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Production of Keratinase by Free and Immobilized Cells of *Bacillus halodurans* Strain PPKS-2: Partial Characterization and Its Application in Feather Degradation and Dehairing of the Goat Skin

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Abstract An extremely alkaliphilic bacterial strain, *Bacillus* sp. PPKS-2, was isolated from rice mill effluents and screened for the production of extracellular keratinase. The maximum production of keratinase occurred after 48 h in shaking culture at pH 11.0 and 37 °C in a medium containing 0.5% soybean flour. The strain grew and produced alkaline keratinase using chicken feather and horn meal as the sole source of carbon and nitrogen. An addition of 0.1% soybean flour or feather hydrolysate and sodium sulfite to feather medium increased the production and complete solubilization of feather took place within 5 days under solid-state fermentation conditions. The partially purified enzyme displayed maximum activity at pH 11.0 and 60 °C in a broad range of NaCl, 0–16%, and was not inhibited by sodium dodecyl sulfate (10%), ethylenediaminetetraacetic acid (10 mM), H_2O_2 (15%), and other commercial detergents. Immobilization of the whole cells proved to be useful for continuous production of keratinase and feather degradation. The enzyme was effectively used to remove hair from goat hide. The strain PPKS-2 can be effectively used for solid waste management of poultry feather in submerged as well as solid-state fermentation.

Keywords *Bacillus halodurans* PPKS-2 · Keratinase · Feather degradation · Dehairing · Immobilization · Solid-state fermentation

Introduction

Keratinases from microorganisms have attracted a great deal of attention in the recent decade, particularly due to their multitude of industrial applications such as in the feed, fertilizers, detergent, leather, and pharmaceutical industries [1]. Bacterial keratinases are of

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particular interest because of their action on insoluble keratin substrates, generally on a broad range of protein substrates [2]. Fibrous proteins such as horn, feather, nail, and hair are abundantly available in nature as waste, and these can be converted into useful biomass, protein concentrate, or amino acids using keratinases. Worldwide, millions of tons of feather waste is generated annually by poultry-processing industries and becomes a part of solid waste management. Until recent years, feathers were baked at high temperature and pressure and used as animal feed supplement in the form of feather meal. The hydrothermal treatment, in addition to being expensive, resulted in the destruction of certain essential amino acids, viz. methionine, lysine, and tryptophan, yielding a product with poor digestibility and variable nutrient quality [3]. Development of enzymatic and/or microbiological methods for the hydrolysis of feather to soluble proteins and amino acids is extremely attractive as it offers a cheap mild reaction condition for the production of valuable products [4–6]. There were some reports in the past on the keratinolytic enzyme-producing microorganisms capable of degrading keratin; some have reached commercial exploitation. Keratinases from *Bacillus* sp., particularly, *Bacillus licheniformis* and *Bacillus subtilis*, have been studied extensively because of their effectiveness in terms of feather degradation and are stable at high pH and temperature [1, 7–11].

Optimizations of cultural conditions are essential for successful production of an enzyme and feather degradation at industrial level. Evaluation of the cost-effective substrates on the yield of enzymes and the optimization of environmental and fermentation parameters such as pH, temperature, aeration, and agitation are critical factors in the production of commercial enzymes. Several reports are available on keratinolytic microorganisms and their biotechnological potential with respect to keratinases and feather meal production at pH 6.0–9.0 [1] but there were very few reports at above pH 11.0 [4, 12, 13]. Different strains of *Bacillus* sp. have been extensively used for the production of keratinases by solid-state fermentation (SSF; between pH 7.0 and 9.0) [1]. There were hardly any reports on keratinase production and feather degradation by *Bacillus* sp. under SSF conditions. SSF has several advantages over submerged fermentation as it is a simple, cost-effective, and environmental friendly process for the effective solid waste management of feather and other keratin substrates. Because of the growing needs of keratinase in various industrial applications, it is important to isolate novel microorganisms producing keratinases with improved biotechnological potential from unexplored habitats. In the present study, we have optimized the conditions for the maximum feather degradation and keratinase production by SSF and whole cell immobilization process. Further, we report some properties and potential industrial applications of keratinase produced by newly isolated alkaline *Bacillus halodurans* strain PPKS-2 at pH 11.0

