Electronic Journal of Biotechnology 74 (2025) 1–10

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Research article

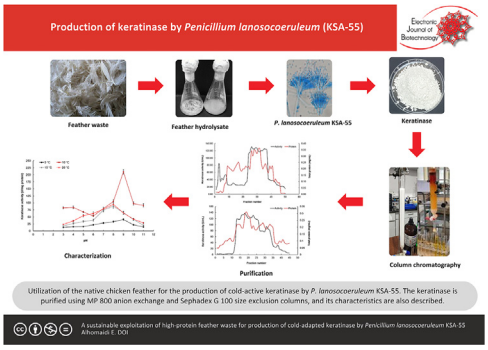
A sustainable exploitation of high-protein feather waste for production of cold-adapted keratinase by *Penicillium lanosocoeruleum* KSA-55 ☆

Eman Alhomaidi

Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia

GRAPHICAL ABSTRACT

A sustainable exploitation of high-protein feather waste for production of cold-adapted keratinase by *Penicillium lanosocoeruleum* KSA-55.



ARTICLE INFO

Article history:
Received 29 July 2024
Accepted 29 October 2024
Available online 20 December 2024

Keywords:
Cold-active
Column chromatography
Feather waste
Fungi
ITS
Keratinase
Keratinous waste
Penicillium
Phylogeny
Protease
Psychrophilic fungi

ABSTRACT

Background: The synthesis of keratinases by psychrophilic and psychrotolerant microorganisms has not received much attention, despite the fact that they can be an effective stand-in for substrate conversion at a low energy cost.

Results: In this study, some psychrophilic fungi isolated from three cold storage locations near Riyadh, Saudi Arabia, were tested for their cold-active keratinase potential. *Penicillium lanosocoeruleum*, a recently isolated fungus from Saudi Arabia, was the potent strain that produced cold-active keratinase. The *Penicillium* species was identified using sequencing of the internal transcribed spacer region (ITS). The generation of cold-active keratinase by *P. lanosocoeruleum* KSA-55 was optimized by two factors at time (TFAT). At pH 9.0 and 15°C, the keratinase activity was 28.9 ± 2.8 U/mL/min which increased to 41.7 ± 3.8 U/mL/min after 6 d of fermentation using peptone as a nitrogen source. The produced keratinase was chromatographed by MP 800 anion exchange resin and Sephadex G 100 size exclusion gel. At pH 9.0 and 10°C, the pure keratinase displayed the maximum specific activity of 210.3 ± 8.4 U/mg. Zn^{2+}, Fe^{2+}, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and phenylmethanesulfonyl fluoride (PMSF), demonstrated severe inhibitory effects on the keratinase activity. Mn^{2+} ions activated the keratinase by 166.85 ± 15.6%. PMSF significantly reduced keratinase activity.

Conclusions: *P. lanosocoeruleum* strain KSA-55 is presented here as a new prospective producer of cold-active keratinase for a variety of biotechnological uses, including the management of keratinous waste in the poultry industry, cosmetics, and medical applications.

☆ Audio abstract available in Supplementary material.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

E-mail address: eaalhomaid@pnu.edu.sa

https://doi.org/10.1016/j.ejbt.2024.10.003

How to cite: Alhomaidi E. A sustainable exploitation of high-protein feather waste for production of cold-adapted keratinase by *Penicillium lanosocoeruleum* KSA-55. Electron J Biotechnol 2025;74. https://doi.org/10.1016/j.ejbt.2024.10.003.

1. Introduction

Sustainable production and consumption support the development of a green economy, improved quality of life, and the planet's long-term recovery [1]. Throughout the recent decades, there has been a substantial demand for innovative renewable products over synthetic ones. Given the opportunity they offer, renewable resources are preferred over synthetic ones in most economies that are looking to biodiversity to strengthen the biotechnology. Nonetheless, because non-renewable resources make up the majority of the available resources, this field is still in its early stages [2]. Within the bioeconomic arena, microbial enzymes are widely used; of these, proteases make up approximately 60% of the overall market. The bio-conversion of recalcitrant keratin-rich wastes and the long-term implementation of cleaner production are two of the many uses for versatile proteases, or microbial keratinases, which are currently gaining traction in biotechnology [2,3,4].

Roughly 3–4 million tons of feather waste are produced annually by the poultry industry, according to reports from the EU. The poultry industry is keenly interested in transforming feathers into valuable products, as it holds significant value for both the environment and mankind. Feathers are thought to be the greatest quantity of keratinous waste in the world because of their high keratin content, which gives them a structure that is resistant to chemicals [5]. Keratin is a structurally fibrous protein that is highly stable and mechanically strong. It is rich in disulfide bonds that cross-link peptide chains and cysteine residues [5]. In nature, there are two main types of keratins: α-keratin, which primarily has an α-helix secondary structure, and β-keratin, which primarily has a β-sheet secondary structure [6].

The traditional techniques for handling chicken feathers, like burning, chemical treatment, and landfilling, are expensive, time-consuming, and environmentally hazardous [3]. The aforementioned methods are insufficient because they: (1) decrease the amount of soil; (2) raise the concentration of harmful substances, like salts and halogens, in water bodies, thereby posing a threat to aquatic life; (3) increase emissions of greenhouse gases, exacerbating global warming; and (4) exacerbate eutrophication in aquatic ecosystems because of the elevated levels of nitrogen and alkalinity in the water [4,7]. In contrast, microbial hydrolysis is currently regarded as the primary environmentally acceptable recycling option [1,4].

Keratinases are enzymes that specifically hydrolyze keratinous substrates, which include feathers, wool, and nails. According to Brandelli et al. [8], microorganisms from the three domains of life produce these enzymes. Regardless of the advantages they provide, the use of these enzymes may be limited because of their high energy consumption, increased risk of byproduct development, and degradation of thermolabile substrates and products. The majority of keratinases that have been described to date come from mesophilic and thermophilic organisms [3,4,9,10,11,12]. Few studies have been done on the production of keratinases by psychrophilic and psychrotolerant microorganisms, although they can be a useful substitute for transforming substrates at a low energy cost [13].

Psychrophile excretion is one of the most promising sources of cold-adapted enzymes with high specific activity at low temperatures. In industry, enzymes are progressively taking the place of chemical processes because they are more environmentally friendly, boost product value, generate fewer waste products, need less energy, and lessen pollution in the environment. Enzymes that have acclimated to the cold can catalyze at as low as 30°C and maintain some catalytic activity at as low as 0°C [14]. Their low reaction energy—the ideal catalytic temperature is typically between 20 and 45°C—their higher substrate affinity, which can reduce the activation energy of enzymatic reactions, and their low thermal stability at high temperatures—which causes them to quickly lose more than half of their activity after 10 min at 50–60°C or several hours at 37°C—set them apart from mesophilic and thermophilic enzymes [15,16,17].

The microbial cold-active enzymes have gained a lot of interest due to their plentiful source, short production cycle, high yield, and facile separation, easily controlled reaction conditions, simplified monitoring and control of production processes and purification [18,19]. The worldwide market for enzymes, taking into account all the many kinds of enzymes used by different industries, was valued at about US$ 5.5 billion in 2018 and was expected to reach more than US$ 7.0 billion by 2025 [20]. As enzymatic processes are more environmentally benign and sustainable than chemical processes, scientists are currently concentrating their efforts on finding new enzymes with the potential to replace industrial chemical catalysis [21,22]. Standard enzymes are denatured under the extremes of pH, temperature, and salinity that are frequently encountered in the industrial manufacture of commodities on a commercial scale [22]. As a result, one must constantly look for novel biocatalysts that can withstand these extreme conditions [15,23,24,25].

As a result, these enzymes have been widely studied and exploited in a wide range of applications, including food processing, detergent production, bioremediation, environmental protection, straw resourcing, and basic molecular biology research [26]. Consequently, the goal of this work is to produce cold-active keratinase from psychrophilic *Penicillium* strain in a way that is both economical and ecologically beneficial.

