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## Production and characterization of keratinase enzymes from *Alcaligenes faecalis* and *Pseudomonas aeruginosa* isolated from decayed fish scales

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### ABSTRACT

Keratinases are enzymes that catalyze the degradation of keratin, a resilient protein found in various biological materials such as: feathers, hair, and fish scales. Two isolates identified from previous work as *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) was used for keratinase production. The enzyme produced were purified using dialysis, ion exchange chromatography and gel filtration chromatography and the purified enzyme was characterized and used to check for keratin substrate specificity. Fermentation studies revealed that *A. faecalis* (SCA A1) displayed optimal growth at pH 9.5 and 10.5, particularly at higher temperatures (48°C, 58°C, and 68°C) when using Mackerel fish scales as the substrate. In contrast, *P. aeruginosa* (FSA A2) demonstrated better growth at pH 7.5 and 8.5, with the highest growth observed at 28°C and 38°C using both Mackerel and Elephant fish scales. Enzyme activity profiles showed that *A. faecalis* exhibited higher activity with Mackerel scales (65.7 U/ml), while *P. aeruginosa* showed greater activity using Elephant scales (68.1 U/ml). Purification of the keratinase enzymes from both isolates resulted in a 7-fold increase in specific activity, reaching 35.16 U/mg for *A. faecalis* and 37.80 U/mg for *P. aeruginosa*. Characterization of the purified enzymes revealed that both exhibited optimal activity at 70°C, with *P. aeruginosa* displaying slightly higher activity. The enzymes also shows good activity over a wide pH range, with optima at pH 7.3 and 6.3 for *A. faecalis* and *P. aeruginosa*, respectively. Substrate specificity analysis indicated that both enzymes had the highest activity towards Mackerel fish scales, followed by Casein, Human Hair, Chicken feathers, and Elephant fish scales. These findings highlight the potential of *A. faecalis* and *P. aeruginosa* for the biodegradation of fish scale waste, offering a sustainable solution for waste management and the production of valuable bioproducts. These results suggest that these keratinolytic bacteria could be utilized in the development of bioremediation strategies for fish processing waste. Additionally, the keratinase enzymes produced by these isolates could be utilized in various industrial applications, such as the production of animal feed supplements, leather processing, and the development of cosmetic and pharmaceutical products.

**Keywords:** Keratinase, Fish scales, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, enzyme activity.

### 1.0 INTRODUCTION

Fish scales are predominantly composed of keratins, which are structural proteins that form protective outer layers in many animals (Zhou *et al.*, 2021). Similar to keratins in hair and nails, scale keratins form tough yet flexible networks through interactions between protein strands (Hassan *et al.*, 2020). Below the mineralized exterior of the scale lies several keratinized layers, with different keratin genes expressed in each region (Hassan *et al.*, 2020).

Keratinases are a class of proteolytic enzymes that specifically degrade keratin, a resilient fibrous protein found in various biological materials, including feathers, hair, skin, and scales. These enzymes play a crucial role

in the biodegradation of keratinous waste, making them significant in environmental and industrial applications (Bansal *et al.*, 2023). The agro-industrial wastes, especially those emanating from leather and poultry processing industries are considered to have little or no economic relevance due to their structural stability, which makes valorization difficult. Nonetheless, several strategies have been employed to harness the locked-up potentials from keratinous wastes, and these strategies have included thermo-energetic processing, and acid or alkaline hydrolysis (Yusuf *et al.*, 2019). Keratin waste valorization with the above-indicated procedures has yielded products which have not been suitable for industrial applications (Yusuf *et al.*, 2019). Keratinases which may bio convert keratin to peptides and amino acids, on the other hand, have not been a front runner in

the valorization of keratinous waste biomass (Sharma *et al.*, 2019).

The bioconversion of keratinous wastes into amino acids or peptides with functional values would be an attractive endeavor for several applications including animal feed formulations. The valorization approach would represent a potentially sustainable strategy for the proper management of keratin-rich agro-wastes (Liaquat *et al.*, 2022) and keratinolytic microorganisms and enzymes would be beneficial from the biotechnological and industrial viewpoint. Consequently, this study evaluated the ability of *Alcaligenes faecalis* and *Pseudomonas aeruginosa* isolated from fish scale dumpsite at Gusau Dam to produce keratinase enzymes and the characterization of the produced enzymes.

## 2.0 MATERIALS AND METHODS

### 2.1 Organisms collection and subculturing

Already identified *Alcaligenes faecalis* and *Pseudomonas aeruginosa* isolated from fish scale dumpsite at Gusau Dam was used for this study. The isolates were sub-cultured in nutrient broth for 18 hrs. and this was used for further studies.

### 2.2 Keratin – Substrate Preparation

The keratin substrate was prepared from Fish scales obtained from local fish sellers. The scales were thoroughly washed and rinsed with distilled water, and subsequently, dried at 60 °C for 48 hours in a hot air oven. The dried scales were milled into fine powder with a pulveriser fitted with a 2 mm mesh and stored in an airtight container at room temperature.

### 2.3 Effect of pH on Microbial Growth

The ability of the isolates to degrade native keratin substrate was investigated at different pH levels. The assay consisted of Mackerel fish scale powder suspended in 100mL of minimal medium ((g/L): K<sub>2</sub>HPO<sub>4</sub>, 0.3; KH<sub>2</sub>PO<sub>4</sub>, 0.4; MgCl<sub>2</sub>, 0.2; CaCl<sub>2</sub>, 0.22). To study the effect of pH, overnight cultures of bacteria were inoculated into the broth containing minimal medium substituted with fish scale and incubated for 120 hrs. at various pH levels: 6.5, 7.5, 8.5, 9.5, and 10.5. Controls were maintained without inoculums for each category, with six replicates. Bacterial growth was estimated by measuring the absorbance at 620nm at every 24-hour interval.

### 2.4 Effect of Temperature on Microbial Growth

To investigate the effect of temperature on the isolates' ability to degrade native keratin substrate, the same assay setup was used. Overnight cultures of bacteria were inoculated into the broth containing minimal medium

substituted with fish scale and incubated for 120 hrs. at different temperatures: 28°C, 38°C, 48°C, 58°C, and 68°C. As with the pH experiment, controls were maintained without inoculums for each category, with six replicates. Bacterial growth was monitored by measuring the absorbance at 620nm every 24 hours.

### 2.5 Effect of Fish Scale Concentration on Microbial Growth

The experiments were conducted in 250mL Erlenmeyer flasks. These flasks contained minimal media composition, which was substituted with fish scale at varying concentrations: 0.1%, 0.5%, 1.0%, 1.5%, and 2.0%. The goal was to observe how different concentrations of fish scale influenced the growth of bacteria. Each flask was inoculated with bacterial isolates. The flasks were then incubated at 37°C with a shaking speed of 120 rpm for 120 hours. The bacterial growth was assessed by measuring the absorbance at 620 nm.

### 2.6 Effect of Different Nitrogen Source on Microbial Growth

To study the effect of different substrates on bacterial growth, the minimal media was externally supplemented with 0.5% each of casein, peptone, and gelatin separately. These substrates were chosen to provide different nutrient sources for the bacteria. Each flask containing the supplemented media was inoculated with bacterial isolates. The flasks were incubated at 37°C with a shaking speed of 120 rpm for 120 hours. The bacterial growth was determined by measuring the absorbance at 620 nm.