Materials and Methods

Feather was collected immediately after slaughtering of the animal and repeatedly washed with distilled water until the effluent was very clear and dried. Elastin–Congo red, keratin, and azocoll were obtained from Sigma Chemicals, USA. All other reagents used were of analytical grade.

Isolation and Screening of the Organism

Strain PPKS-2 was isolated employing enrichment culture technique from rice mill effluents of Raichur City, Karnataka, India. Individual colonies showing clear zones of casein hydrolysis on the casein agar plate at pH 11.0 were picked and grown in liquid culture. The isolate producing maximum keratinase was identified as *B. halodurans* strain PPKS-2 by 16S ribosomal DNA

(rDNA) analysis as it has 98% similarity with *B. halodurans*, accession number AM 295056, and the sequence is deposited in the National Center for Biotechnology Information gene bank with accession number EU118675. The bacterium is deposited in the National Chemical Laboratory, Pune, India with accession number NCIM 5292.

Growth and Media Composition

The cells of strain PPKS-2 were aerobically cultured at 37 °C in a basal salt medium containing 0.5% peptone, 0.2% yeast extract, 1% glucose, 0.1% K_2HPO_4, 0.5% NaCl, and 0.02% $MgSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to pH 11.0 by adding separately autoclaved 1% Na_2CO_3.

Production of Enzyme

The strain PPKS-2 was grown in 250-ml Erlenmeyer flask containing 50 ml of the above fermentation medium in which peptone was substituted by 10 g/l of chicken feather with or without supplements. Flasks were inoculated with 1 ml of 24-h-old culture of *Bacillus* sp. PPKS-2 and incubated at 37 °C in a rotary shaker at 180 rpm for 48 h. SSF was carried out in 250-ml Erlenmeyer flasks containing 1 g of feather or human hair mixed with 10 ml of basal salt solution and 0.5 ml of inoculum added with or without 0.1% peptone or soybean flour or feather hydrolysate and sodium sulfite and fermented at 37 °C. The flask was removed at regular intervals; the contents were centrifuged and the supernatant was used as source of enzyme. Alternatively, feather in sample was harvested by filtration with Whatman number 3 filter paper, washed twice with distilled water, and dried at 65 °C to constant weight. The percentage of feather degradation was calculated from the difference in residual feather dry weight between a control (feather without bacterial inoculation) and treated sample.

Enzyme Assay

The caseinolytic activity of the enzyme was determined by using casein as a substrate as described earlier [14]. In brief, 2 ml of reaction mixture contained 1 ml of 1% casein (Hammerstein) and 0.5 ml of enzyme in the presence of 50 mM glycine–NaOH buffer of pH 11.0 and incubated for 15 min at 60°C. One unit of caseinolytic activity is defined as the amount of enzyme that liberates 1 μg of tyrosine per minute under assay condition. Keratinolytic activity was measured using 1% keratin or elastin–Congo red as a substrate in place of casein as in the above. One unit of keratinolytic activity is expressed in keratin units (KU), defined as an increase of 0.01 OD at 660 nm for keratin or 495 nm for elastin–Congo red in 1 h. Collagenase activity was measured by incubating the enzyme with 4 mg of azocoll in 1 ml of 50 mM glycine–NaOH buffer (pH 11.0) at 60 °C for 1 h with constant agitation using a rotary shaker at 250 rpm. The samples were centrifuged at 12,000×*g* for 10 min and the absorbance of the supernatant was measured at 520 nm [15]. One unit is defined as the amount of enzyme bringing about an increase in 0.1 absorbance per minute at 60 °C. Protein concentration was determined by the method of Lowry et al. [16] using bovine serum albumin as standard.