2. Materials and methods

2.1. Isolation and identification of psychrophilic fungi

At three distinct locations for cold storage near to Riyadh, Saudi Arabia, 10 Petri plates containing Czapek's autolysate yeast extract agar (CYA) [27] were separately exposed to refrigerator air for 60 min before being incubated at 10°C for 15 d in order to isolate psychrophilic fungi. According to the macroscopic and microscopic characteristics, *Penicillium* species were identified using CYA medium and *Cladosporium* species were identified using potato dextrose agar (PDA) [27]. The developing fungal colonies were isolated and purified using a single spore isolation technique [28]. The resultant isolates were then used in the keratinolytic activity test.

2.2. Fermentation medium

Feather mineral liquid medium (FMM) [29]) was employed as the fermentation medium in this study. The FMM was made up of (g/L): 10 g of chicken feathers per liter of mineral liquid medium (pH 6.0), K_2HPO_4, 2.486; NaH_2PO_4, 0.496; $MgCl_2$, 0.01; $FeCl_3$, 0.016; $CaCl_2$, 0.0001; and $ZnCl_2$, 0.013.

2.3. Screening of keratinolytic ability in submerged fermentation (SmF)

Using FMM, 62 fungal isolates in total were examined for cold-active keratinolytic activity. A total of 250 mL Erlenmeyer flasks were separately filled with 50 mL each of FMM. A 7-day-old culture of the tested fungi were used to prepare spore suspensions that contained 1.5×10^8 spore/mL. 2.0 mL of the fungal spore suspensions were used separately to inoculate the flasks. The cultures remained at 10°C and 150 rpm of shaking until no more feather degradation was apparent. The isolate that proved to be the most effective in eradicating chicken feathers was selected as the strong cold-active keratinase producer for further investigation.

2.4. Extraction of keratin powder

A sample of native chicken feathers was gathered from Amal Al Khair poultry farm near to Riyayadh, Saudi Arabia. The feather sample was defatted in a chloroform–methanol (1:1) mixture for 24 h while being constantly stirred. It was then repeatedly cleaned with distilled water and oven-dried at 50°C. Keratin was extracted from 100 g of native chicken feathers using the procedure described by Alwakeel et al. [3]. The keratinase assay procedures used the keratin powder that was produced.

2.5. Morphological and molecular identification of the potent keratinase-producing isolate

Using appropriate references [30], the morphological characteristics of the *Penicillium* isolate employed in this investigation were determined. On Czapek's agar (Cz), malt extract agar (MEA), and Czapek's yeast Autolysate agar (CYA) [27], the growth rates of the novel species were investigated. According to Samson et al. [31], spore suspensions produced in a 0.2% agar and 0.05% Tween 80 solution were used to make inoculations. After inoculating the plates in a three-point pattern with an inoculum size of 1.0 µL/spot, the plates were incubated for 7 d at 25°C in the dark. The colony characteristics from the MEA culture were examined. For molecular identification of the *Penicillium* isolate KSA-55 in this study, the procedure outlined by Moubasher et al. [32] was used to isolate DNA. SolGent EF-Taq polymerase was used to carry out the PCR reaction [33,34]. For amplification of the ITS region, ITS1 and ITS4 primer pair were used [35]. The continuous sequence of the *Penicillium* isolate KSA-55 used in this investigation was created using DNASTAR (version 5.05). The 21 sequences in the whole ITS dataset utilized for phylogenetic analysis included an outgroup sequence for *Talaromyces pinophilus* KUMCC 18-0203, one sequence for *Penicillium* isolate KSA-55 in this work, and 19 sequences from the genus *Penicillium* uploaded from GenBank. Using MAFFT (version 6.861b) and the default settings, all sequences were aligned collectively in this analysis [36]. All sequences in this study were aligned using MAFFT (version 6.861b) using the default parameters. BMGE [37] improved alignment gaps and parsimony of uninformative characters. MEGA X (version 10.2.6) was used to perform maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analyses [38], and 1000 replications were employed to assess the robustness of the most parsimonious trees [39]. The best nucleotide substitution model for ML analysis was found using Modeltest 3.7′s Akaike Information Criterion (AIC) [40].

2.6. Optimizing fermentation conditions for cold-active keratinase production

Two factors at a time (TFAT) conditions were applied, allowing for variations in pH, temperature, nitrogen supply, and fermentation duration. The experiments were conducted in 250 mL Erlenmeyer flasks using 50 mL of FMM. As the only carbon source, 1.0% of the native chicken feathers were added. The flasks were inoculated with a 2.0% spore suspension made from a 7 d old culture of *Penicillium* isolate KSA-55 in a 10% Tween 80 solution. Following that, the flasks were incubated under a range of operating parameters, including pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0), temperature (10, 15, and 20°C), and incubation duration (1–7 d). The nitrogen source options included peptone, yeast extract, beef extract sodium nitrate, ammonium chloride, and ammonium sulfate, all of which were tested at 2 g/L. Three distinct experiments were carried out.

2.7. Keratinase assay and protein estimation

The reaction included 0.01 g of powdered keratin and 0.01 g of the enzyme, both of which were dissolved in 1.0 mL of pH 8.0 phosphate buffer solution. The reaction was stopped after 60 min at 10°C by adding 2.0 mL of 10% trichloroacetic acid (TCA), and the precipitate was then decanted by centrifugation at 10,000 rpm for 10 min. After diluting 0.2 mL of the supernatant to 1.0 mL, 5.0 mL of alkaline copper reagent was added. Afterwards, 0.5 mL of the Folin–Ciocalteau reagent was applied and the tubes were kept in the dark for 30 min to allow the blue color formation at 660 nm. Keratinase activity was calculated following Alwakeel et al. [3]. One unit of keratinase (U) was defined as the amount of enzyme needed to release 1 µmol tyrosine per min.

2.8. Cold-active keratinase production in submerged fermentation (SmF)

Each of the 500 mL Erlenmeyer flasks used for the submerged fermentation process held 100 mL of FMM that had been enhanced with 1% native chicken feather as a fermentation substrate. As a source of nitrogen, peptone was added to the fermentation medium, and the medium's pH was brought to 3.0. A 5 mL spore suspension (1.5×10^8 spore/mL) from a 7-day-old *Penicillium* isolate KSA-55 culture was separately added to each flask after they had been sterilized (121°C for 20 min). In addition, 150 rpm and 15°C were the incubation conditions for six days. Centrifugation was used to extract the cell-free supernatant after the fermentation period, with 10,000 rpm and 4°C for 10 min.

2.9. Ammonium sulfate precipitation and dialysis of the cold-active keratinase

Protein was extracted in its entirety at 4°C using a 70% saturated ammonium sulfate solution. The precipitated protein was separated and lyophilized using a freeze dryer. Salts and other tiny molecules were eliminated from the lyophilized protein by dissolving it in phosphate buffer (pH 8.0), dialyzing it twice for two hours at room temperature (cutoffs: 12–14 kD), and then, cooling it for an overnight stay at 4°C. After being partially purified and lyophilized, the dialyzed protein's fungal keratinase was employed in enzyme characterization experiments.

2.10. Purification of the cold-active keratinase

2.10.1. Ion-exchange column

A glass column (60 cm × 2.5 cm) with a bed volume of 200 cm^3 held a Lewatit MonoPlus (MP 800) anion exchanger inside. After equilibrating the column with 100 mM of pH 8.0 phosphate buffer, a 10.0 mL sample was injected. At NaCl concentrations of 0, 0.1, 0.25, 0.5, 1.0, and 1.5 M, the enzyme was eluted using 100 mM phosphate buffer (pH 9.0). The volume of the fractions was 6.0 mL, and the column flow rate was set at 0.25 mL/min. The cold-active keratinase activity was measured, and the most active fractions were pooled, concentrated, and prepared for injection into the size-exclusion column.

2.10.2. Size-exclusion column

A bed volume of 200 cm^3 Sephadex G 100 was packed in a glass column (60 cm × 2.5 cm) and pre-equilibrated with 100 mM of pH 9.0 phosphate buffer. A 10.0 mL sample was injected, and 6.0 mL fractions of the bounded protein was then eluted using NaCl concentrations of 0, 0.1, 0.25, 0.5, 1.0, and 1.5 M in 100 mM phosphate buffer (pH 9.0) at a flow rate of 0.25 mL/min. The cold-active keratinase activity was measured by the previously described method.

