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SHORT COMMUNICATION

Chicken feathers: a complex substrate for the co-production of α-amylase and proteases by *B. licheniformis* NH1

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Abstract This study is concerned with the co-production of alkaline proteases and thermostable α-amylase by some feather-degrading *Bacillus* strains: *B. mojavensis* A21, *B. licheniformis* NH1, *B. subtilis* A26, *B. amyloliquefaciens* An6 and *B. pumilus* A1. All strains produced both enzymes, except *B. pumilus* A1, which did not exhibit amylolytic activity. The best enzyme co-production was obtained by the NH1 strain when chicken feathers were used as nitrogen and carbon sources in the fermentation medium. The higher co-production of both enzymes by *B. licheniformis* NH1 strain was achieved in the presence of 7.5 g/l chicken feathers and 1 g/l yeast extract. Strong catabolic repression on protease and α-amylase production was observed with glucose. Addition of 0.5% glucose to the feather medium suppressed enzyme production by *B. licheniformis* NH1. The growth of *B. licheniformis* NH1 using chicken feathers as nitrogen and carbon sources resulted in its complete degradation after 24 h of incubation at 37°C. However, maximum protease and amylase activities were attained after 30 h and 48 h, respectively. Proteolytic activity profiles of NH1 enzymatic preparation grown on chicken feather or casein-based medium are different. As far as we know, this is the first contribution towards the co-production of α-amylase and proteases using keratinous waste. Strain NH1 shows potential use for biotechnological processes involving keratin hydrolysis and industrial α-amylase and proteases co-production. Thus, the utilization of chicken feathers may result in a cost-effective process suitable for large-scale production.

Keywords *Bacillus licheniformis* NH1 · Thermostable α-amylase · Alkaline proteases · Co-production · Chicken feathers

Introduction

A million tons of chicken feathers from poultry-processing plants are produced as waste products annually throughout the world. Considering its high protein (90%) content, this waste could have great potential as a source of protein and amino acids for animal feed and for many other applications. However, feather wastes have been utilized on a limited basis as a dietary protein supplement for animal feedstuffs because of poor digestibility and because feather meal production is an expensive process. In fact, in its native state, keratin is not degradable by common proteolytic enzymes such as trypsin, pepsin and papain because of their high degree of cross-linking by disulphide bonds, hydrogen bonding and hydrophobic interactions [1].

Prior to being used, the feather is stream pressure cooked or chemically treated to make it more digestible. However, these treatment processes require significant energy and result in the loss of some essential amino acids [2].

Proteolysis of keratin by enzymatic and/or microbiological methods represents an alternative method for improvement of the nutritional value of feather waste and for avoidance of the destruction of certain amino acids [2]. Keratinolytic activity has been reported in many fungi and various bacteria, e.g., *Streptomyces* [3], *Actinomyces* [4], *Vibrio* [5] and *Bacillus* [6]. The most effective keratin-degrading strains in the *Bacillus* genus belong to

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the *B. licheniformis* [7] species. Keratinase may find potential applications in different fields where keratins should be hydrolysed, such as medicine, cosmetics, waste bioconversion, leather and detergent industries, but also for obtaining specific amino acids and peptides from proteins [8]. The bioconversion of keratinous residues is attracting increasing biotechnological interest since it might represent an alternative method of waste management that could be coupled with the production of valuable products [9]. These waste materials can be converted into economically useful feather meal, nitrogenous fertilizers, biodegradable films and glues [10]. Feathers may also find important applications in the fermentation industry for the production of commercial hydrolytic enzymes.