Effect of Temperature, pH, and NaCl Concentrations

The effect of temperature, pH, and NaCl concentrations on the growth and enzyme production was studied. This was carried out by cultivating the organism at different

temperatures (20–50 °C), different initial pH values using adequate buffers (4.0–6.0 acetate buffer, 7.0–8.0 Tris–HCl buffer, and 9.0–12.0 glycine–NaOH) at 50 mM concentration, and NaCl concentrations 0–20% (*w/v*). The growth was measured by measuring absorbance at 660 nm in a spectrophotometer (UV-6405, Jenway, UK). The enzyme activity and biomass were measured at optimum growth (48 h).

Effect of Carbon and Nitrogen Sources

The bacterial strain PPKS-2 was grown in the basal salt medium containing different carbon sources (1%) to study their effect on the production of keratinase. The various carbon sources tested were glucose, starch, glycerol, mannitol, and maltose. To determine the basic nutritional requirements of the strain, the cells were inoculated into different media designed by combination of components of the basal salt medium. The nitrogen sources tested were ammonium sulfate, tryptone, peptone, casein, and beef and yeast extract. Low-cost protein-rich flours, chicken feather, and horn meal were used as alternative carbon and nitrogen sources for enzyme production.

Immobilization of *Bacillus* sp. PPKS-2 for Keratinase Production

Strain PPKS-2 cells were immobilized in sodium alginate beads for the continuous production of keratinase similar to as per previous procedures [17, 18]. The mid-log phase cells of strain PPKS-2 (4 g wet weight) were added to 100 ml of 2% (*w/v*) sodium alginate and kept for 30 min. To this, glutaraldehyde (2.5% *w/v*) was added to give a final concentration of 0.1% (*w/v*), followed by gentle mixing and then kept for 90 min. Beads were formed by dropping this solution into 0.05 M $CaCl_2$. They were washed twice in 50 mM glycine–NaOH buffer of pH 9.5 (at pH 10.0 and above beads were unstable) and placed in the same buffer containing 1% peptone or feather hydrolysate (sterilized). The production of keratinase was monitored at different intervals in shaking conditions.

Hydrolysis of Raw Chicken Feather by Keratinase

Chicken feather (1 g) was added to 100-ml (550 KU/ml) enzyme secreted by immobilized cells with or without additives (listed in Table 4) and incubated at pH 11.0 and 45 °C under shaking conditions (200 rpm). The percentage of feather degradation was calculated.

Dehairing of Goat Skin

The cell-free crude enzyme (10 ml) was applied to a piece of goat skin (5×5 cm), presoaked in tap water from flesh side, and kept at room temperature in a dry place. Loosening of hair and epidermis was observed by mechanical means at hourly interval.

Partial Purification of Enzyme

Five hundred milliliters of cell-free keratinase secreted by immobilized cells was concentrated with a VIVA cell 250 (30 kDa) protein concentrator to 20 ml, which was extensively dialyzed against 50 mM glycine–NaOH buffer of pH 11.0. This partially purified enzyme was used as source for studying the effect of NaCl, pH, temperature, and stability.

Results

The cells of *Bacillus* sp. PPKS-2 were Gram positive and rod-shaped and had no pigmentation. The colonies were filamentous with elevation of opaque circle and size of 4 to 5 μm in diameter. They were spore-forming catalase and oxidase positive, and Tween 20, 60, and 80 were hydrolyzed. Indole production and methyl red and Voges–Proskauer tests are negative. In liquid medium, at pH 11.0, growth produced homogeneous turbidity. Optimum growth was obtained at pH 11.0 and 37 °C. It was able to hydrolyze gelatin, casein, keratin, starch, and utilized urea. Glucose and maltose fermented without gas production. Based on the above biochemical test and 16S rDNA analysis, the strain PPKS-2 was identified as *B. halodurans*.