### 2.7 Purification of Keratinase

Dialysis was performed to remove small molecular weight impurities from the crude enzyme from both *Alcaligenes faecalis* and *Pseudomonas aeruginosa*. Two hundred (200) mls of the crude enzyme from both organisms was dialyzed against a dialysis buffer containing 5M sucrose using a dialysis membrane with an appropriate molecular weight cutoff and the dialysis was done in 4°C refrigerator for 18 hours. The high sucrose concentration aided in maintaining the stability of the keratinase enzyme during the dialysis process.

Ion exchange chromatography was employed for further purification of the dialyzed keratinase enzyme. A diethylaminoethyl (DEAE) cellulose resin was used as the ion exchange matrix (Daroit, 2010). The dialyzed keratinase enzyme was loaded onto a DEAE column, and the bound proteins were eluted using a gradient of increasing pH. Keratinase, having a specific charge, selectively bound to the DEAE resin and was eluted from

the column using a gradient of 0.5M NaCl in Tris-HCl and tris-HCl and 10ml fractions were collected and analyzed for keratinase activity. The fractions with activity was further concentrated by dialyzing with 5M sucrose solution after which gel filtration chromatography was done.

Gel filtration chromatography, also known as size exclusion chromatography, was utilized as a final purification step (Gupta *et al.*, 2013). A column packed with Sephadex G-150, a gel filtration matrix, was used. The keratinase-containing fraction from the ion exchange chromatography step was loaded onto the Sephadex G-150 column after concentration by dialysis. Proteins were eluted based on their size, with keratinase eluting early in the chromatogram due to its exclusion from the gel beads.

### 2.8 Characterization of the Keratinase

To determine the optimal temperature for keratinolysis, enzyme reactions were carried out at different temperatures for 30 min. and then the residual activity was measured. The optimum pH was determined at 30 °C and 70°C using the following buffers (50 mmol/L): sodium phosphate buffer (pH 6.0~7.5), Tris-HCl buffer (pH 7.5~9.0), and Glycine/NaOH buffer (pH 9.0~10.0).

### 2.9 Substrate Specificity of Keratinase

To determine the keratinase specificity on different keratin substrates, the following substrates were used: casein (Sigma, USA), BSA (Sigma, USA), Fish scales, chicken feathers and human hair. The incubation procedures were the same as described for keratinase activity determination except that every incubation contained 20 mg substrate and 50 µl purified keratinase. The extent of hydrolysis was determined spectrophotometrically at 280 nm by measuring TCA-soluble peptides released from the substrates during incubation.

## 3.0 RESULTS

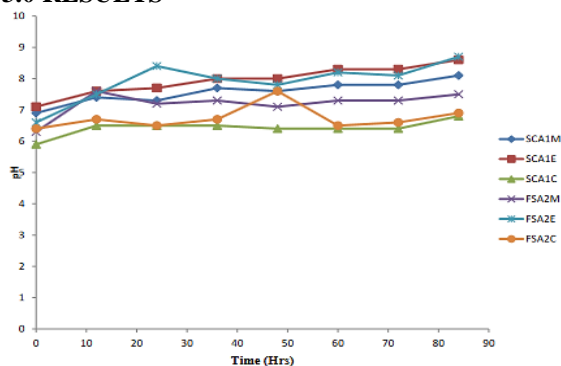


Figure 3:1 pH of fermentation broth at different time intervals

SCA1M – fermentation with mackerel fish scale powder  
 SCA1E – fermentation with elephant fish scale powder  
 SCA1C – control without any scale  
 FSA2M – fermentation with mackerel fish scale powder  
 FSA2E – fermentation with elephant fish scale powder  
 FSA2C – control with any scale  
 SCA1 – microbial isolate  
 FSA2 – microbial isolate

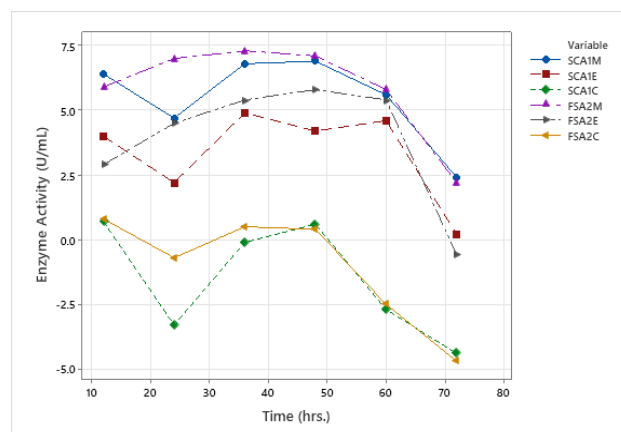


Figure 3.2: Enzyme activity at different time intervals using different fish scales

Where;

SCA1M = Fermentation broth containing *Alcaligenes faecalis* isolates with Mackerel fish scale powder.

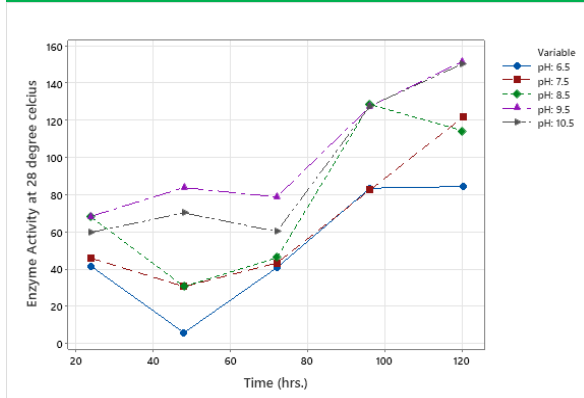
SCA1E = Fermentation broth containing *Alcaligenes faecalis* isolates with Elephant fish scale powder.

SCA1C = control sample containing fermentation broth with only *Alcaligenes faecalis* isolate without any fish scale powder.

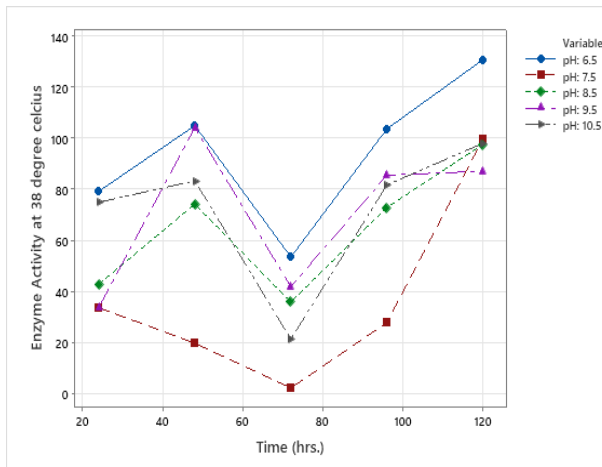
FSA2M = Fermentation broth containing *Pseudomonas aeruginosa* isolates with Mackerel fish scale powder.

FSA2E = Fermentation broth containing *Pseudomonas aeruginosa* isolate with Elephant fish scale powder.

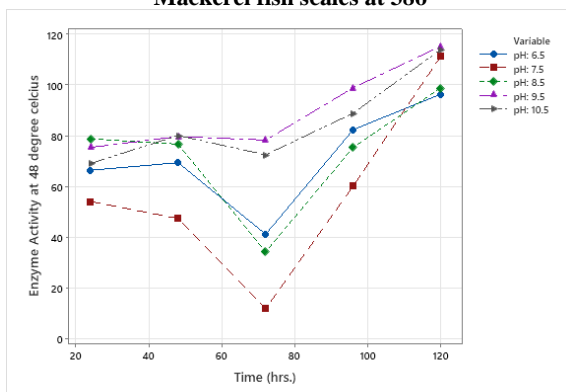
FSA2C = control sample containing fermentation with only *Pseudomonas aeruginosa* isolate without any fish scale powder.