2.11. Impact of pH, temperature and some ions and inhibitors on the cold-active keratinase activity

In a water bath, 0.01 g of enzyme powder and 0.01 g of keratin powder (both dissolved in 1.0 mL of 50 mM buffer solution) were included in the reaction mixture. The effects of pH (3.0–11.0) and temperature (5–20°C) on keratinase activity have been determined. Some monovalent and divalent ions, such as NaCl, KCl, $CaCl_2$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $FeSO_4$, $MnSO_4$, $MgSO_4$, and $ZnSO_4$, were tested by introducing them at 5.0 mM [3,41]. An enzyme inhibitor was tested using a 5 mM solution of ethylenediaminetetraacetic acid (EDTA) or sodium dodecyl sulfate (SDS). The impact of phenylmethanesulfonyl fluoride (PMSF) at a concentration of 0.01 mM was also tested. The activity of the keratinase in the absence of ions, EDTA, or SDS was measured under normal conditions to define 100% activity. Three repeats of the different tests were set up.

2.12. Statistical analysis

All data were expressed using the mean and standard deviation (SD) of the preliminary study, which was carried out in triplicate. Stahle and Wold's method of statistical significance analysis was followed [42]. At $p \leq 0.05$, it was considered significant.

3. Results

3.1. Isolation of psychrophilic fungi

From the three locations under investigation, 62 fungal colonies could be isolated. Along with two isolates of dark fungi, the majority of the isolated fungi belonged to the two main genera: *Cladosporium* (43 isolates) and *Penicillium* (17 isolates). Two species of *Cladosporium*, *C. cladosporioides* (17 isolates) and *C. sphaerospermum* (26), comprised the 43 isolates. Five species —*Penicillium lanosocoeruleum* (2 isolates), *P. chrysogenum* (7), *P. citrinum* (4), *P. crustosum* (3), and *P. glabrum* (1)— represented *Penicillium* (17 isolates). Two isolates were used to represent *Alternaria alternata*.

3.2. Screening of keratinolytic activity of psychrophilic fungi

After 7 d of incubation at 10°C and 150 rpm, the majority of the examined fungi were found to be able to hydrolyze the native chicken feathers, albeit to varying degrees, in the screening test. The isolate KSA-55 was the most effective *Penicillium* isolate, completely hydrolyzing the feather waste. This isolate was chosen for molecular identification and cold-active keratinase production optimization.

3.3. Morphological and molecular identification of the Penicillium isolate KSA-55

The *Penicillium isolate* KSA-55 in this study displayed the identical morphological characteristics of the reference *Penicillium lanosocoeruleum* strain. Conidiophores asymmetrical, terverticillate, biverticillate, or quaterverticillate. Metulae 10–15 μm, bearing 4–8 phialides. Phialides 6–10 μm, ampulliform, with apices abruptly tapering to a short neck. Conidia globose to subglobose, smooth, 2.5–4 μm (Fig. 1).

Utilizing *Penicillium* KSA-55′s ITS sequence, the GenBank database revealed that *Penicillium lanosocoeruleum* CBS 334.48 and IHEM 27477 [(GenBank accession numbers MH856372 and OW988441; identities = 559/559 (100%); Gaps = 0/559 (0%)] were the most similar sequences. The isolate used in this study was placed in the *Penicillium lanosocoeruleum* clade in the evolutionary tree. It was also placed on the same branch as *P. lanosocoeruleum* CBS 334.48 and CBS 484.84, which endorsed a strong support clade with 71% ML/85% MP (Fig. 2). Consequently, *Penicillium* isolate KSA-55 used in this study was identified as *Penicillium lanosocoeruleum*. ITS sequence of *Penicillium lanosocoeruleum* KSA-55 was deposited in GenBank as OR856115 (Fig. 2).

3.4. Optimization of the cold-active keratinase production

Incubation at pH 9.0 and 15°C exhibited the keratinase activity peak (28.92 ± 2.8 U/mL) produced by *P. lanosocoeruleum* KSA-55 (Fig. 3). After 6 d of fermentation, all investigated nitrogen sources increased the keratinase output, with peptone being significantly better than yeast extract and beef extract, boosting keratinase activity to 41.7 ± 3.8 U/mL (Fig. 4).

3.5. Purification of the cold-active keratinase

The enzyme was anion-exchange chromatographed on MP 800 gel, yielding nine fractions (Fr. 27-Fr. 35) with the highest active keratinase and protein peaks (Fig. 5A). This step was repeated until the produced keratinase was completely purified. The fractions with the greatest activity from the MP 800 column were collected and concentrated, and used in further purification using Sephadex G 100 size exclusion column. The most active fraction (Fr. 13-Fr. 33) obtained from Sephadex G 100 column (Fig. 5B) were pooled, lyophilized, and used as pure keratinase preparation in the characterization tests.

3.6. Yield and activity of the pure cold-active keratinase

After two-stage column chromatography, it can be purifying the cold-active keratinase in this study by 3.48-fold yielding a specific activity of 210.3 U/mg protein at pH 9.0 and 10°C (Table 1; Fig. 6).

3.7. Effect of some ions, activators, and inhibitors on the activity of the cold-active keratinase

When tested under the ideal conditions observed (pH 9 at 10°C), all of the tested ions, including EDTA, SDS, and PMSF, exhibited severe inhibitory effects on the activity of the keratinases produced, with the exception of Mn, which activated the keratinase by 166.85 ± 15.6. Zn had the strongest inhibitory effect, followed by

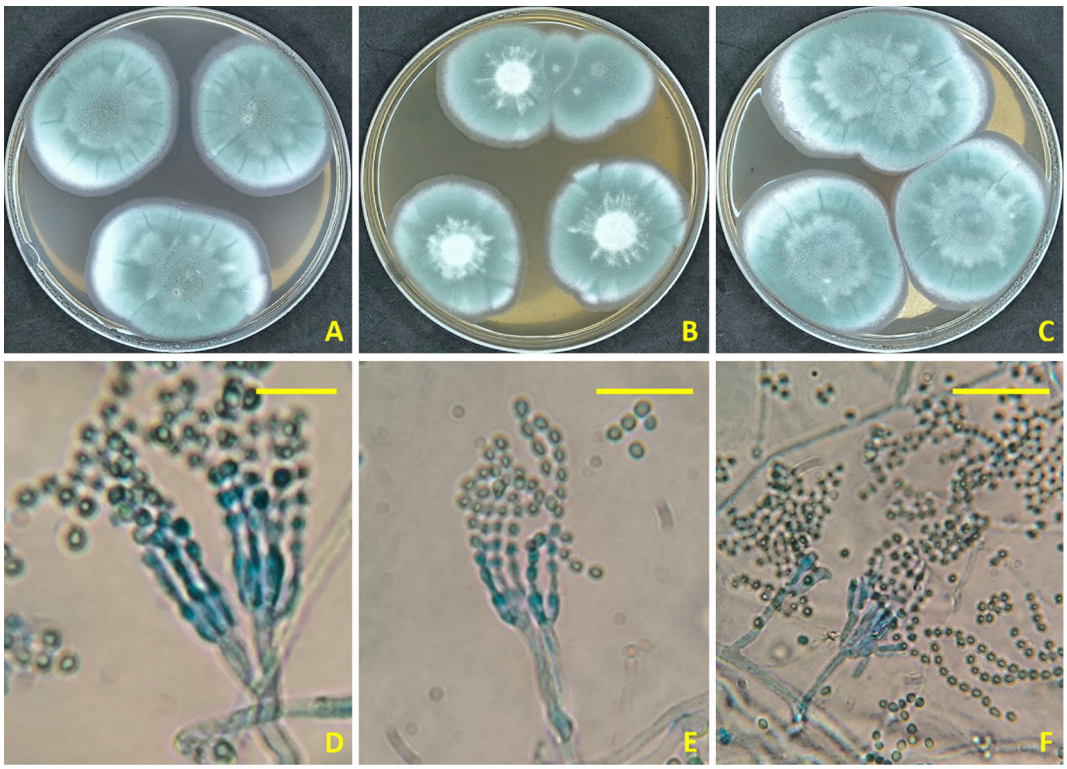


Fig. 1. *P. lanosocoeruleum* KSA-55. (A-C) Seven-day-old colonies on Cz, MEA, and CYA at 25°C. (D-F) Conidiophores and conidia (Scale bars: D = 10 μm. E-F = 20 μm).