Growth and Keratinase Production

Keratinase production was observed in the fermentation broth as soon as the bacterium entered the exponential phase (18 h) and reached maximum in the stationary phase (48 h) as shown in Fig. 1. The optimum cultural conditions for growth and protease production were up to 48 h of incubation which will remain more or less stable until 52 h and then decrease with increase in incubation time. This is because of the continuing depletion of nutrients and buildup of metabolic wastes results in death of the cells at a rapid and uniform rate.

Effect of Temperature, pH, and NaCl Concentrations on the Growth and Production of Alkaline Keratinase

Maximum growth and enzyme secretion were observed in the temperature range of 35–45 °C with optimum at 37 °C. No growth was observed at 20 °C, whereas low growth and enzyme secretion were observed at 50 °C. Highest growth and enzyme production were observed in alkaline pH (9.0–12.0) with an optimum at 11.0 (data not shown). There was no growth at pH 5, whereas low growth and enzyme secretion were noticed at pH 7.0–8.0. The *Bacillus* sp. strain PPKS-2 was able to grow up to 16% NaCl and produce the extracellular keratinase, active in a broad range of NaCl concentration (0–14%). This clearly indicates the halo-tolerant nature of the strain PPKS-2 (Fig. 2).

Effect of Carbon and Nitrogen Sources

Different carbon and nitrogen sources were employed in preliminary studies to determine the growth and production of extracellular alkaline keratinase after incubation for 48 h. The

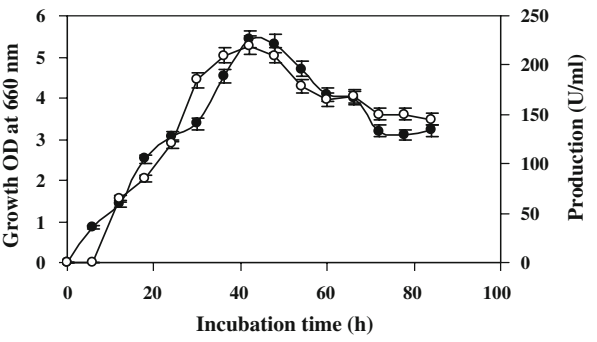

Fig. 1 The effect of incubation time on growth (*empty circles*) and alkaline keratinase production (*filled circles*) by *Bacillus* sp. PPKS-2 at pH 11.0 and temperature 37 °C in the presence of 1% casein under submerged fermentation conditions

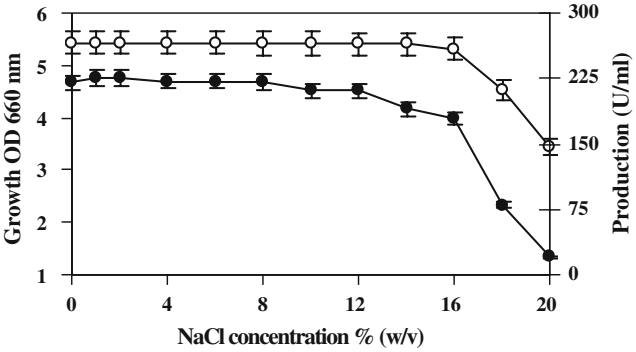

Fig. 2 The effect of NaCl on growth (*empty circles*) and alkaline keratinase production (*filled circles*) by *Bacillus* sp. PPKS-2 at pH 11.0 and temperature 37 °C in the presence of 1% casein under submerged fermentation conditions

strain PPKS-2 grew well in all the media, but the production of the enzyme was different in different media. Among the organic nitrogen sources used, casein, peptone, and skimmed milk powder had significant effect on the production of extracellular keratinase and the highest level of production was achieved when the cells were grown in a medium containing 0.5% gelatin (*w/v*; 692 U/ml) and the least in 1% urea (85 U/ml). Raw chicken feather, horn meal with 0.1% feather hydrolysate supplement, and other protein-rich flours proved to be the best low-cost alternative of carbon and nitrogen source for enzyme production (Fig. 3). Complete feather degradation under SSF conditions without any supplement took 8 days but the addition of 0.1% soybean flour or peptone or feather hydrolysate and sodium sulfite to the fermentation medium speeded up the process and reduced it to 5 days (Table 1).