The most important industrial enzymes in use today belong to the hydrolase group, including proteases and amylases enzymes. These enzymes have most widely been reported to occur in microorganisms, although they are also found in plants and animals. The genus *Bacillus* is known to produce a wide range of hydrolytic enzymes. Among the genus *Bacillus*, *B. licheniformis* and *B. amyloliquefaciens* are the two species used most frequently in the commercial production of thermostable amylases and alkaline proteases. Amylolytic enzymes play an important role in the degradation of starch and represent about 25–33% of the world enzyme market [11]. These enzymes are of great significance in present day biotechnology and find applications in the food, baking, textile, paper and detergent industries. Proteolytic enzymes account for nearly 60% of the industrial enzyme market and are used in the industry for biotechnological applications involving the hydrolysis of protein substrates. The use of alkaline proteases has increased significantly in various industrial processes, such as detergent, feed additives, dehairing, decomposition of gelatin on X-ray films and peptide synthesis [12].

Several studies have been undertaken to define ideal culturing and nutritional conditions for obtaining higher yields of α-amylase and/or proteases. Complex fermentation media, which give optimum production of α-amylases or proteases, have been reported. Considering the wide range of applicability of amylases and proteases, the development of a low-cost fermentation medium is necessary. To date, a limited number of studies have been reported on the isolation of feather-degrading microorganisms with potential application in the fermentation industry for the production of commercial enzymes. Keratinolytic substrates were tested only for the production of proteolytic enzymes. No studies have been reported on the use of chicken feathers as a growth substrate for amylase production.

In this paper, we report the co-production of a thermostable α-amylase and alkaline proteases by a *B. licheniformis* NH1 strain grown on chicken feathers as carbon and nitrogen sources.

In a previous work, El Hadj-Ali et al. [13] reported the production of an extracellular alkaline serine protease from *B. licheniformis* NH1 and its potential employment as a detergent additive. In this paper, we report the co-production of a thermostable α-amylase and alkaline protease by *B. licheniformis* NH1 grown on chicken feather-based medium. To the best of our knowledge, this is the first report on α-amylase and protease co-production using feathers as a fermentation substrate.

Materials and methods

Microorganisms

All the strains used in this report were isolated in our laboratory. *B. licheniformis* NH1 is an alkaliphilic bacterium, which was isolated from an activated sludge reactor treating fishery wastewater [13]. *B. mojavensis* A21 was isolated from marine water in Sfax city, Tunisia [14], and *Bacillus subtilis* A26 was isolated from marine water in Sfax city, Tunisia [15]. *B. amyloliquefaciens* An6 was isolated from the soil near the detergent industry [16]. *B. pumilus* A1 was isolated from polluted water from the local slaughter house [17].

Media and culture conditions

Inoculum was routinely grown in Luria-Bertani (LB) broth medium composed of (g/l): peptone, 10; yeast extract, 5; NaCl, 5.

The initial medium used for α-amylase and protease co-production had the following composition (g/l): chicken feathers 10, K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1 and NaCl 0.5; the pH was adjusted to 7 prior to sterilization. Media were autoclaved at 120°C for 20 min.

Cultivations were conducted in 25 ml of medium in 250-ml conical flasks maintained at 37°C. Incubation was carried out with agitation at 200 rpm for 24 and 48 h. The cultures were centrifuged at 12,000 rpm for 15 min, and cell-free supernatant was used for estimation of α-amylase and protease activities.

Substrate preparation

Chicken feathers were supplied by a local poultry processing factory in Sfax, Tunisia. It was washed with tap water and finally with distilled water until the effluent was very clear, then dried at 60°C overnight. After drying, feathers were manually cut into small pieces.

Enzyme assay

Protease activity

Protease activity was measured by the method of Kembhavi et al. [18] using casein as a substrate. A 0.5-ml aliquot of the culture supernatant, suitably diluted, was mixed with 0.5 ml of 100 mM glycine-NaOH (pH 10.0) containing 1% casein, and incubated under optimal pH and temperature. The reaction was stopped by addition of 0.5 ml trichloroacetic acid (20%, w/v). The mixture was allowed to stand at room temperature for 15 min and then centrifuged at 13,000 rpm for 15 min to remove the precipitate. The acid-soluble material was estimated spectrophotometrically at 280 nm. A standard curve was generated using solutions of 0–50 mg/l tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μg of tyrosine in 1 min under the experimental conditions used.