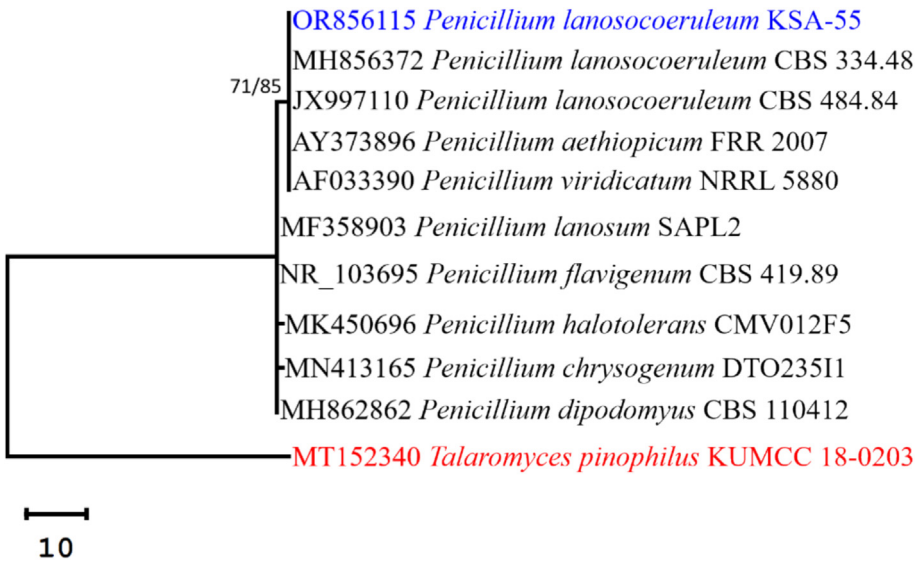


Fig. 2. Maximum parsimony phylogenetic tree produced by ML/MP analysis using *P. lanosocoeruleum* KSA-55′s ITS sequence data (blue color) in comparison to the most comparable *Penicillium* ITS sequences found in GenBank. Near the corresponding nodes, bootstrap support values (1000 replications) for ML/MP ≥50% are displayed. *Talaromyces pinophilus* KUMCC 18-0203, the outgroup (highlighted in red), is the root of the tree. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

EDTA, Fe, and PMSF. PMSF markedly decreased the keratinase activity (Table 2).

4. Discussion

Microbial keratinases are thought to be very beneficial for several biotechnological applications such as, treating keratinous wastes from the agriculture and leather industries, detergent and textile industry, decontamination of prions, and treatment of dermatophytic and nail diseases, scars and epithelial regeneration [7]. In addition, it has been demonstrated that keratinase has a stimulating effect on the growth of plants. In light of this, the germination rate of seeds (87.5% success rate) and the growth of Bengal gram (*Cicer arietinum*) seedlings was greatly enhanced by the

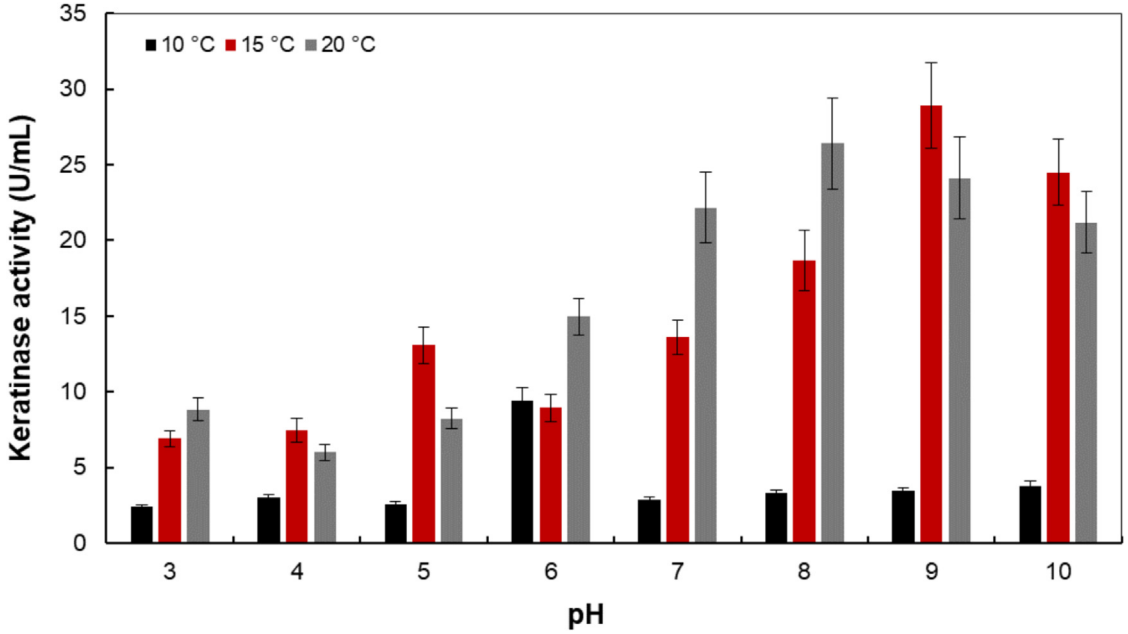


Fig. 3. Effect of medium's pH and temperature on the activity of keratinase produced by *P. lanosocoeruleum* KSA-55 in SmF.

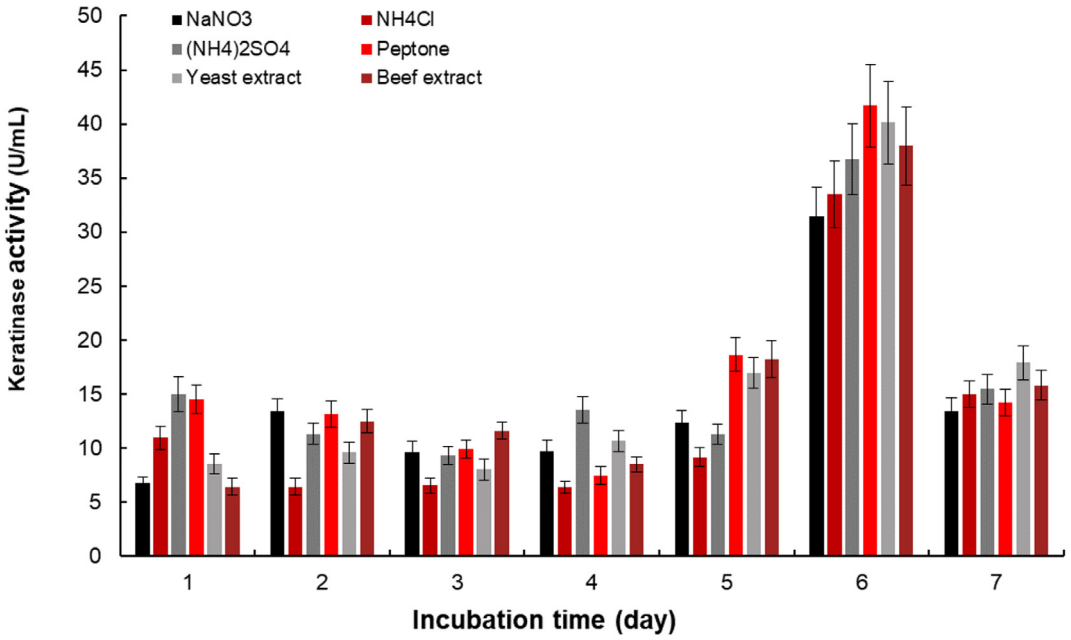


Fig. 4. Effect of medium's nitrogen source and fermentation time on the activity of keratinase produced by *P. lanosocoeruleum* KSA-55 in SmF.

filter-sterilized fermented hydrolysate of *Paenibacillus woosongensis* TKB2 [43]. The germination energy and germination index for the group supplemented with 3% Bacillus pumilus JYL feather hydrolysate were 94.22% and 87.52%, respectively, both considerably exceeding those of the control group [44]. The impact of *Bacillus subtilis* PF1 and chicken feathers on the development of *Vigna radiata* in soil was examined, revealing significant plant growth promotion activity. The elevated carbon-to-nitrogen ratio in soil further facilitated the plant growth-promoting effects of keratinases [45].