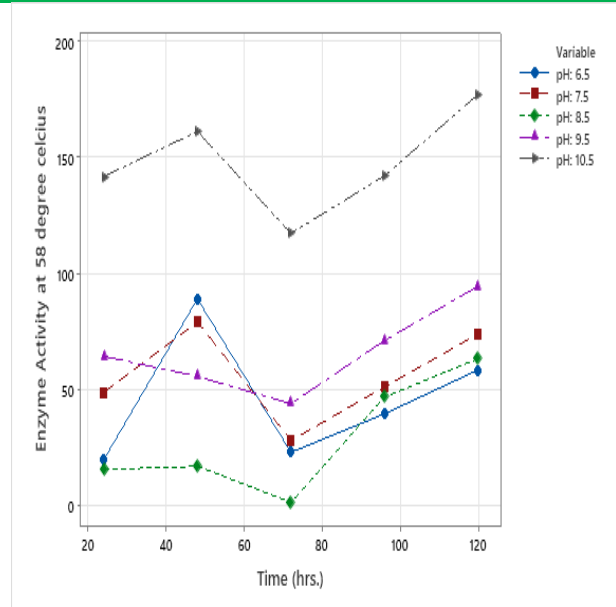
**Figure 3.3: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Mackerel fish scales at 28°C**



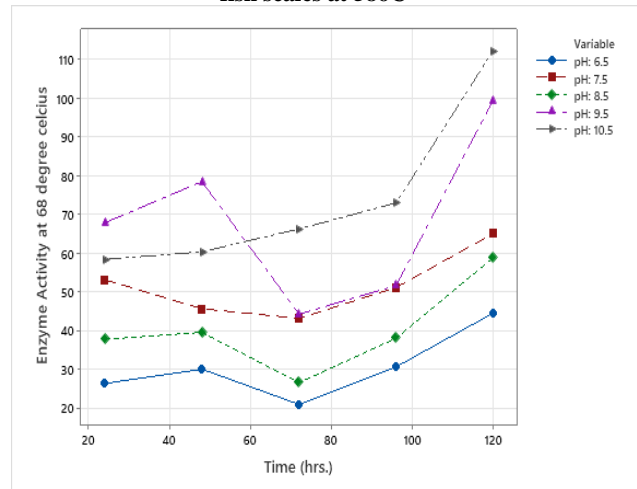
**Figure 3.4: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Mackerel fish scales at 38o**



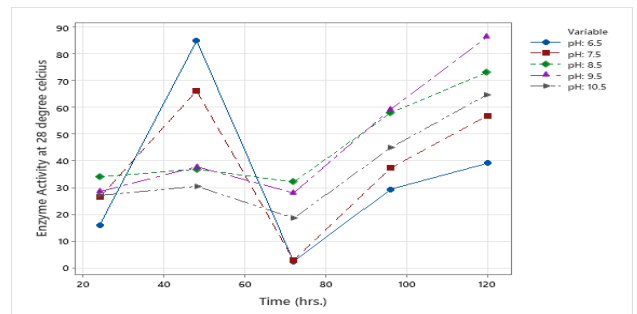
**Figure 3.5: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Mackerel fish scales at 48oC**



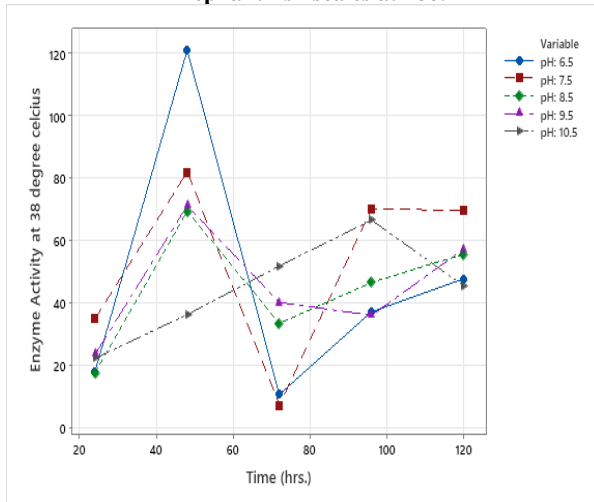
**Figure 3.6: Effect of different pH on the growth of *Alcaligenes faecalis* at different intervals using Mackerel fish scales at 58oC**



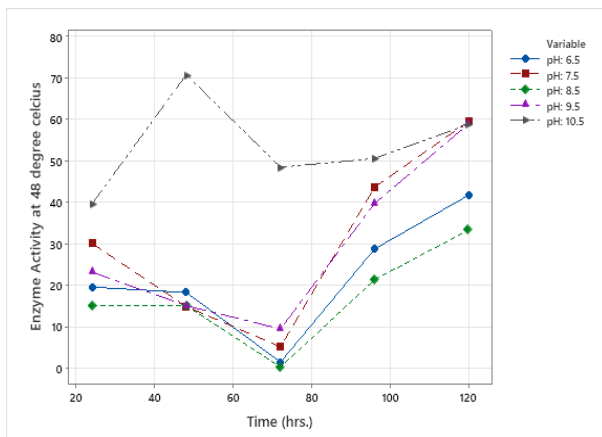
**Figure 3.7: Effect of different pH on the growth of *Alcaligenes faecalis* at different intervals using Mackerel fish scales at 68oC**



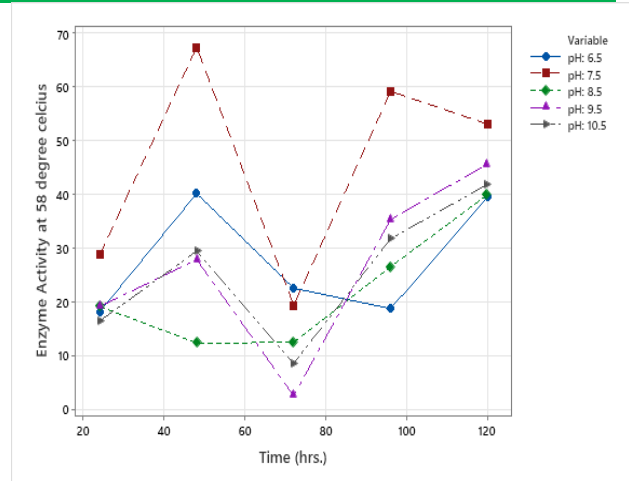
**Figure 3.8: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Elephant fish scales at 28o.**



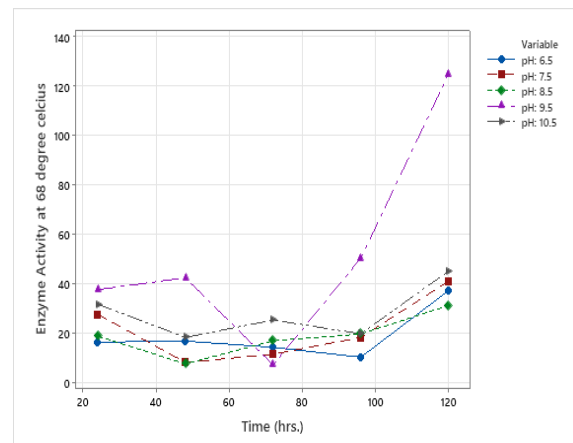
**Figure 3.9: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Elephant fish scales at 38oC**