α-Amylase activity

α-Amylase activity was estimated by determination of the reducing sugars liberated from starch hydrolysis, determinated by the dinitrosalicylic method [19]. One unit (U) of enzyme activity was defined as the amount of enzyme releasing 1 μmol of reducing end groups per minute in 0.1 M acetate buffer (pH 6.5) with 1% (w/v) potato starch (Sigma) as substrate at optimal temperature. D-Glucose was used as standard.

Detection of enzyme activity by zymography

Protease activity staining was performed on sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) electrophoresis after gel running, and the sample was not heated. After electrophoresis, the gel was submerged in 100 mM glycine-NaOH buffer (pH 10.0) containing 2.5% Triton X-100, with shaking for 30 min to remove SDS. Triton X-100 was removed by washing the gel three times with 100 mM glycine-NaOH buffer (pH 10.0). The gel was then incubated with 1% (w/v) casein in 100 mM glycine-NaOH buffer (pH 10.0) for 40 min at 50°C. Finally, the gel was stained with 0.25% Coomassie Brilliant Blue R-250 in 45% ethanol-10% acetic acid and destained with 5% ethanol-7.5% acetic acid. The development of clear zones on the blue background of the gel indicated the presence of protease activity.

Determination of feather degradation percentage

The percentage of feather degradation was measured using the weight loss method. The dry mass of feathers was obtained by washing the residue twice with distilled water, and the residue was dried at 105°C until constant weight. Feather degradation percentage was determined by subtracting the weight of feathers before the inoculation from the weight of the residue after degradation.

Determination of biomass

Bacterial growth was determined by the plate count method on nutrient agar.

Methods of analysis

All data presented are the average of at least two measurements that deviated by not more than 5%.

Results and discussion

Co-production of α-amylase and protease by some *Bacillus* strains

The effect of chicken feathers, as the sole nitrogen and carbon source, on the co-production of α-amylase and proteases by some *Bacillus* strains was first investigated in medium containing 10 g/l chicken feathers as the sole nitrogen and carbon source (Table 1).

All tested strains grew well and degraded chicken feathers (Fig. 1). Chicken feather degradation percentages were 80, 88, 70, 100 and 97, respectively, for the strains *B. mojavensis* A21, *B. subtilis* A26, *B. amyloliquefaciens* An6, *B. licheniformis* NH1 and *B. pumilus* A1. Chicken feather degradation suggests the presence of the keratin-degrading enzyme(s) or keratinase(s) secreted in extracellular medium. In fact, keratin is not degradable in its native state by common proteolytic enzymes such as trypsin, pepsin and papain because of their high degree of cross-linking by disulphide bonds, hydrogen bonding and hydrophobic interactions [1]. Feather degrading microorganisms are almost ubiquitous in nature, although preferentially thriving on keratinous substrates, and are known to have the capacity to solubilize keratinous substrates. Keratinolytic activity has been reported for different genera, especially *Streptomyces* [4], *Bacillus* [7, 9], *Chryseobacterium*, *Flavobacterium* and some other microorganisms.

The best feather degradation was achieved by NH1 strain, and complete feather degradation was observed after 24 h of cultivation. The degradation of whole chicken feather by *B. megaterium* F7-1 was observed after incubation at 30°C for 2 days, and most of the feather was degraded after 4 days except the feather shaft, which was completely degraded after 7 days of cultivation [20]. Williams et al. [7] demonstrated that *B. licheniformis*

Table 1 Effect of chicken feathers on the co-production of α-amylase and protease by some *Bacillus* strains

	α-Amylase activity (U/ml)	Protease activity (U/ml)	Biomass (CFU/ml)	Final pH value	Feather degradation (%)
B. subtilis A26					
24 h	1.31	200	26×10^7	–	–
48 h	2.62	221	88×10^7	8.79	88
B. mojavensis A21					
24 h	2.3	258	43×10^6	–	–
48 h	1.17	295	30×10^6	8.65	80
B. pumilus A1					
24 h	0	186	35×10^8	–	–
48 h	0	196	38×10^8	8.7	97
B. licheniformis NH1					
24 h	3.68	2,270	6×10^7	–	–
48 h	6.04	2,840	31×10^7	8.91	100
B. amyloliquefaciens An6					
24 h	1.23	171	149×10^7	–	–
48 h	2.16	349	202×10^7	8.76	70