The goal of studying keratinases is to breakdown various waste materials, and finding effective keratin degraders gives opportunities to remediate waste from the cattle, poultry, and leather sectors [45]. The cheap cost and harmless consequences of employing natural keratinophilic microorganisms to produce enzymes are benefits [46]. A variety of intriguing new uses for byproducts, including detergent additives, vitamins, amino acids, and polypeptides, have the potential to increase agriculture's sustainability [47,48]. It is still necessary to investigate the mechanisms underlying the decomposition of keratinous materials. One keratinase can cat-

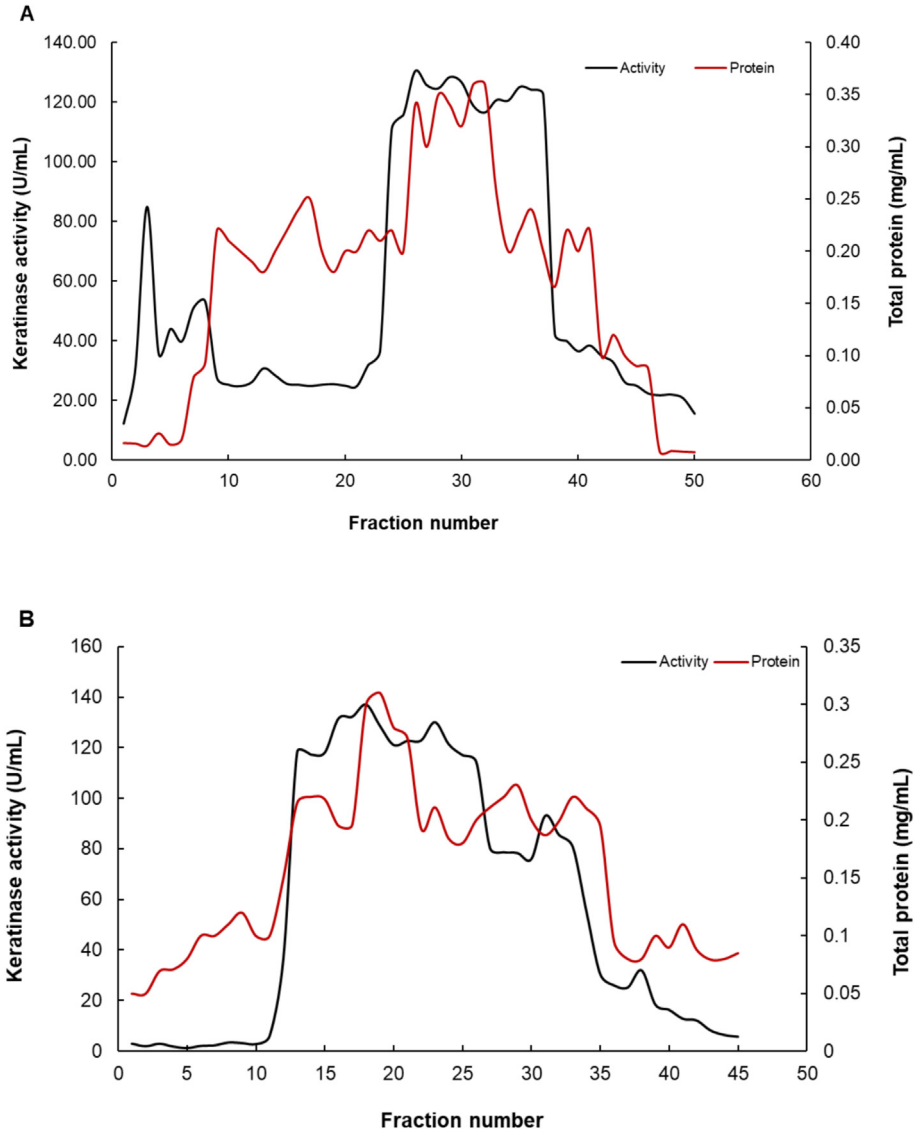


Fig. 5. (A), Anion exchange on MP 800 and (B), size exclusion on Sephadex G 100 of the major active keratinase fractions obtained from *P. lanosocoeruleum* KSA-55 at pH 9.0 and 10°C.

Table 1
Purification profile of cold-active keratinase produced by *P. lanosocoeruleum* KSA-55.

Purification steps	Volume mL	Activity U/mL	Total activity U	Protein mg/mL	Total protein mg	Specific activity U/mg	Yield %	Purification fold
Fermentation medium	1800	41.7	75,060	0.69	1242	60.434	100	1
70% ammonium sulfate	70	74.1	5187	1.18	82.6	62.8	6.65	1.04
MP 800	15	118.62	1779.3	0.932	13.98	127.27	1.125	2.1
Sephadex G 100	15	134.6	2019	0.64	9.6	210.3	0.773	3.48

alyze keratinolysis on its own [48] or, for maximum effect, in combination with additional enzymes such as collagenase [4], or disulfide reductases [49] which are responsible for the breakdown of disulfide bonds.

In this work, chicken feather waste was hydrolyzed at low incubation temperature using *P. lanosocoeruleum* KSA-55, which was isolated from a cold environment in Saudi Arabia, to create cold-active keratinase. In this paper, *P. lanosocoeruleum* is re-reported here for the first time from Saudi Arabia. It has never been documented that *P. lanosocoeruleum* produces keratinase. Some psychrophiles, such as *Penicillium* species, have been shown to exhibit high metabolic rates as a physiological response to cold temperatures, which serves to offset the poor biochemical rates at low temperatures, even if they grow slowly. Because psychrophiles need a lot of ATP to sustain a high metabolic rate, they create cold-adapted enzymes that can effectively function at low

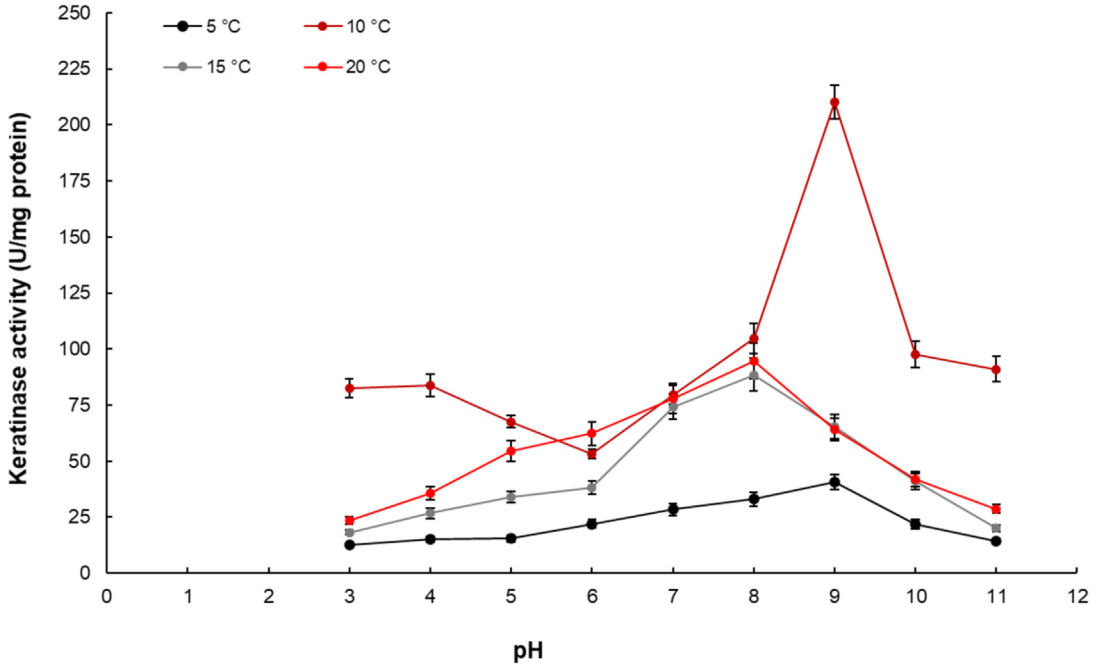


Fig. 6. Effect of pH and temperature on the specific activity of the cold-active keratinase produced by *P. lanosocoeruleum* KSA-55 in SmF.

Table 2
Effect of monovalent and divalent ions, EDTA, and SDS (5 mM/mL), in addition to PMSF (0.01 mM/mL) on keratinase activity produced by *P. lanosocoeruleum* KSA-55. The results are expressed as the proportion of the activity in the tested inhibitory conditions, from the keratinase activity in the control without inhibitors. Mean values (±SD) with different letters are significantly different ($p < 0.05$; $n = 3$).