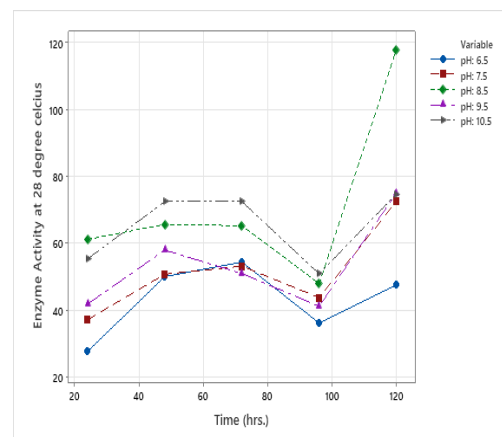
**Figure 3.10: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Elephant fish scales at 48oC**



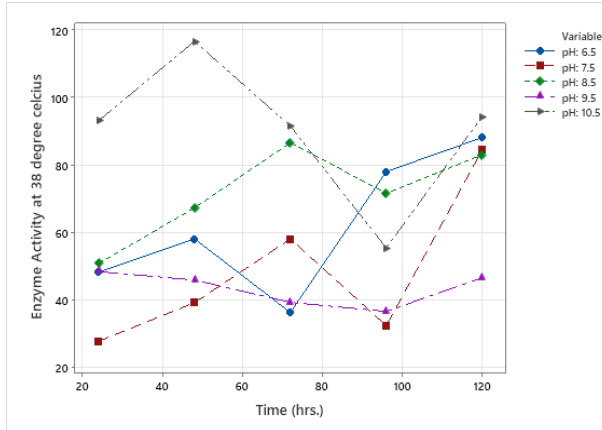
**Figure 3.11: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Elephant fish scales at 58oC**



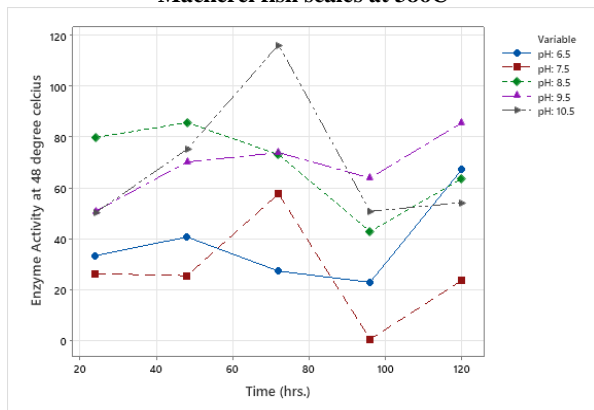
**Figure 3.1: Effect of different pH on the growth of *Alcaligenes faecalis* at different time intervals using Elephant fish scales at 68oC**



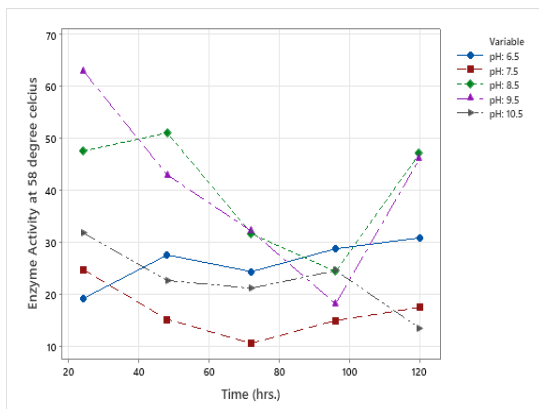
**Figure 3.2: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Mackerel fish scales at 28oC**



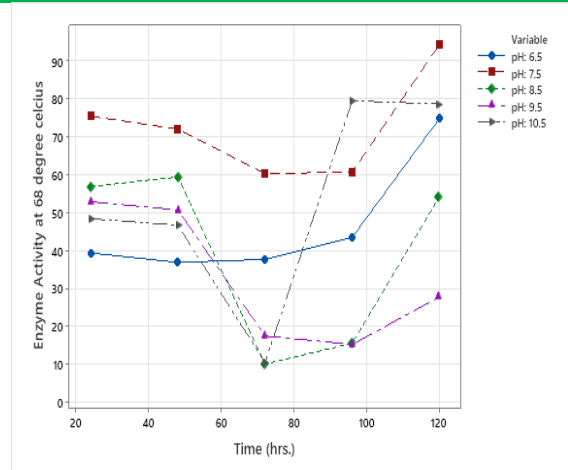
**Figure 3.3: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Mackerel fish scales at 38oC**



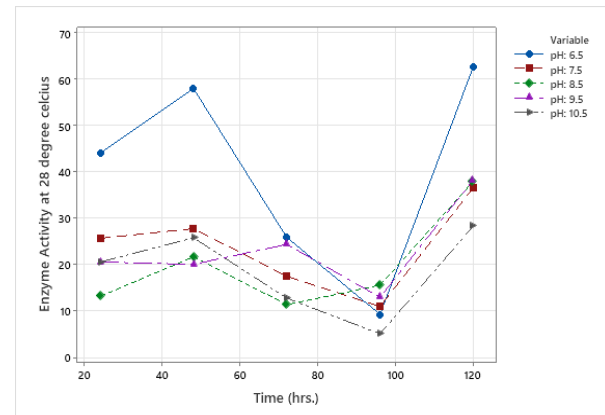
**Figure 3.15: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Mackerel fish scales at 48oC**



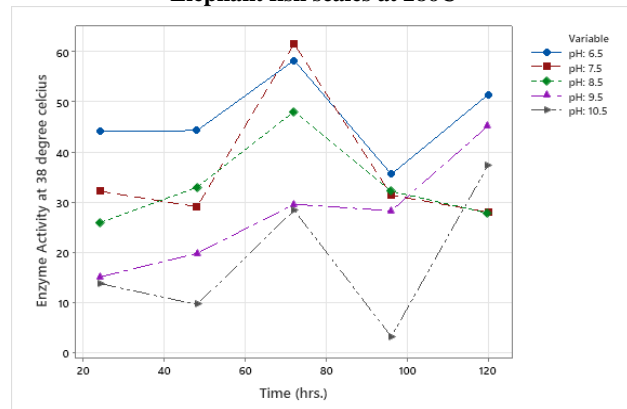
**Figure 3.4: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Mackerel fish scales at 58oC**



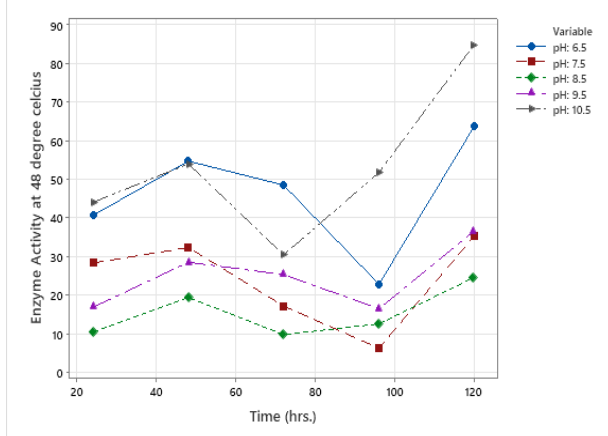
**Figure 3.5: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Mackerel fish scales at 68oC**