Cultivations were performed for 24 and 48 h at 37°C in media consisting of (g/l): chicken feathers 10, K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1, NaCl 0.5

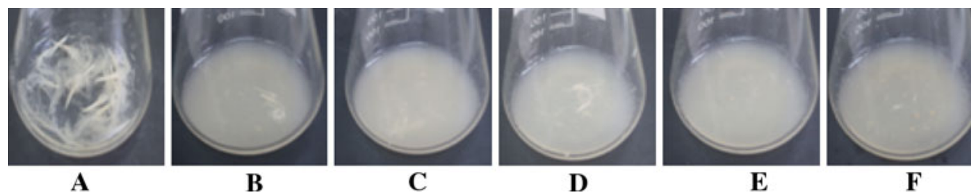

Fig. 1 Raw chicken feather degradation by some *Bacillus* strains. Cultivation was performed for 48 h at 37°C in media consisting of (g/l): raw chicken feathers 10, K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1 and NaCl 0.5. **a** Control (*uninoculated medium*); cultivation with strain *B. mojavensis* A21 (**b**), *B. subtilis* A26 (**c**), *B. amyloliquefaciens* An6 (**d**), *B. licheniformis* NH1 (**e**), *B. pumilus* A1 (**f**)

PWD-1 degraded chicken feather completely at 50°C in 6 days.

All tested strains, excepted *B. pumilus* A1 strain, exhibited protease and α-amylase production when chicken feathers was used as sole carbon and nitrogen source. However, higher α-amylase and protease activities were achieved with *B. licheniformis* NH1 strain. In contrast to the reports of other researchers suggesting that starch induces α-amylase activity, biosynthesis of α-amylase by *B. licheniformis* NH1 studied in this work appeared to be independent of starch availability. The NH1 strain produces a thermostable bleach-stable alkaline protease [13] and was also found to produce a thermostable α-amylase, which is highly active at higher temperatures, with an optimum at 90°C and active over a wide range of pH [21].

The carbon sources such as glucose and maltose have been utilized for the production of α-amylase; however, the use of starch remains promising and ubiquitous. A number of other substrates such as lactose [22], casitone [23], fructose [24] and oilseed cakes [25] have been described for production of α-amylase by *Bacillus* species. The flours of different grains such as wheat, barley and rice have also been used in the fermentation medium to increase the productivity of α-amylase [26, 27].

The induction of α-amylase activity in medium containing chicken feathers as the carbon source may be due to the effect of inducible peptides or free amino acids resulting from feather degradation by extracellular proteases produced by the strain. Amino acids in conjunction with vitamins were reported to affect α-amylase production. The production of α-amylase by *B. amyloliquefaciens* ATCC 23,350 increased by a factor of 300 in the presence of glycine [28].

Hence, an interesting potential application of the *B. licheniformis* NH1 strain is that chicken feathers, a cheap and readily available substrate, can be used for the production of alkaline proteases and thermostable α-amylase at the industrial level. In the production of industrial enzymes, up to 30–40% of the production cost is for the growth substrate [29]. Therefore, the use of chicken feathers could result in a substantial reduction in the cost of enzyme production.

Effect of chicken feather concentration and different nitrogen sources on α-amylase and protease co-production by *B. licheniformis* NH1

The effect of different chicken feather concentrations on alkaline proteases and thermostable α-amylase co-production

Table 2 Effect of chicken feather concentration on the co-production of α-amylase and protease by *B. licheniformis* NH1

Chicken feather concentration (g/l)	Final pH value	Biomass (CFU/ml) × 10^7	Protease activity (U/ml)	α-Amylase activity (U/ml)
0	7.95	3	252	0.62
2.5	8.46	14	2,461	2.69
5	8.72	16	2,998	5.97
7.5	8.86	21	3,156	6.57
10 (control)	8.96	33	2,870	6.04
15	9.06	50	2,808	5.45