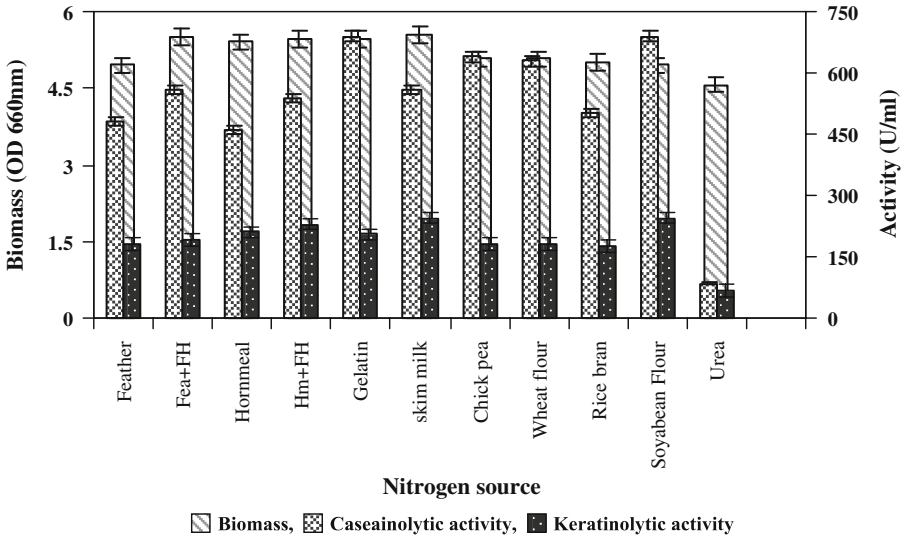

Fig. 3 Effect of different nitrogen sources (1% *w/v*) on the growth and production of alkaline keratinase by *Bacillus* sp. PPKS-2 at pH 11.0 and temperature 37 °C after 48 h

Table 1 Production of keratinase by *Bacillus* sp. PPKS-2 at pH 11 and 37 °C under SSF.

Keratin substrate (1 g)	Caseinolytic activity (U/ml)[a]	Keratinolytic activity (KU/ml)[a]	Time taken for 100% degradation of feather (in days)
Feather	525±2.0	386±2.9	8
Horn meal	480±2.8	362±5.2	ND
Human hair	530±1.7	396±4.5	7
Feather + 0.1% beef extract	535±2.3	368±4.9	5
Feather + 0.1% peptone	670±3.1	385±2.1	5
Feather + 0.1% soya	780±4.1	482±1.9	5
Feather + 0.1% sodium sulfite	485±5.1	350±2.6	5
Feather + 0.1%peptone + 0.1% sodium sulfite	730±4.5	435±3.7	5
Feather + 0.1% soya + 0.1% sodium sulfite	785±3.8	625±4.1	5
Feather + feather hydrolysate (1 ml)	830±3.5	720±4.2	5
Feather + feather hydrolysate (1 ml) + 0.1% sodium sulfite	860±3.8	780±3.7	4
Human hair + feather hydrolysate (1 ml) + 0.1% sodium sulfite	872±3.5	800±4.4	4

ND not detected

[a] Enzyme activity was determined after 4 days of incubation. Each value is an average of triplicate determination

Effect of Glucose Concentration on Growth and Production of Alkaliphilic Keratinase

Positive correlation of glucose 0–1% (*w/v*) was observed on growth (data not shown). The enzyme production increased from 40 (U/ml) to 225 (U/ml) with an increase in glucose concentration from 0% to 1% (*w/v*). However, the activity decreased beyond 1%, suggesting the threshold level of glucose for optimum keratinase production.