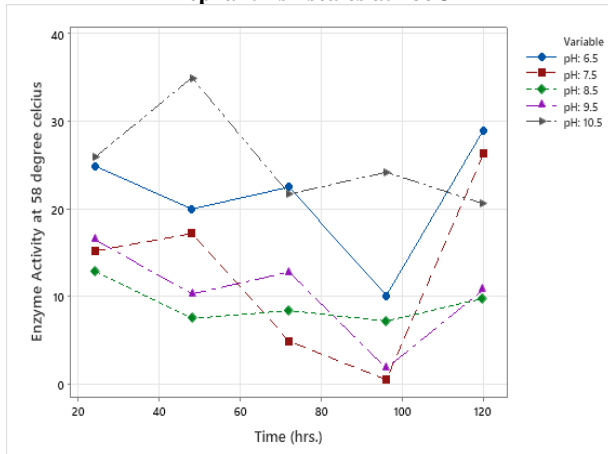
**Figure 3.6: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Elephant fish scales at 28oC**



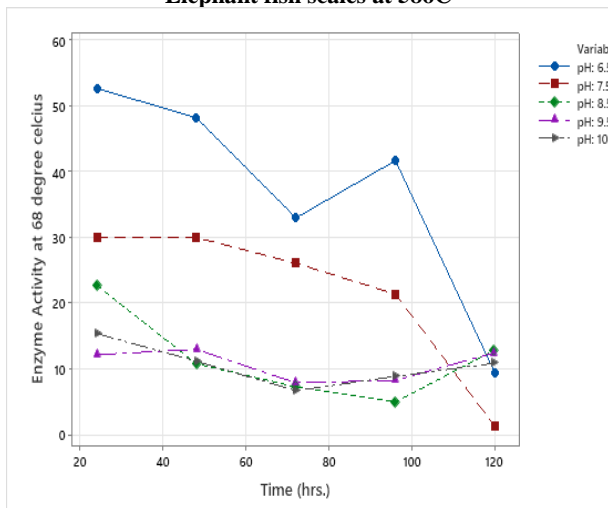
**Figure 3.19: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Elephant fish scales at 38oC**



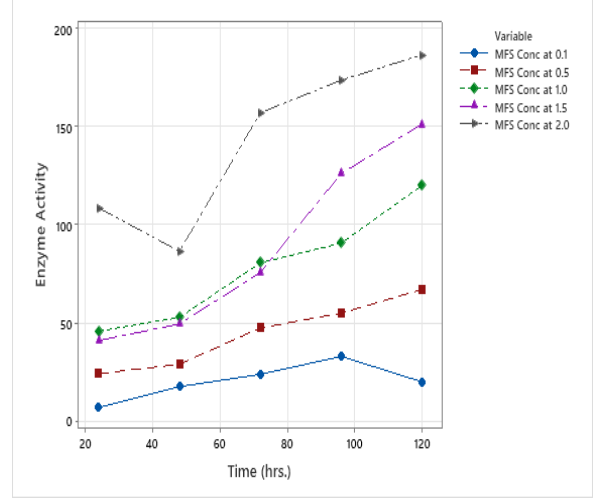
**Figure 3.20: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Elephant fish scales at 48oC**



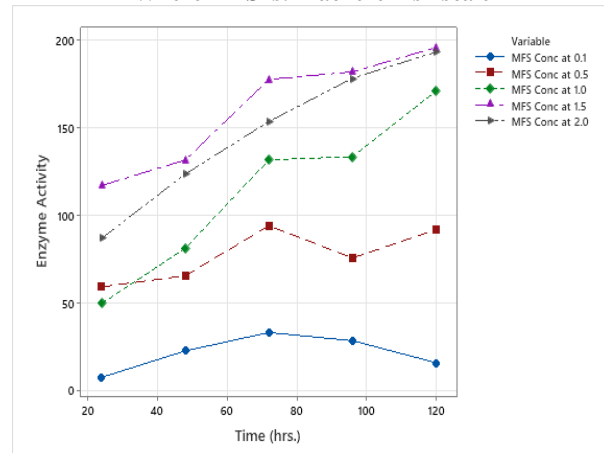
**Figure 3.7: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Elephant fish scales at 58oC**



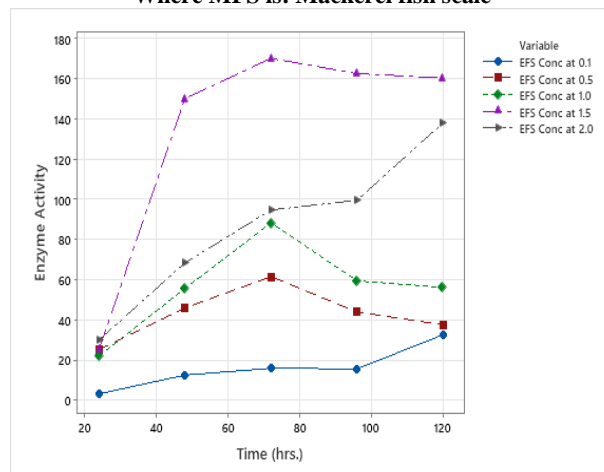
**Figure 3.8: Effect of different pH on the growth of *Pseudomonas aeruginosa* at different time intervals using Elephant fish scales at 68oC**



**Figure 3.9: Effect of mackerel fish scale concentration on enzyme production by *Alcaligenes faecalis* (SCA) Where MFS is: Mackerel fish scale**

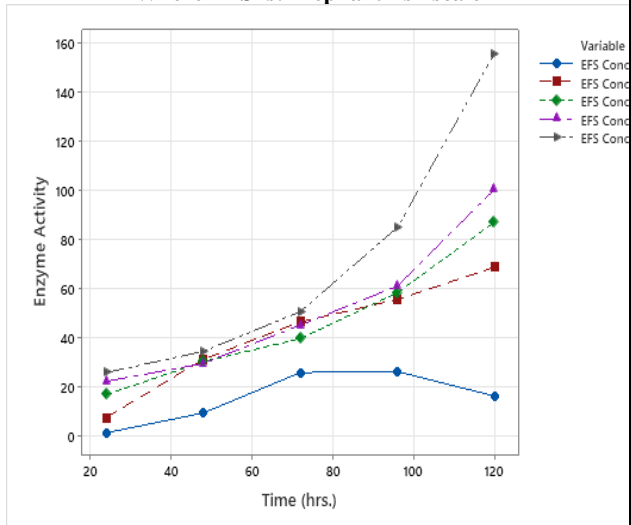


**Figure 3.10: Effect of mackerel fish scale concentration on enzyme production by *Pseudomonas aeruginosa* (FSA) Where MFS is: Mackerel fish scale**