Cultivations were performed for 48 h at 37°C in media consisting of (g/l): K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1, NaCl 0.5 and different concentrations of chicken feathers

Table 3 Effect of different nitrogen sources supplemented to the chicken feathers on the co-production of α-amylase and protease by *B. licheniformis* NH1

Nitrogen sources	Final pH value	Biomass (CFU/ml) × 10^7	Protease activity (U/ml)	α-Amylase activity (U/ml)
None (control)	8.86	21	3,156	6.57
$(NH_4)_2SO_4$	8.94	10	2,632	5.98
Casein	8.83	18	3,152	6.12
Yeast extract	8.79	31	3,960	9.52
NH_4Cl	8.84	5	2,923	6.00
Pastone	8.72	17	3,294	6.46

Cultivations were performed for 48 h at 37°C in media consisting of (g/l): chicken feathers 7.5, K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1, NaCl 0.5 and different nitrogen sources at 1 g/l concentration

by *B. licheniformis* NH1 was tested. As shown in Table 2, the co-production of both enzymes was mainly favored in the presence of chicken feather concentrations ranging from 2.5 to 15 g/l, while maximum enzyme activity was achieved only when 7.5 g/l chicken feathers was added to the fermentation medium. It was also noted that bacterial growth increased with the increase of chicken feather concentration.

In microorganisms, nitrogen (both organic and inorganic forms) is metabolized to produce primarily amino acids, nucleic acids, proteins and cell wall components. Alkaline protease production heavily depends on the availability of both carbon and nitrogen sources in the medium and has a regulatory effect on the enzyme synthesis. *B. licheniformis* NH1 was grown in liquid medium in the presence of various nitrogen sources supplemented to chicken feathers. Pastone, casein, yeast extract, ammonium sulphate and ammonium chlorure were screened as additive nitrogen sources. Protease and α-amylase activities were assayed after 48 h incubation. In these experiments, chicken feathers were taken at a constant level of 7.5 g/l.

According to the results shown in Table 3, the higher enzyme co-production of α-amylase and protease was obtained with yeast extract. However, none of the investigated nitrogen sources exerted a favorable effect on both enzymes' production. Yeast extract supplementation resulted in maximum growth, which was higher than the medium without nitrogen source supplementation. Maximum protease and α-amylase co-production was observed in culture supplemented with yeast extract, which showed growth-associated enzyme production.

Organic nitrogen sources have been preferred for the production of α-amylase, such as yeast extract [30], beef extract [23] and peptone [25]. The amylase synthesis by several microorganisms has been correlated to the presence or absence of various amino acids and complex nitrogenous sources in the culture medium [31]. On the contrary, Mai et al. [32] observed stimulation of amylase activity by ammonium salts. Yeast extract was the best nitrogen source, followed by casein, while beef extract/peptone was a poor organic nitrogen source for protease production by *Bacillus* sp. JB-99 [33].

The positive effect on growth exhibited by yeast extract in this study may be because it contains other nutrients, such as vitamins, carbohydrates and growth cofactor sources, which supported the growth of NH1 strain.

To investigate the best yeast extract concentration on the protease and α-amylase co-production, yeast extract was added to basal medium containing chicken feather (0.75%, w/v) at a concentration ranging from 0.1 to 0.5% (w/v). An optimal protease and α-amylase co-production was observed at a concentration of yeast extract of 0.1% (w/v).

Table 4 Effect of addition of glucose at different concentrations on the co-production of α-amylase and protease by *B. licheniformis* NH1

Glucose concentration (g/l)	Final pH value	Biomass (CFU/ml)	Protease activity (U/ml)	α-Amylase activity (U/ml)
0 (control)	9.07	32×10^7	3,774	9.72
1	8.90	45×10^7	3,727	9.86
2.5	8.29	35×10^5	801	0.60
5	5.00	50×10^4	0	0
7.5	4.71	31×10^4	0	0

Cultivations were performed for 48 h in media consisting of (g/l): chicken feathers 7.5, yeast extract 1, K_2HPO_4 1.4, KH_2PO_4 0.7, $MgSO_4$ 0.1 and NaCl 0.5 in the presence of different glucose concentrations ranging from 0 to 7.5 g/l

It should be noted that a higher concentration of yeast extract resulted in a slight reduction in the production of both enzymes.

