply concentration. In order to build the enzyme, it is required for the production of proteins and amino acids (Mukherjee et al. 2023). These results are consistent with the findings of Barman et al. (2017), who were able to produce keratinase enzyme from Arthrobacter sp. bacteria at a nitrogen source concentration of 0.1g/mL medium. El-Ghonemy and Ali (2017) were able to produce keratinase enzyme from Aspergillus sp. bacteria at a nitrogen source concentration of 0.5 g/mL medium.

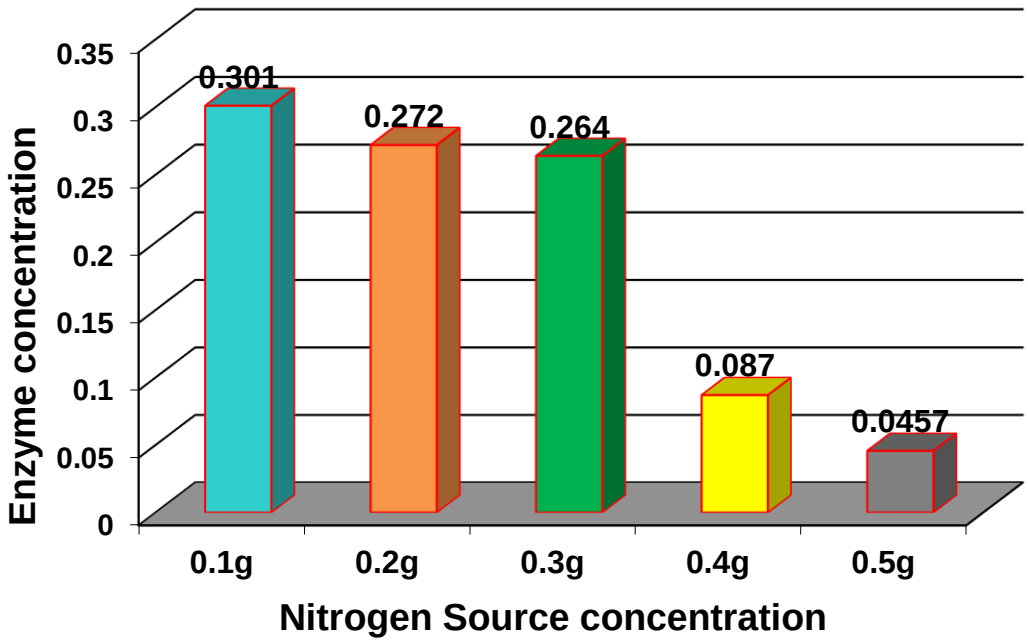

Figure 8. Effect of nitrogen source concentration on keratinase enzyme production

Period of incubation: The optimal keratinase enzyme synthesis occurred after 72 hours of incubation, as shown in Figure 9. By the time the temperature reached 30°C, and the pH hit 8, the enzyme concentration had reached 0.301U/mL. Also, as the incubation period got longer, the enzyme production got lower. Due to food depletion and toxin release, enzyme synthesis decreases as the incubation duration increases. In addition to the potential for cell autolysis and the subsequent release of metabolic chemicals that adversely impact enzyme synthesis, lengthening the incubation time causes changes in the medium's environmental conditions, including changes in pH and osmotic pressure (Omer et al. 2018). These results are consistent with what was indicated by Barman et al. (2017), who obtained the best production of keratinase enzyme from Arthrobacter sp bacteria after an incubation period of 72 hours. These results differ from those of Dhiva et al. (2020), who obtained the best production of keratinase enzyme from Pseudomonas aeruginosa SU-1 bacteria after an incubation period of 96 hours.