Effect of pH, Temperature, and NaCl Concentration on Partially Purified Enzyme

The keratinase was active in a broad range of pH 8.0–12.0 at an optimum of 11.0. The maximum keratinase activity was recorded between 45 and 60 °C, while it decreased rapidly above 65 °C. The enzyme is stable and active for more than 5 days at room temperature to 45 °C and retained 100% activity at 60 °C for 2 h. Further, the enzyme activity was reduced by 50% at 60 °C for 6 h. The enzyme was active over a broad range of NaCl (0–16%) by retaining 100% activity at 14% NaCl and 80% of activity at 16% NaCl under experimental conditions (data not shown). The keratinase was completely inhibited by 1 mM phenylmethylsulfonyl fluoride, a serine protease inhibitor, and not affected by sodium dodecyl sulfate (SDS), ethylenediaminetetraacetic acid (EDTA), and H_2O_2 (Table 2).

Immobilization

The immobilized cells started to secrete the enzyme after 24 h and reached maximum at 48 h. However, the enzyme production increased by more than 20% in immobilized state when compared with free cells. When the immobilized cells are supplemented with 0.1%

Table 2 Effect of different inhibitors on the alkaline keratinase of *Bacillus* sp. PPKS-2.

Inhibitors	Concentration	Remaining activity (%)[a]
None	–	100±1.2
1.10 phenanthroline	10 mM	95±1.6
Phenyl thiourea	10 mM	98±1.9
EDTA	10 mM	93±2.1
CTAB	10 mM	95±2.9
PMSF	1 mM	00±1.1
SDS	5 mM	100±2.3
SDS	10 mM	99±1.8
H_2O_2	5, 10, 15 (%)	98±1.9
Rin	1, 2 (%)	98±1.7
Surf excel	1, 2 (%)	98±2.2

[a] Each value is an average of triplicate determination. The enzyme was first incubated with the inhibitor for 1 h at room temperature (28±2 °C). Residual activity was measured following standard assay procedure

feather hydrolysate, enzyme production was increased by another 15%. No further increase in enzyme production was observed with longer duration. Whole-cell immobilized beads were stable and could be used for more than ten cycles with negligible cell leaching (Table 3).

Feather Hydrolysis by Cell-Free Keratinase

Feather degradation was studied by using keratinase secreted by immobilized cells at 45 °C as the enzyme was stable and active at 45 °C under shaking conditions. The cell-free enzyme hydrolyzed the feather or human hair more efficiently in presence of added additives. More than 80% grinded feather or 90% of human hair was hydrolyzed at pH 11.0 in 48 h when the media were supplemented with 0.1% sodium sulfite or dithiothreitol; SDS and Triton X-100, however, had no effect on feather degradation (Table 4). Elastin–Congo red was also hydrolyzed to 82% (data not shown). Keratinase did not hydrolyze azocoll, indicating the absence of collagenase activity.

Table 3 The production of alkaline keratinase by immobilized *Bacillus* sp. PPKS-2 supplemented with (a) 1% peptone and (b) feather hydrolysate at 37 °C and pH 9.5.

Incubation time (h)	Keratinase activity (KU/ml)	
	Peptone (1%)	Feather hydrolysate (1%)
12	00	00
24	195±2.8	105±4.2
36	205±3.1	290±3.5
48	380±4.2	550±3.7
60	510±3.3	500±3.8
72	470±3.1	480±2.9

[a] Enzyme activity was determined after 48 h of incubation. Each value is an average of triplicate determination

Table 4 Hydrolysis of feather and hair (1%) by keratinase secreted by immobilized cells supplemented with 1% feather hydrolysate at pH 11 and 45 °C.