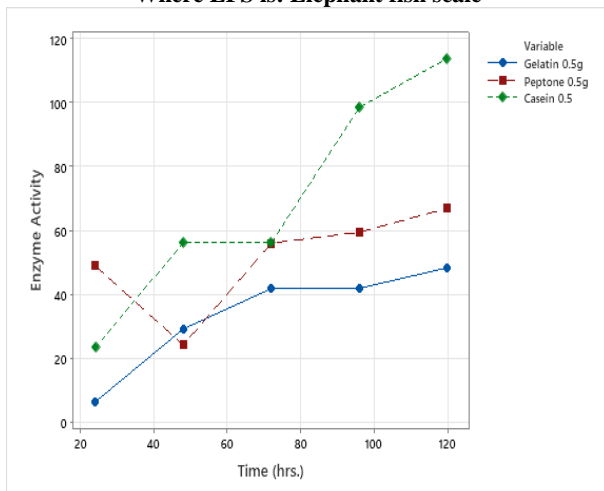


**Figure 3.25 Effect of elephant fish scale concentration on enzyme production by *Alcaligenes faecalis* (SCA)**

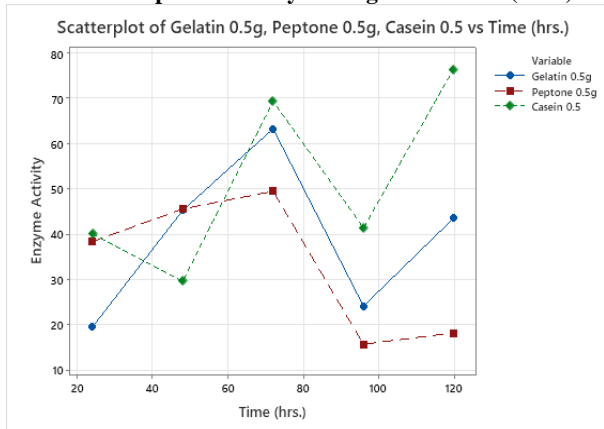
Where EFS is: Elephant fish scale



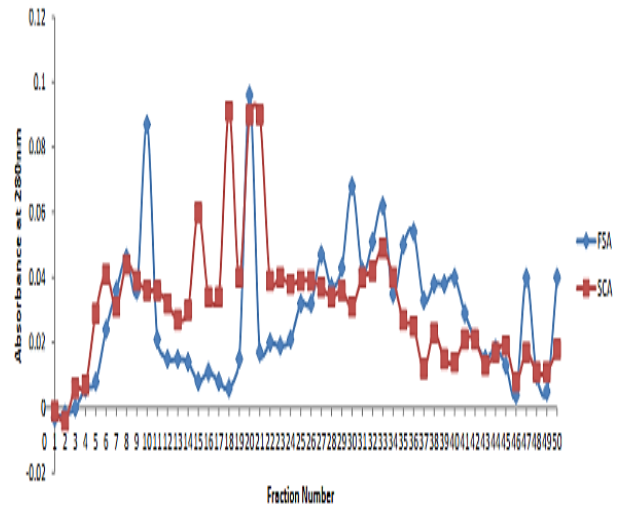
**Figure 3.11: Effect of elephant fish scale concentration on enzyme production by *Pseudomonas aeruginosa* (FSA)**  
Where EFS is: Elephant fish scale



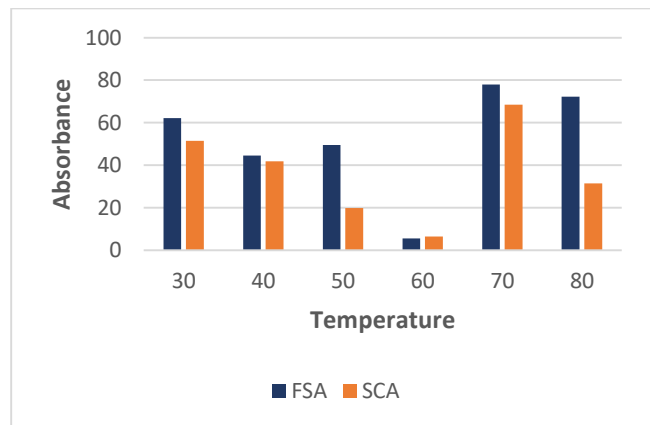
**Figure 3.12: Effect of different nitrogen sources on keratinase production by *Alcaligenes faecalis* (SCA)**



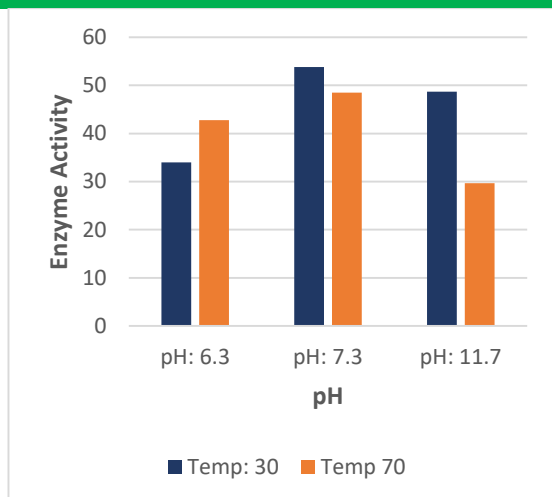
**Figure 3.13: Effect of different nitrogen sources on the growth of *Pseudomonas aeruginosa* (FSA)**



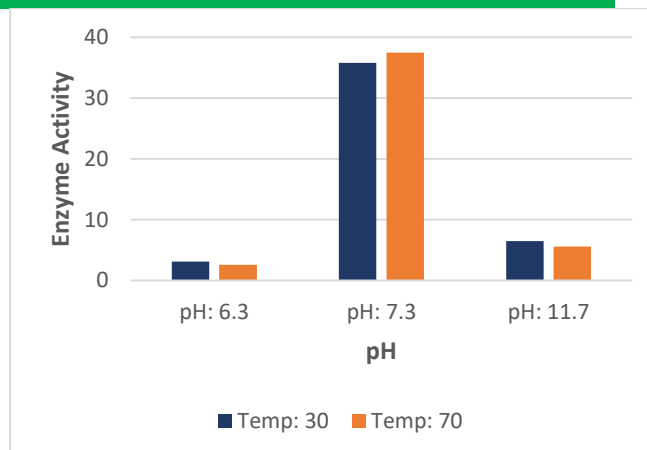
**Figure 3.14: Elution profile of FSA and SCA from DEAE-cellulose**



**Figure 3.30: Effect of temperature on keratinase activity**  
Where:  
SCA – enzyme from *Alcaligenes faecalis*  
FSA – enzyme from *Pseudomonas aeruginosa*



**Figure 3.31: Effect of pH on keratinase enzyme activity from *Alcaligenes faecalis* at different temperatures**



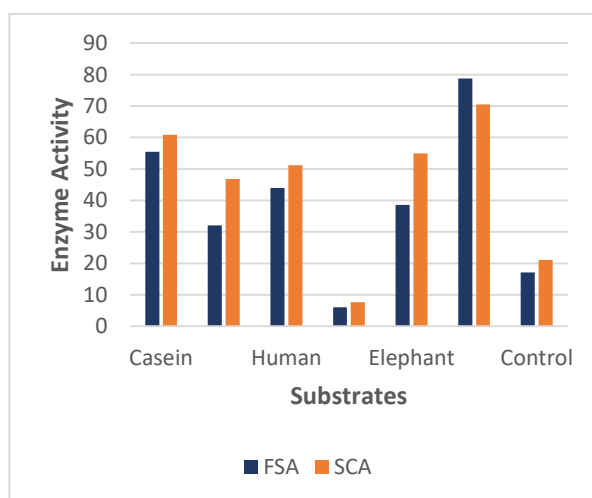
**Figure 3.32: Effect of pH on keratinase enzyme activity from *Pseudomonas aeruginosa* at different temperatures**