Effect of initial pH values of fermentation medium on α-amylase and protease co-production by *B. licheniformis* NH1

Bacillus licheniformis NH1 was grown in the basal medium containing chicken feathers at 7.5 g/l, and the pH values of culture media were adjusted to 6.0–12.0 with diluted HCl or NaOH solution prior to sterilization. The effect of the initial pH of culture media on the co-production of α-amylase and protease by *B. licheniformis* NH1 was studied.

The highest α-amylase and protease co-production was obtained with a starting pH of 7.0. The maximum production of α-amylase was achieved when the initial pH of the fermentation was adjusted between 7.0 and 8.0. As the pH of the medium was increased or decreased to 7.0 or 8.0, there was gradual reduction in α-amylase formation. The final pH of the media after 48 h of cultivation was near pH 9. These results agree with previous reports, which indicated that the feather-degrading enzyme produced by *Bacillus* species could be classified as an alkaline protease and was most active under neutral to basic conditions [34]. The increase in pH during cultivation is an important characteristic accompanying keratin hydrolysis and the keratinolytic potential of microorganisms. Organisms with higher keratinolytic activity turn the media more alkaline in comparison with other organisms exhibiting lower keratinolytic activity [35]. This observation is based on the fact that keratin degradation involves deamination reactions, which result in an increase in pH.

Repression study of α-amylase and protease co-production with glucose

The observed co-production of α-amylase and protease on chicken feather medium by *B. licheniformis* NH1 strain may be due to the absence of catabolite repression caused by starch hydrolysis products such as glucose. Indeed, it has been reported that the synthesis of carbohydrate-degrading enzymes in most species of *Bacillus* is subject to catabolite repression by readily metabolizable substrates, such as glucose. In order to investigate further, the repressive effect of glucose on α-amylase and protease co-production from *B. licheniformis* NH1, a different glucose concentration was added to the basal medium containing 7.5 g/l chicken feathers and 1 g/l yeast extract. Carbohydrate inhibition of protease and α-amylase production was observed, indicating that this strain has a catabolic repression regulatory mechanism. As shown in Table 4, if glucose is present in high concentrations of more than 2.5 g/l, productions of α-amylase and protease are repressed, even if chicken feathers are present in the growth medium.

Similarly, it has been reported that synthesis of bacterial proteases and carbohydrate-degrading enzyme leads to catabolic repression by readily metabolizable substrates such as glucose and fructose.

Growth of the NH1 strain was slightly increased up to 0.1% (w/v) glucose and repressed in the presence of high glucose concentrations of more than 2.5 g/l. A similar relationship is reported in the case of alkaline protease production by an alkaliphilic *B. peudofirmus* [36], where glucose repressed both growth and protease production.

Pattern of α-amylase and proteases co-production by *B. licheniformis* NH1

The pattern of α-amylase and protease co-production, as well as bacterial growth, was followed with time (Fig. 2). The growth of *B. licheniformis* NH1 using chicken feathers as a nitrogen and carbon source resulted in its complete degradation after 24 h of incubation at 37°C. However, maximum enzyme productivities were attained after 30 h incubation for protease activity and 48 h for α-amylase activity. The stationary growth phase was reached after 48 h, coinciding with maximum α-amylase activity.