Ions and inhibitors	Specific activity (U/mg)	Residual activity (%)
Control	210.3 ± 13.65^{b}	100 ± 6.49^{b}
NaCl	93.7 ± 5.44^{f}	44.56 ± 2.59^{f}
KCl	100.27 ± 6.31^{d}	47.68 ± 3^{d}
$CaCl_2$	106.77 ± 7.57^{c}	50.77 ± 3.6^{c}
$MgSO_4$	97 ± 7.27^{e}	46.12 ± 3.46^{e}
$FeSO_4$	43.57 ± 5.1^{l}	20.72 ± 2.42^{l}
$CuSO_4$	79.53 ± 6.54^{h}	37.82 ± 3.11^{h}
$ZnSO_4$	25 ± 1.8^{m}	11.9 ± 0.86^{m}
$MnSO_4$	350.88 ± 32.8^{a}	166.85 ± 15.6^{a}
$CoCl_2$	67.55 ± 5.17^{j}	32.12 ± 2.46^{j}
$NiCl_2$	90.43 ± 5.46^{g}	43.0 ± 2.6^{g}
EDTA	42.48 ± 3.99^{l}	20.2 ± 1.9^{l}
SDS	70.83 ± 4.88^{i}	33.68 ± 2.32^{i}
PMSF	52.3 ± 3.64^{k}	24.87 ± 1.73^{k}

temperatures in order to make use of whatever nutrition sources are present in their surroundings [50].

Penicillium species, as psychrophilic fungi, have been isolated from Antarctic soil probes [51]. Cold ecosystems span more than 70% of the ecosphere, including oceans, soils, glaciers, lakes, and sea ice. Antarctica, the southernmost continent, is also the coldest, with ice sheets covering 90% of the continent [52]. Extremely durable macromolecules are necessary for the modern biotechnological sectors. Numerous biotechnological uses exist for psychrophilic and psychrotolerant microorganisms as well as for the proteins and enzymes they have evolved to withstand cold. Because microorganisms adapt to various habitats, their molecular machinery evolves as a result. It is known that the cold-adapted microorganisms generate cold-active enzymes [53,54].

In this investigation, a cold-active keratinase was provided with peptone as a nitrogen source, and optimal conditions were set for six days of incubation at pH 9.0 and 15°C. The digestive enzymes generated by microorganisms are mostly extracellular and are significantly influenced by nutritional and physicochemical variables. In an operation based on the manufacture of bioactive chemicals by microbes, optimizing different media components can have a significant impact on production costs and can result in profit or loss. Investigating the best conditions for biotechnology solutions is therefore essential. The usage of each component is determined by the proper balance of multiple media components. Optimization of diverse media components is required for a cost-effective approach of enzyme synthesis [4]. The production of keratinase was significantly influenced by the nutrient medium's composition and the culture conditions. Physical and chemical parameters such as pH, temperature, incubation duration, as well as nitrogen sources have been demonstrated to influence keratinase production [3,4].

Numerous fungi and bacteria have been identified as possible keratinase producers, with widely divergent ideal conditions. However, very rare data were found about the production of cold-active keratinase. For instance, pH 8 and 40°C were the ideal conditions for a 25 d incubation period for *Aspergillus terreus* [55]. *Aspergillus niger* strains produced varied levels of keratinases with the maximum activity reached at pH 5 after 7 d of incubation [56]. *Aspergillus* sp. DHE7 had the highest keratinase activity of 199 IU/mL after 4 d at pH 6.0 and 30°C [57]. The highest amount of alkaline keratinase was developed by *Cochliobolus hawaiiensis* after 15 d at pH 9.5 and 30°C [58]. At 25°C for 21 d, *Chrysosporium tropicum* produced keratinase at its maximum level [59]. At 30°C, *Trichophyton ajelloi* exhibited its greatest level of enzyme activity [60]. On 20th day of incubation, *Microsporum gypseum* and *M. canis* showed the highest activity [61]. At 50°C and pH 9.0, *Bacillus thuringiensis* exhibited its highest activity [62]. Due to small methodological variations, it is generally challenging to compare the values of enzyme activity between different research. As such, one should proceed cautiously when making comparisons.

The produced cold-active keratinase displayed its activity peak at pH 9.0 and 10°C, indicating that it was a cold-active and alkaline enzyme. Except for Mn^{2+}, which stimulated the cold-active keratinase, all of the examined ions in this work showed severe inhibitory effects on the activity of the keratinases produced when

evaluated under the optimal conditions observed (pH 9 at 10°C). Zn^{2+} exhibited the greatest inhibitory impact, followed by EDTA, Fe^{2+}, and PMSF. The keratinase activity was significantly reduced by PMSF, suggesting that the enzyme in question was a member of the serine protease family. Many studies have established the impact of metals, activators, and inhibitors on microbial keratinase activity [3,4,63,64]. This was also true of our strain *P. lanosocoeruleum*. No previous information regarding our species has been found. The effect of metal ions on the keratinase activity was reported in many studies. Regarding this concern, a feather-degrading culture of *Aspergillus oryzae* has been stimulated by Ca^{2+} and Ba^{2+} ions while inhibited by EDTA and Pb^{2+} ions [65]. EDTA, Hg^{2+}, and Fe^{3+} significantly decreased *A. flavipes* keratinase activity, while Zn^{2+}, Mg^{2+}, and Cu^{2+} had no effect on *A. flavipes* keratinase [66]. According to the research, different fungi have extremely varying optimal circumstances. This means that the ideal culture conditions and inhibitory substances must be investigated.

By utilizing psychrophiles to produce the enzymes at lower temperatures and reducing processing temperatures, cold-active enzymes are thought to be able to slow down climate change [67]. The affinity of cold-adapted enzymes for their substrate is increased by their low activation energy requirements for the enzyme-substrate complex. In order to do this, the flexibility of the enzyme structure is changed. This minimizes energy consumption by raising the rate of enzymatic activity at low temperatures. As of right now, cold-adapted enzyme catalysis is explained by two theories: The enzyme requires little energy at low temperatures because: (1) its structure is more flexible than that of mesophilic or thermophilic enzymes; and (2) it activates with less energy than other molecules [17].

5. Concluding remarks

Fungi from three cold storage locations near Riyadh, Saudi Arabia, were tested for their ability to hydrolyze chicken feather waste and produce cold-active keratinase. *Penicillium lanosocoeruleum* was isolated for the first time from Saudi Arabia and has been found to be a powerful strain capable of hydrolyzing feather waste and creating cold-active keratinase. *Penicillium lanosocoeruleum* KSA-55 produced the peak of keratinase at pH 9.0 and 15°C after 6 d of employing peptone as nitrogen supply. The enzyme produced the most specific activity at pH 9.0 and 10°C after chromatographed by MP 800 anion exchange and Sephadex G 100 columns. Zn^{2+}, Fe^{2+}, EDTA, SDS, and PMSF all had a strong inhibitory effect on the keratinase activity. Mn^{2+} ions significantly increased keratinase activity, but PMSF markedly decreased keratinase activity. *Penicillium lanosocoeruleum* strain KSA-55 is reported here as a new prospective producer of cold-active keratinase for a number of biotechnological applications. Given the existing lack of understanding and availability of cold-active proteases with varying features, it is vital to conduct additional research in pursuit of suitable cold-active keratinases. Addressing the functional properties of these enzymes may open up new avenues for developing keratinases with desired functions.

CRediT authorship contribution statement

Eman Alhomaidi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

Financial support

This work was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R317), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declaration of competing interest

There is no conflict of interest.

Supplementary material

https://doi.org/10.1016/j.ejbt.2024.10.003.

Data availability

Data will be made available on request.