**Table Error! No text of specified style in document..1: Purification summary of keratinase enzymes from *Alcaligenes faecalis* (SCA)**

Steps	Vol (ml)	Total Protein (mg)	Total Activity	Specific Activity (U/mg)	Purification fold	% yield
Homogenate	200	191	940.86	4.93	1	100
Ion exchange	70	74	888	12	2.2	35
Gel Filtration	9	0.60	21.10	35.16	6	19

**Table Error! No text of specified style in document..2: Purification summary of keratinase enzymes from *Pseudomonas aeruginosa* (FSA)**

Steps	Vol (ml)	Total Protein (mg)	Total Activity	Specific Activity (U/mg)	Purification fold	% yield
Homogenate	200	208	1024.6	4.93	1	100
Ion exchange	74	64	768.17	12.00	2.4	37
Gel Filtration	14	0.61	23.06	37.80	7	22



**Figure 3.15: Substrate specificity of the enzyme**  
Where:  
SCA – enzyme from *Alcaligenes faecalis*

#### FSA – enzyme from *Pseudomonas aeruginosa*

#### 4.0 DISCUSSION

The isolation of keratinolytic organisms from various sources has garnered significant interest due to their potential applications in waste management and biotechnological processes. Microorganisms capable of degrading keratin have been successfully isolated from diverse environments, including soil, compost, and decaying organic matter, with notable studies identifying keratinolytic bacteria from sources such as chicken feathers and fish scales (Bansal *et al.*, 2023; Khan *et al.*, 2021, Oluchukwu *et al.*, 2024). The exploration of keratinolytic microorganisms from these different sources not only enhances our understanding of microbial diversity but also opens avenues for developing sustainable methods for keratin waste degradation (Mishra *et al.*, 2021).

This study explored production and characterization of keratinase enzymes using *Alcaligenes faecalis* and *Pseudomonas aeruginosa* isolated and identified from a previous study by Oluchukwu *et al.*, 2024 from decaying fish scales at Gusau dam, Zamfara State. The fermentation studies conducted in the present study provide valuable insights into the growth and metabolic characteristics of the two identified isolates, *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2), in the presence of Mackerel and Elephant fish scales. The pH profiles of the fermentation broths containing the two isolates in Figure 4.1 showed that the pH increased over time, reaching values of around 8.0-8.6 for the *Alcaligenes faecalis* (SCA A1) isolate and 7.5-8.7 for the *Pseudomonas aeruginosa* (FSA A2) isolate. This pH increase can be attributed to the production of ammonia and other alkaline compounds during the degradation of the fish scales by the keratinase enzyme (Lakshmi *et al.*, 2013).

The enzyme activity profiles of the two isolates in Figure 4.2 revealed that the *Pseudomonas aeruginosa* (FSA A2) isolate exhibited higher enzyme activity when grown with Mackerel fish scales, while the *Alcaligenes faecalis* (SCA A1) isolate showed higher enzyme activity with Elephant fish scales. These differences in enzyme activity could be due to variations in the keratin composition and structure between the two types of fish scales, which may influence the substrate specificity and catalytic efficiency of the keratinases produced by the respective isolates. These findings are in agreement with those reported by Lakshmi *et al.* (2013), who observed a similar increase in pH and the production of various amino acids during the degradation of feathers by their *Bacillus subtilis* and *Bacillus cereus* isolates. Moreover, the variations in enzyme activity observed between the two isolates in the present study are consistent with the reports of Nnolim *et al.* (2020), who found differences in the keratinolytic potential among their isolated *Bacillus* and *Arthrobacter* species.

The present study investigated the effect of different pH values (6.5, 7.5, 8.5, 9.5, and 10.5) and temperatures (28°C, 38°C, 48°C, 58°C, and 68°C) on the growth of the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates using Mackerel and Elephant fish scales as substrates. The results on Figure 4.3 to 4.12 showed that the *Alcaligenes faecalis* (SCA A1) isolate exhibited optimal growth at pH 9.5 and 10.5, particularly at higher temperatures of 48°C, 58°C, and 68°C when using Mackerel fish scales as the substrate. In contrast in Figure 4.13 to 4.22, the *Pseudomonas aeruginosa* (FSA A2) isolate displayed better growth at pH 7.5 and 8.5, with the highest growth observed at 28°C

and 38°C when using either Mackerel or Elephant fish scales.

These findings suggest that the two isolates have different pH and temperature preferences for their growth and metabolism, which may be related to their distinct physiological characteristics and adaptations to their respective environmental niches. The ability of the *Alcaligenes faecalis* (SCA A1) isolate to grow well under alkaline conditions and at higher temperatures could be advantageous for its application in the degradation of keratinous waste materials, as many industrial processes often require enzymes that can function under such conditions. Similar observations have been reported in previous studies. Cai *et al.* (2008) found that their mutant *Bacillus subtilis* strain KD-N2 exhibited optimal keratinase production at an initial pH of 7.5 and a cultivation temperature of 23°C. Barman *et al.* (2017) reported that their *Arthrobacter* sp. NFH5 isolate had the highest keratinase activity at pH 8.0 and 40°C. These studies highlight the importance of optimizing the growth conditions for individual keratinolytic isolates to enhance their enzyme production and catalytic efficiency.

The present study in Figure 4.23 to 4.26 showed the influence of different concentrations of Mackerel and Elephant fish scales (0.1, 0.5, 1.0, 1.5, and 2.0 g) on the growth of the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates. The results showed that the *Alcaligenes faecalis* (SCA A1) isolate exhibited the highest growth at a Mackerel fish scale concentration of 2.0 g, while the *Pseudomonas aeruginosa* (FSA A2) isolate displayed the best growth at a Mackerel fish scale concentration of 1.5 g. When Elephant fish scales were used as the substrate, both isolates exhibited higher growth at the higher concentrations of 1.5 and 2.0 g. These findings suggest that the two isolates have different preferences and optimal concentrations of the fish scale substrates for their growth and metabolism. The variations in growth patterns could be attributed to factors such as the availability of nutrients, the ease of accessibility of the keratin substrate, and the potential inhibitory effects of higher substrate concentrations.

Similar studies have reported the influence of substrate concentration on the growth and keratinase production of keratinolytic microorganisms. Lakshmi *et al.* (2013) observed that their *Bacillus subtilis* and *Bacillus cereus* isolates exhibited the highest keratinase activity and feather degradation at a feather concentration of 1% (w/v). Nnolim *et al.* (2020) also found that their isolated *Bacillus* and *Arthrobacter* species showed varying degrees of chicken feather degradation depending on the

substrate concentration. The findings from the present study and the literature highlight the importance of optimizing the substrate concentration for individual keratinolytic isolates to achieve maximum growth and enzyme production, which is crucial for the efficient degradation of keratinous waste materials.

Figure 4.27 and 4.28 in the present study showed the influence of various substrates, including gelatin, peptone, and casein, on the growth of the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates. The results showed that the *Alcaligenes faecalis* (SCA A1) isolate exhibited the highest growth when casein was used as the substrate, followed by peptone and gelatin. In contrast, the *Pseudomonas aeruginosa* (FSA A2) isolate displayed the best growth with gelatin, followed by peptone and casein.