Supplement (0.1%)	% of degradation at 48 h		
	Grinded feather	Raw feather	Human hair
None	60	50	70
Sodium sulfite	80	75	90
Dithiothreitol	82	78	90
β-mercaptoethanol	82	79	90
Glutathione	80	76	90
Cysteine	80	77	90
SDS	80	75	93
Triton X-100	82	76	92

Dehairing of Goat Skin

The cell-free crude enzyme dehaired the 5×5-cm goat hide in 16 h at pH 11.0 and at an ambient temperature of 28±2 °C (Fig. 4).

Discussion

The growth and keratinase production of *Bacillus* sp. PPKS-2 (Fig. 1) indicate that there is distinct growth associated with enzyme production. The bacterium can grow maximally between 35 and 45 °C, suggesting mesophilic properties. In the current work, biomass and enzyme production were found to be higher at 37 °C. Although keratinolytic bacteria often display optimal growth and activity at higher temperatures, this is consistent with optimum values described for keratinolytic *Bacillus* sp. FK46 [10], *Lysobacter* sp. [19], and *Stenotrophomonas* sp. D-1 [4], which showed optimum temperature for growth and

Fig. 4 Dehairing of goat skin by alkaline keratinase of PPKS-2. **a** Treated with keratinase; **b** treated with distilled water. Other experimental details were given in "Materials and Methods" section

keratinolytic enzyme production ranging from 20 to 37 °C. Maximum biomass and keratinase production were observed at pH 9.0–12.0 which agrees well with those described earlier [4, 12, 13].

Carbohydrate inhibition of keratinase production was observed (data not shown), indicating that this strain has a catabolite repression regulatory mechanism, a common control mechanism for biosynthesis of bacterial proteases [18]. The low-cost substrates were screened for the maximum production of keratinase. Some cost-effective substrates such as soybean meal have been successfully used [20]. The strain grew well and produced appreciable level of alkaline keratinase using feather as sole source of nitrogen. Microbial keratinase is predominately extracellular when grown on keratinous substrates; however, a few cells have been found and intracellular keratinases have also been reported [1, 21]. In most cases, keratin serves as the inducer; however, soymeal is also known to induce enzyme production [9, 22]. Keratinolysis takes place in two-step sulfitolysis or reduction in disulfide bonds and proteolysis [1]; sulfitolysis requires either the presences of live cells [23, 24] or reductants like sodium sulfite, dichlorodiphenyltrichloroethane, mercaptoethanol, glutathione, cysteine, and thioglycolate [25, 26] or disulfide reductase [26], which act in conjunction with keratinolytic proteases to bring about complete degradation of keratin. Keratinases have been produced under submerged shaking condition, except for a few thermophilic bacteria [21] where static submerged fermentation has been reported. De Azeredo et al. [27, 28] have successfully employed SSF using feather meal for the production of keratinase by *Streptomyces* sp. 594 at pH 10.0. The strain PPKS-2 reported here outstands as regard to pH, temperature, enzyme production, and feather degradation in short period by SSF compared with the reported organisms.

The bacterium is able to grow and produce appreciable levels of alkaline keratinase using raw feather or human hair as substrates and could offer tremendous potential for the development of biotechnological methods for the hydrolysis of feather or hair. Especially, the high level of keratinase production by strain PPKS-2 even in the absence of any supplement makes it extremely interesting. The potential application of the present study is that feather is a cheap and readily available substrate and can be used without any chemical/mechanical treatment for the production of keratinase. Biodegradation of feathers by microorganisms is one method for increasing their use as feed protein for example. As they are environmentally friendly, keratinolytic enzymes are used more and more often in the production of amino acids and peptides for biotechnological applications. With the help of these enzymes, feathers may be converted into specific products such as the rare amino acids serine, cysteine, and proline. Recently, feeding experiments with chickens and other animals demonstrated a significantly better growth response when the bacterial fermentation product feather lysate was added to the diet compared to when untreated feathers or commercial feather meal was added [1, 4, 6, 29]. This feature was also observed for other streptomycetes [30] as well as for other bacteria [27, 28, 31, 32].