References

[1] Al Mousa AA, Moubayed NM, Al Jaloud AM, et al. Chicken feathers waste management by microbial as a sustainable and tool environmental friendly. J Environ Prot 2021;12(9):639–53. https://doi.org/10.4236/jep.2021.129039.
[2] Nnolim NE, Udenigwe CC, Okoh AI, et al. Microbial keratinase: Next generation green catalyst and prospective applications. Front Microbiol 2020;11:. https://doi.org/10.3389/fmicb.2020.580164. PMid: 33391200580164.
[3] Alwakeel SS, Ameen F, Al Gwaiz H, et al. Keratinases produced by *Aspergillus stelliformis*, *Aspergillus sydowii*, and *Fusarium brachygibbosum* isolated from human hair: Yield and activity. J Fungi 2021;7(6):471. https://doi.org/10.3390/jof7060471. PMid: 34200943.
[4] Al-Bedak OAM, Moharram AM, Hussein N-A-G, et al. Microbial exploitation of feather wastes for sustainable production of keratinase and collagenase enzymes by *Didymella keratinophila* AUMC 15399 in submerged fermentation. Fermentation 2023;9(6):507. https://doi.org/10.3390/fermentation9060507.
[5] Smirnova M, Bolaño Losada C, Akulava V, et al. New cold-adapted bacteria for efficient hydrolysis of feather waste at low temperature. Bioresour Technol Rep 2023;23:. https://doi.org/10.1016/j.biteb.2023.101530101530.
[6] Fraser RB, Parry DA. Filamentous structure of hard β-keratins in the epidermal appendages of birds and reptiles. In: Parry D, Squire J, editors. Fibrous proteins: Structures and mechanisms. Subcellular Biochemistry. Cham: Springer; 2017. p. 231–52. https://doi.org/10.1007/978-3-319-49674-0_8. PMid: 28101864.
[7] Vidmar B, Vodovnik M. Microbial keratinases: Enzymes with promising biotechnological applications. Food Technol Biotechnol 2018;56(3):312–28. https://doi.org/10.17113/ftb.56.03.18.5658. PMid: 30510475.
[8] Brandelli A, Daroit DJ, Riffel A. Biochemical features of microbial keratinases and their production and applications. Appl Microbiol Biotechnol 2010;85:1735–50. https://doi.org/10.1007/s00253-009-2398-5. PMid: 20039036.
[9] Sittipol D, Rodpan S, Ajingi YUS, et al. Identification, overexpression, purification, and biochemical characterization of a novel hyperthermostable keratinase from *Geoglobus acetivorans*. 3 Biotech 2021;11(1):2. https://doi.org/10.1007/s13205-020-02538-1. PMid: 33269186.
[10] Derhab N, Mabrouk ME, El-Metwally MM, et al. Thermostable keratinase from *Bacillus cereus* L10: Optimization and some potential biotechnological applications. Biomass Convers Biorefinery 2023;1–17. https://doi.org/10.1007/s13399-023-04887-2.
[11] Kokwe L, Nnolim NE, Ezeogu LI, et al. Thermoactive metallo-keratinase from *Bacillus* sp. NFH5: Characterization, structural elucidation, and potential application as detergent additive. Heliyon 2023;9(2):. https://doi.org/10.1016/j.heliyon.2023.e13635. PMid: 36852054e13635.
[12] Pei X-D, Li F, Yue S-Y, et al. Production and characterization of novel thermo- and organic solvent–stable keratinase and aminopeptidase from *Pseudomonas aeruginosa* 4–3 for effective poultry feather degradation. Environ Sci Pollut Res 2023;30(2):2480–93. https://doi.org/10.1007/s11356-022-22367-4. PMid: 35930154.
[13] Joshi S, Satyanarayana T. Biotechnology of cold-active proteases. Biology 2013;2(2):755–83. https://doi.org/10.3390/biology2020755. PMid: 24832807.
[14] Liu Y, Zhang N, Ma J, et al. Advances in cold-adapted enzymes derived from microorganisms. Front Microbiol 2023;14:. https://doi.org/10.3389/fmicb.2023.1152847. PMid: 371802321152847.
[15] Santiago M, Ramírez-Sarmiento CA, Zamora RA, et al. Discovery, molecular mechanisms, and industrial applications of cold-active enzymes. Front Microbiol 2016;7:1408. https://doi.org/10.3389/fmicb.2016.01408.
[16] Debroy A, George N. Psychrophiles and psychrozymes: Structural adaptations and applications. In: Singh S, Singh Chohan JS, Kumar R, editors. Conference Proceedings: Advancements in Civil Engineering COSMEC – 2021 Sept 23-24; Mohali, India. AIP Publishing. https://doi.org/10.1063/5.0120019.
[17] Moharram AM, Zohri A-NA, Hesham AE-L, et al. Production of cold-active pectinases by three novel *Cladosporium* species isolated from Egypt and application of the most active enzyme. Sci Rep 2022;12(1):15599. https://doi.org/10.1038/s41598-022-19807-z. PMid: 36114347.