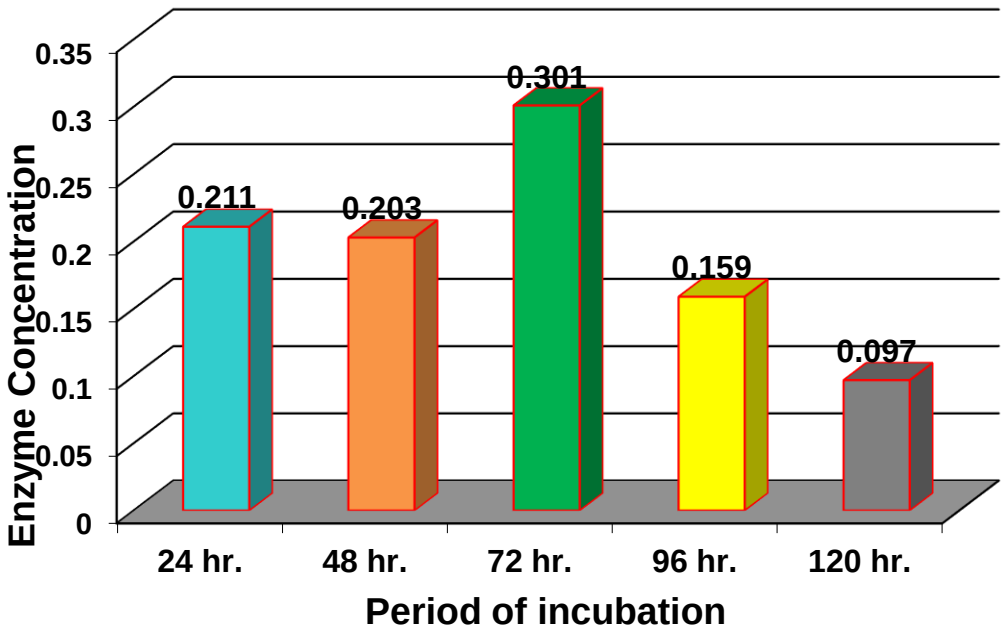

Figure 9. Effect of incubation duration on keratinase enzyme production

Conclusions: This study successfully demonstrated the production of keratinase enzyme using the local bacterial isolate *Kocuria rosea*, employing environmentally abundant and cost-effective waste materials as substrates. Optimal production conditions were established, including a temperature of 30°C, pH 8, 3 g/100 mL sheep hoof powder as the carbon source, urea as the nitrogen source at 0.1 g/100 mL, and a 72-hour incubation period. Under these parameters, the enzyme activity reached 0.301 U/mL, highlighting the feasibility of utilizing such waste materials for industrial-scale enzyme production. These findings underscore the potential of *Kocuria rosea* in bioindustrial applications, particularly in processes involving keratin waste recycling and sustainable waste management.

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Conflict of Interest: There is no conflict of Interest.

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مجله بیوتکنولوژی کشاورزی
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تولید آنزیم کراتیناز از جدایه محلی Kocuria rosea با استفاده از زباله های محیطی

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چکیده

هدف: کراتیناز آنزیمی است که به همراه بسیاری دیگر از آنزیم‌های تجزیه کننده پروتئین به گروه متالوپروتئازها تعلق دارد. کراتیناز یک آنزیم تخصصی است که روی سوبسترا (کراتین) عمل می‌کند و پیوندهای شیمیایی قوی کراتین را می‌شکند. این مطالعه با هدف تولید آنزیم کراتیناز با استفاده از جدایه‌های باکتری محلی به‌دست‌آمده از نمونه‌های خاک و ضایعات طیور از مناطق مختلف استان الانبار عراق با استفاده از مواد زاید مختلف مانند سم، شاخ و پوست انجام شد.

مواد و روش‌ها: از ۲۰ نمونه خاک و ضایعات طیور، ۱۷ جدایه باکتریایی به دست آمد که توانایی بالایی برای رشد در محیط کشت پر آگار مورد استفاده برای جداسازی را نشان دادند. پنج ایزوله باکتریایی کارآمد بر اساس قطر کلنی‌های رشد کرده روی محیط کشت پر آگار انتخاب شدند. در این میان، ایزوله باکتری با کد محلی A2 پس از کشت پنج ایزوله منتخب بر روی محیط کراتینه خالص، به عنوان کارآمدترین جدایه در تجزیه کراتین انتخاب شد. جدایه باکتری بر اساس ویژگی‌های مورفولوژیکی، فرهنگی، میکروسکوپی و آزمایش‌های بیوشیمیایی شناسایی شد. برای تایید هویت از دستگاه VITEK 2 Compact استفاده شد.

نتایج: نتایج نشان داد که جدایه Kocuria rosea بود. شرایط بهینه برای تولید آنزیم از Kocuria rosea شامل دما، pH، اندازه تلقیح، منبع کربن و غلظت آن، منبع نیتروژن و غلظت آن و زمان انکوباسیون مورد بررسی قرار گرفت. بر اساس آزمایش‌ها و نتایج آن‌ها، محیط تهیه‌شده با سم‌های گوسفندی برای رشد کوکوریا رزا و تولید کراتیناز انتخاب شد، به‌ویژه که این ضایعات در دسترس و ارزان قیمت در محیط محلی است. نتایج مطالعه شرایط بهینه نشان داد که بهترین تولید آنزیم کراتیناز در دمای ۳۰ درجه سانتی گراد، pH برابر ۰/۸، سرعت تکان دادن ۱۵۰ دور در دقیقه، با ۳ گرم در ۱۰۰ میلی لیتر سم گوسفند در محیط، ۵ میلی لیتر در ۱۰۰ میلی لیتر تلقیح بود. با استفاده از اوره به عنوان منبع نیتروژن در غلظت ۰/۱ گرم در ۱۰۰ میلی لیتر و زمان انکوباسیون ۷۲ ساعت فعالیت آنزیمی به ۰/۳۰۱ U/ml رسید.

نتیجه‌گیری: این یافته‌ها بر پتانسیل Kocuria rosea در کاربردهای بیوصنعتی، به ویژه در فرآیندهای مربوط به بازیافت زباله‌های کراتین و مدیریت زباله پایدار تأکید می‌کند.

واژه‌های کلیدی: باکتری‌های کراتینولیتیک، سیستم ویتک ۲، گروه متالوپروتئاز، صنعت زیستی، عراق

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