Bacillus sp. strain PPKS-2 cells were immobilized in alginate beads for the continuous production of keratinase similar to that of *Chromohalobacter* sp. TVSP101 [18] and *Halobacterium salinarium* [17]. Continuous enzyme production by immobilized cells can be coupled with continuous feather degradation. In this process, enzyme is totally free from bacteria and can be used directly to treat the feather for every 48 h once. Maximum feather degradation (more than 80%) takes place at 48-h interval (Table 4). Part of the hydrolyzed feather can be used as supplement for immobilized cells and the process can operated continuously without any inputs for the enzyme production and feather degradation for more than ten cycles. This could result in a substantial reduction of time and cost for the production of enzyme and feather meal at the industrial level. Considering that feather

protein has shown to be an excellent source of nitrogen, the strain PPKS-2 could be used to produce animal feed protein.

The enzyme exhibited unusual stability in the presence of SDS, H_2O_2, EDTA, and commercial detergents. In general, detergent-compatible enzymes are alkaline thermostable in nature with a high pH optima because the pH of the laundry detergent is generally in the range of 9.0–11.0 and varying thermostability at laundry temperatures (50 to 60 °C) [33]. However, the protease from the subtilisin Carlsberg, used in commercial detergents, has a half-life of 2.5 min at 60 °C [34]. Besides pH and temperature stability, bleach stability is important because bleach-stable enzymes are not generally available except for a few reports [33, 35, 36]. Very few published reports are available on the compatibility of the alkaline proteases with detergents [1, 8]. Thus, the reported keratinase of *Bacillus* sp. PPKS-2 outstands with respect to pH, temperature, stability, detergent compatibility, and above all bleach stability for its future application in detergent formulation. Keratinases with high activity and stability in alkaline range and high temperature are interesting for biotechnological applications. Since the keratinase secreted by *Bacillus* sp. strain PPKS-2 was stable up to 45 °C for 5 days, inactivation of the enzyme during storage and transportation does not arise. Further, this enzyme does not require much sophistication for its storage and transportation, as they are the limiting factors in the industrial applications.

Another potential application of the keratinase is in the leather industry. In general, the dehairing process required elastolytic and keratinolytic activity, offering an effective treatment of leather, especially the dehairing and bating of skin and hides. The alkaline conditions enable the swelling of hair roots and subsequent attack of proteases on the hair follicle protein allows for easy removal of the hair. Despite the strong alkaline conditions, this process is feasible and safer than the traditional methods using sodium sulfide treatment, which contributes to 100% of sulfide and over 80% of the suspended solids in tannery effluents [8]. The bating following the dehairing process involves the degradation of elastin and keratin, removal of hair residues, and the deswelling of collagen which produces good soft leather. The lack of collagenase activity is of advantage in the leather industry because collagen, the major leather-forming protein, would not be significantly degraded. This criterion was satisfied by the keratinase of strain PPKS-2 and is thus suitable for dehairing. Dayanandan et al. [37] have reported an alkaline protease from *Aspergillus tamarri*, which dehaired the goat skin at pH 9.0–11.0 and 30–37 °C with 1% enzyme concentration and incubation period of 18–24 h. Similarly, an enzyme isolated from *Bacillus* sp. was used for dehairing of goat skin with 2–3% concentration which was active in pH 7.5–9.0 at 37 °C [38].

From these results, it is possible to conclude that the *B. halodurans* PPKS-2 can be a potential source of alkaline keratinase as it produced the enzyme using cost-effective medium. The keratinase produced by free and immobilized cells was active over a broad pH and NaCl and was thermostable. Its ability to hydrolyze keratinaceous substrates makes extremely attractive for the production soluble proteins that can be used as animal feed supplements. Further, this study also demonstrated the potential usefulness of keratinase in detergent and leather industry.

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