[18] Kim H-D, Kim S-M, Choi J-I. Purification, characterization, and cloning of a cold-adapted protease from Antarctic *Janthinobacterium lividum*. J Microbiol Biotechnol 2018;28(3):448–53. https://doi.org/10.4014/jmb.1711.11006. PMid: 29212294.
[19] Ge J, Jiang X, Liu W, et al. Characterization, stability improvement, and bread baking applications of a novel cold-adapted glucose oxidase from *Cladosporium neopsychrotolerans* SL16. Food Chem 2020;310:. https://doi.org/10.1016/j.foodchem.2019.125970. PMid: 31838375125970.
[20] Bhatia RK, Ullah S, Hoque MZ, et al. Psychrophiles: A source of cold-adapted enzymes for energy efficient biotechnological industrial processes. J Environ Chem Eng 2021;9(1):. https://doi.org/10.1016/j.jece.2020.104607104607.
[21] Bruno S, Coppola D, di Prisco G, et al. Enzymes from marine polar regions and their biotechnological applications. Mar Drugs 2019;17(10):544. https://doi.org/10.3390/md17100544. PMid: 31547548.
[22] Sarmiento F, Peralta R, Blamey JM. Cold and hot extremozymes: Industrial relevance and current trends. Front Bioeng Biotechnol 2015;3:148. https://doi.org/10.3389/fbioe.2015.00148. PMid: 26539430.
[23] Lee C, Jang S-H, Chung H-S. Improving the stability of cold-adapted enzymes by immobilization. Catalysts 2017;7(4):112. https://doi.org/10.3390/catal7040112.
[24] Kuddus M. Cold-active enzymes in food biotechnology: An updated mini review. J Appl Biol Biotechnol 2018;6:58–63. https://doi.org/10.7324/JABB.2018.60310.
[25] Dhaulaniya AS, Balan B, Kumar M, et al. Cold survival strategies for bacteria, recent advancement and potential industrial applications. Arch Microbiol 2019;201:1–16. https://doi.org/10.1007/s00203-018-1602-3. PMid: 30478730.
[26] Yadav P, Singh RP, Rana S, et al. Mechanisms of stress tolerance in cyanobacteria under extreme conditions. Stresses 2022;2(4):531–49. https://doi.org/10.3390/stresses2040036.
[27] Smith D, Onions AH. The preservation and maintenance of living fungi: CAB International; 2nd ed. 1994; 132 p. ISBN: 978-0-85198-902-0. https://doi.org/10.1079/9780851989020.0000.
[28] Noman E, Al-Gheethi A, Rahman N, et al. Single spore isolation as a simple and efficient technique to obtain fungal pure culture. IOP Conference Series: Earth and Environmental Science; 4th International Conference on Civil and Environmental Engineering for Sustainability - IConCEES 2017 4–5 Dec; Langkawi, Malaysia. IOP Publishing 2018;140:012055. https://doi.org/10.1088/1755-1315/140/1/012055.
[29] Rios P, Bezus B, Cavalitto S, et al. Production and characterization of a new detergent-stable keratinase expressed by *Pedobacter* sp. 3.14.7, a novel Antarctic psychrotolerant keratin-degrading bacterium. J Genetic Eng Biotechnol 2022;20(1):81. https://doi.org/10.1186/s43141-022-00356-x. PMid: 35612674.
[30] Moubasher AH. Soil fungi in Qatar and other Arab countries. Econ Bot 1996;50:242. https://doi.org/10.1007/BF02861455.
[31] Samson RA, Visagie CM, Houbraken J, et al. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol 2014;78(1):141–73. https://doi.org/10.1016/j.simyco.2014.07.004. PMid: 25492982.
[32] Moubasher AH, Ismail MA, Al-Bedak OA, et al. *Ramophialophora chlamydospora*, a new species from an alkaline lake of Wadi-El-Natron. Egypt Asian J Mycol 2019;2(1):110–7. https://doi.org/10.5943/ajom/2/1/5.
[33] Al-Bedak OA, Moubasher AH. *Aspergillus gaarensis*, a new addition to section *Circumdati* from soil of Lake El-Gaar in Wadi-El-Natron. Egypt Stud Fungi 2020;5(1):59–65. https://doi.org/10.5943/sif/5/1/5.
[34] Al-Bedak OA, Moubasher AH, Ismail MA, et al. *Aspergillus curvatus*, a new species in section Circumdati isolated from an alkaline water of Lake Khadra in Wadi-El-Natron. Egypt Asian J Mycol 2020;3(1):325–34. https://doi.org/10.5943/ajom/3/1/7.
[35] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications 1990;18(1):315-22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
[36] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 2013;30(4):772–80. https://doi.org/10.1093/molbev/mst010. PMid: 23329690.
[37] Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): A new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evol Biol 2010;10(1):210. https://doi.org/10.1186/1471-2148-10-210. PMid: 20626897.
[38] Kumar S, Stecher G, Li M, et al. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018;35(6):1547–9. https://doi.org/10.1093/molbev/msy096. PMid: 29722887.
[39] Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 1985;39(4):783–91. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x. PMid: 28561359.
[40] Posada D, Crandall KA. MODELTEST: Testing the model of DNA substitution. Bioinformatics 1998;14(9):817–8. https://doi.org/10.1093/bioinformatics/14.9.817. PMid: 9918953.
[41] Ameen F. Purification and characterization of xylanase produced by *Aspergillus fumigatus* isolated from the northern border region of Saudi Arabia. Fermentation 2023;9(7):595. https://doi.org/10.3390/fermentation9070595.
[42] Ståhle L, Wold S. Analysis of variance (ANOVA). Chemom Intel Lab Syst 1989;6(4):259–72. https://doi.org/10.1016/0169-7439(89)80095-4.
[43] Paul T, Halder SK, Das A, et al. Exploitation of chicken feather waste as a plant growth promoting agent using keratinase producing novel isolate *Paenibacillus woosongensis* TKB2. Biocatal Agric Biotechnol 2013;2(1):50–7. https://doi.org/10.1016/j.bcab.2012.10.001.
[44] Sun Z, Li X, Liu K, et al. Optimization for production of a plant growth promoting agent from the degradation of chicken feather using keratinase producing novel isolate *Bacillus pumilus* JYL. Waste Biomass Valoriz 2021;12:1943–54. https://doi.org/10.1007/s12649-020-01138-7.
[45] Bhange K, Chaturvedi V, Bhatt R. Ameliorating effects of chicken feathers in plant growth promotion activity by a keratinolytic strain of *Bacillus subtilis* PF1. Bioresour Bioprocess 2016;3:13. https://doi.org/10.1186/s40643-016-0091-y.
[46] de Menezes CLA, Santos RdC, Santos MV, et al. Industrial sustainability of microbial keratinases: Production and potential applications. World J Microbiol Biotechnol 2021;37(5):86. https://doi.org/10.1007/s11274-021-03052-z.
[47] Kang D, Herschend J, Al-Soud WA, et al. Enrichment and characterization of an environmental microbial consortium displaying efficient keratinolytic activity. Bioresour Technol 2018;270:303–10. https://doi.org/10.1016/j.biortech.2018.09.006. PMid: 30236907.
[48] Kalaikumari SS, Vennila T, Monika V, et al. Bioutilization of poultry feather for keratinase production and its application in leather industry. J Clean Prod 2019;208:44–53. https://doi.org/10.1016/j.jclepro.2018.10.076.
[49] Kang D, Huang Y, Nesme J, et al. Metagenomic analysis of a keratin-degrading bacterial consortium provides insight into the keratinolytic mechanisms. Sci Total Environ 2021;761:. https://doi.org/10.1016/j.scitotenv.2020.143281. PMid: 33190895143281.
[50] Al-Maqtari QA, Al-Ansi W, Mahdi AA. Cold-active enzymes and their applications in industrial fields-A review. Int J Res Agric Sci 2019;6(4):2348–3997.
[51] Litova K, Gerginova M, Peneva N, et al. Growth of Antarctic fungal strains on phenol at low temperatures. J BioSci Biotechnol 2014(SE/ONLINE):43–6.
[52] Kuddus M, Roohi, Bano N, et al. Cold-active microbial enzymes and their biotechnological applications. Microb Biotechnol 2024;17(4). https://doi.org/10.1111/1751-7915.14467.
[53] Rawat M, Chauhan M, Pandey A. Extremophiles and their expanding biotechnological applications. Arch Microbiol 2024;206(6):247. https://doi.org/10.1007/s00203-024-03981-x. PMid: 38713374.
[54] Sarsan S, Rodhe AV, Roy KVV, et al. Biotechnological potential of cold-adaptive extremozymes. In: Singh RP, Manchanda G, Sarsan S, editors. Developments in Applied Microbiology and Biotechnology, Microbial Essentialism. Academic Press; 2024. p. 265–99. https://doi.org/10.1016/B978-0-443-13932-1.00021-0.
[55] Koutb M, Morsy FM, Bagy MMK, et al. Optimization of extracellular keratinase production by *Aspergillus terreus* isolated from chicken's litter. J Adv Lab Res Biol 2012;3(3):210–6.
[56] Mazotto AM, Couri S, Damaso MC, et al. Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. Int Biodeter Biodegr 2013;85:189–95. https://doi.org/10.1016/j.ibiod.2013.07.003.
[57] El-Ghonemy DH, Ali TH. Optimization of physico-chemical parameters for hyper keratinase production from a newly isolated *Aspergillus* sp. DHE7 using chicken feather as substrate-management of biowaste. J Appl Pharm Sci 2017;7(9):171–8. https://doi.org/10.7324/JAPS.2017.70923.
[58] Isaac GS, Abu-Tahon MA. Dehairing capability of alkaline keratinase produced by new isolated *Cochliobolus hawaiiensis* AUMC 8606 grown on chicken feather. Rom Biotechnol Lett 2016;22(6):12147–54.
[59] Menon S, Savur B, Kasat D, et al. Management and utilization of keratin waste–A review. Int J Adv Res Ideas Innov Technol 2020;6(3):511–5.
[60] Kačinová V, Kolčáková V, Petranová D. Axiocentric media education as a strategy for the cultivation of media recipients. Eur J Sci Theol 2014;10(1):103–16.
[61] Peng Z, Mao X, Zhang J, et al. Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis. Biotechnol Biofuels 2020;13(1):61. https://doi.org/10.1186/s13068-020-01700-4. PMid: 32266007.
[62] Hassan MA, Taha TH, Hamad GM, et al. Biochemical characterisation and application of keratinase from *Bacillus thuringiensis* MT1 to enable valorisation of hair wastes through biosynthesis of vitamin B-complex. Int J Biol Macromol 2020;153:561–72. https://doi.org/10.1016/j.ijbiomac.2020.03.032. PMid: 32151720.
[63] Li K, Li G, Liang Y, et al. Structural and enzymatic characterization of a novel metallo-serine keratinase KerJY-23. Int J Biol Macromol 2024;260:. https://doi.org/10.1016/j.ijbiomac.2024.129659. PMid: 38266845129659.
[64] Ramalingum N, Bhagwat P, Permaul K, et al. Production, characterization, and application of *Pseudomonas aeruginosa* S-04 keratinase for feather utilization. Biomass Convers Biorefin 2024;14(10):11683–95. https://doi.org/10.1007/s13399-022-03218-1.
[65] Farag AM, Hassan MA. Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae*. Enzyme Microb Technol 2004;34(2):85–93. https://doi.org/10.1016/j.enzmictec.2003.09.002.
[66] El-Ayouty YM, El-Said A, Salama A. Purification and characterization of a keratinase from the feather-degrading cultures of *Aspergillus flavipes*. Afr J Biotechnol 2012;11(9):2313–9. https://doi.org/10.5897/AJB11.686.
[67] Sahay S, Hamid B, Singh P, et al. Evaluation of pectinolytic activities for oenological uses from psychrotrophic yeasts. Lett Appl Microbiol 2013;57(2):115–21. https://doi.org/10.1111/lam.12081. PMid: 23574042.