As shown in Fig. 2, the maximum of proteolytic activity was about 4,000 U/ml after 30 h of cultivation. Maximum protease activity was observed in the logarithmic phase and

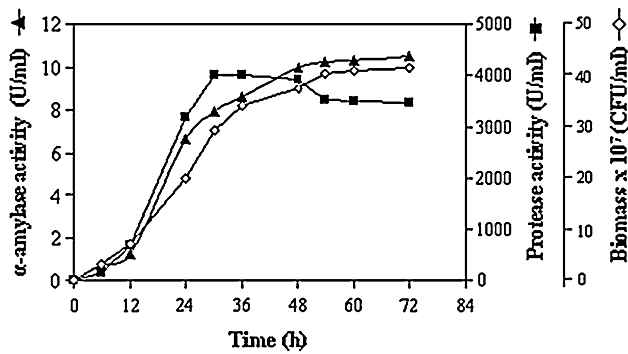

Fig. 2 Time course of alkaline proteases and thermostable α-amylase co-production and growth of *B. licheniformis* NH1. Shaking cultivation was carried out at 37°C. Protease and α-amylase activities were determined in culture filtrate obtained after removal of cells by centrifugation, as described in "Materials and methods" section

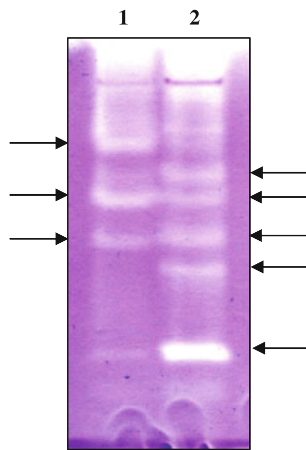

Fig. 3 Proteolytic activity staining on SDS-PAGE (12%) of the culture supernatant of *B. licheniformis* NH1 grown on casein (*lane 1*) and chicken feather (*lane 2*) based medium

was decreased slightly at the beginning of the stationary phase. Lin et al. [37] reported that *B. licheniformis* PDW-1 secreted proteolytic enzyme in the late exponential growth phase. Sangali and Brandelli [5] also reported that proteolytic activity increased with cultivation time and observed a maximum activity at the beginning of the stationary phase.

To give more information about the diversity of extracellular proteases secreted by *B. lichenifomis* NH1, zymogram analysis was done as described in the "Material and methods" section. Many enzymes, such as proteases and α-amylases, have proven to be renaturable after electrophoresis in the presence of SDS. Proteases of *B. licheniformis* NH1 were renatured by the removal of SDS after polyacrylamide gel electrophoresis, and proteolytic activity was visualised as a clear zone. As shown in Fig. 3, proteolytic activity profiles of cell-free enzymatic preparation of *B. licheniformis* NH1 grown on chicken feather- or casein-based medium are different. Interestingly, five major proteases were observed when the strain was cultivated in feather-based medium; however, only three major clear zones were observed in casein-based medium.

The fermentation broth of keratinolytic *Bacillus* strains contains not only specific keratinases, but frequently also trypsin- and chymotrypsin-like serine proteases and subtilisin-like proteases [38]. The role of the non-keratin-specific proteases includes participation in the degradation of oligopeptides produced by keratinases from keratin and promotion of the utilization of other proteins present in the habitats of the bacteria.

A detailed knowledge of the proteolytic enzymes' properties and sequences will be important for further studies. Isolation of each protease type for N-terminal sequencing could give insight as to whether they are products of different genes, aggregates or products of proteolytic degradation.

Conclusion

The production of enzymes in synthetic growth media often promotes exuberant growth and high enzyme yields, but their expensive cost makes them unsuitable for a large-scale production.

Based on the present study, it appears that chicken feathers, by-products of poultry processing, can serve as basal and standardized medium for obtaining high yields of α-amylase and proteases from *Bacillus* strains. Chicken feathers are very inexpensive, readily available, and a naturel substrate, and could be used as nitrogen and carbon sources for growth of microorganisms and production of commercial enzymes such as proteases and α-amylase. The study of co-production of α-amylase and protease by *B. licheniformis* NH1 shows that chicken feathers could replace the commercial and more expensive substrates. Thus, the utilization of such a substrate could result in a cost-effective process suitable for large-scale production. The absence of carbohydrates in chicken feathers could also be beneficial by avoiding catabolite repression, which is often observed in extracellular protease or α-amylase production.

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