These differences in substrate preferences between the two isolates can be attributed to their distinct metabolic capabilities and the availability of specific nutrients required for their growth and enzyme production. Casein, a dairy-based protein, may have provided the *Alcaligenes faecalis* (SCA A1) isolate with a favourable source of nitrogen and other essential nutrients for its growth and keratinase production. On the other hand, the *Pseudomonas aeruginosa* (FSA A2) isolate might have been better adapted to utilize the gelatin substrate, which is a common source of protein in various media formulations.

Similar observations have been reported in previous studies. Cai *et al.* (2008) found that their *Bacillus subtilis* mutant strain KD-N2 exhibited higher keratinase production when feathers were used as the substrate compared to other protein sources, such as hair and silk. Nnolim *et al.* (2020) also reported differences in the keratinolytic potential of their isolated *Bacillus* and *Arthrobacter* species when grown on various keratin-rich substrates, including chicken feathers and cow hair. The findings from the present study and the literature suggest that the growth and enzyme production of keratinolytic microorganisms can be influenced by the type of substrate provided, and the optimization of the substrate composition is crucial for enhancing the degradation of keratinous waste materials.

The purification summary of keratinase enzymes from *Alcaligenes faecalis* (SCA) and *Pseudomonas aeruginosa* (FSA) in table 4.1 and 4.2 provides valuable insights into the isolation and characterization of these enzymes. Both organisms demonstrated significant keratinase production, with purification steps yielding enzymes of increased specific activity and purity. For *A. faecalis*, the initial homogenate had a total protein

content of 191 mg and a total activity of 940.86 units. Through ion exchange and gel filtration chromatography, the enzyme was purified 6-fold, resulting in a specific activity increase from 4.93 U/mg to 35.16 U/mg. However, this process resulted in a significant loss of total activity, with only 19% yield retained in the final purified enzyme. This trade-off between purity and yield is common in enzyme purification processes.

Similarly, *P. aeruginosa* showed promising initial keratinase production, with a homogenate containing 208 mg total protein and 1024.6 units of total activity. The purification process increased the specific activity from 4.93 U/mg to 37.80 U/mg, also achieving a 7-fold purification. The final yield was slightly higher at 22%, suggesting that the *P. aeruginosa* enzyme may be somewhat more stable during the purification process. These results are comparable to findings from previous studies. For instance, Cai *et al.* (2008) reported a purified keratinase from *Bacillus subtilis* KD-N2 with a molecular mass of 30.5 kDa, which is in the same range as many bacterial keratinases. The optimum pH and temperature for that enzyme (pH 8.5 and 55°C) are also similar to those often reported for bacterial keratinases, including those from *Bacillus* species (Haq *et al.*, 2020; Cai *et al.*, 2008).

The specific activities achieved in this study (35.16 U/mg for *A. faecalis* and 37.80 U/mg for *P. aeruginosa*) are noteworthy. While they may not be the highest reported in the literature—for example, *Bacillus* sp. FPF-1 reported by Nnolim *et al.* (2020) showed a keratinase concentration of 1698.18 U/mL—they are still significant and suggest good potential for practical applications. The purification fold of 7 achieved for both enzymes indicate a substantial increase in purity, which is crucial for characterizing the enzymes and understanding their properties. This level of purification is comparable to many studies in the field, such as the work by Lakshmi *et al.* (2013) on *Bacillus* species. The yield percentages (19% for *A. faecalis* and 22% for *P. aeruginosa*) are relatively low, which is a common challenge in enzyme purification. Improving these yields could be a focus for future optimization studies. For comparison, Akhter *et al.* (2020) reported yields of 74.66 U/mL for *B. cereus* and 57.66 U/mL for *Pseudomonas* sp., highlighting the variability in keratinase production across different species and strains.

The use of ion exchange and gel filtration chromatography for purification aligns with standard practices in the field. These techniques have been successfully employed by many researchers, including Cai *et al.* (2008) and Li (2019), to obtain pure keratinase enzymes. The higher total protein and activity in the *P.*

*aeruginosa* homogenate (208 mg and 1024.6 units) compared to *A. faecalis* (191 mg and 940.86 units) indicates that *P. aeruginosa* might be a slightly more efficient keratinase producer under the given conditions. This aligns with findings from various studies that have identified *Pseudomonas* species as potent keratinase producers (Riffel & Brandelli, 2006; Qiu *et al.*, 2020).

The present study also focused on the characterization of the keratinase enzymes produced by the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates. The results in Figure 4.30 to 4.32 showed that the keratinase enzymes from both isolates exhibited optimal activity at a temperature of 70°C, with the *Pseudomonas aeruginosa* (FSA A2) isolate displaying a slightly higher enzyme activity compared to the *Alcaligenes faecalis* (SCA A1) isolate. The enzymes also showed good activity over a wide pH range, with the *Alcaligenes faecalis* (SCA A1) isolate exhibiting optimal activity at pH 7.3 and the *Pseudomonas aeruginosa* (FSA A2) isolate showing the highest activity at pH 6.3. These findings are consistent with reports from previous studies on the characteristics of keratinase enzymes. Haq *et al.* (2020) described a *Bacillus licheniformis* K-19 strain that produced a keratinase with optimal activity at 60°C and pH 7.5-8.0, highlighting the thermostability and broad pH tolerance of this enzyme. Cai *et al.* (2008) also reported the purification and characterization of a keratinase from a *Bacillus subtilis* strain, which showed optimal activity at 55°C and pH 8.5.

The substrate specificity analysis in Figure 4.33 revealed that the keratinase enzymes from both the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates exhibited the highest activity towards Mackerel fish scales, followed by casein, human hair, chicken feathers, and elephant fish scales. This suggests that the enzymes have a preference for the keratin present in Mackerel fish scales, which may be due to the specific structural and compositional features of this substrate. Similar substrate preferences have been reported in previous literature. Riffel and Brandelli (2006) found that the keratinases produced by their isolated *Burkholderia*, *Chryseobacterium*, and *Pseudomonas* strains were active towards a range of keratinous substrates, including feather meal, raw feathers, chicken nails, hair, and wool. Lakshmi *et al.*, (2013) also observed that the keratinase enzymes from their *Bacillus subtilis* and *Bacillus cereus* isolates exhibited higher activity towards feather substrates compared to other protein sources. The thermostability, broad pH tolerance, and substrate specificity observed in the keratinase enzymes from the *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) isolates suggest their potential for

various industrial applications, such as the degradation of keratinous waste materials, leather processing, and the production of feather-based feed supplements.

## 5.0 CONCLUSION

This study highlights the potential of *Alcaligenes faecalis* (SCA A1) and *Pseudomonas aeruginosa* (FSA A2) as effective keratinolytic microorganisms for the degradation of fish scale waste. The findings demonstrate that these isolates can produce keratinase enzymes capable of hydrolyzing keratin, which is prevalent in fish scales. Both isolates exhibited distinct physiological characteristics and substrate preferences, suggesting their adaptability and efficiency in utilizing different keratinous materials.

The keratinases produced showed promising properties, including thermostability and broad pH tolerance, indicating their suitability for various industrial applications, such as bioremediation, animal feed production, and the cosmetics industry. Furthermore, the ability to effectively degrade fish scales, a significant waste product from the fishing industry, underscores the environmental benefits of utilizing these microorganisms.

As such, the research contributes valuable insights into sustainable waste management strategies, emphasizing the importance of optimizing growth conditions and substrate utilization to enhance keratinase production. Future studies should focus on the scalability of these processes and the exploration of additional applications, ultimately promoting a circular economy and reducing environmental pollution in the fishing industry.

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