Production of feather hydrolysate by *Elizabethkingia meningoseptica* KB042 (MTCC 8360) in submerged fermentation

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Abstract A keratinolytic bacterium *Elizabethkingia meningoseptica* KB042 was isolated from dropped off feathers. The bacterium showed $82.50 \pm 0.3\%$ feather degradation when grown on medium containing 10 g/l chicken feathers with initial pH 7.0 at 37°C, 150 rpm in 6 days. The pH of the medium was increased up to 10.02 ± 0.10 during 6 days of incubation. Soluble protein and amino acids concentration in the culture fluid was also found increased until the end of incubation. During the cultivation of strain KB042 on feather as sole source of carbon and nitrogen, the maximum cysteine release was noted on the 3rd day. Varying feather concentration 1.0–2.0% in basal medium resulted in soluble protein release between 1814.42 and 1954.61 µg/ml. The amino acid concentration was found to be maximum, i.e. 937.85 ± 11.9 µg/ml in the cultures grown with 2% feather. The hydrolysate was also found rich in essential amino acids valine, tryptophan, threonine, leucine and cysteine and contains minor amount of methionine and arginine. These data indicate a potential biotechnology for biotransformation and utilization of feather keratin as a source of protein which can be used as animal feed after successful animal trials.

Keywords Poultry waste · Feather · *Elizabethkingia meningoseptica* KB042 · Soluble protein · Amino acids

Introduction

Feathers are composed of 91% keratin protein, 8% water and 1% lipid. Keratin belongs to the class of sulfur containing fibrous protein that serves as structural unit for various living tissue. The close conformational packing in α -helical or β -pleated sheets, presence of hydrophobic amino acid residues and disulfide linkages make them resistant to degradation by common proteases [1]. In mature chicken, feather accounts up to 5–7% of the live weight. The poultry industries are generating large amounts of feathers as waste. The localized accumulation of feather waste around poultry processing sites creates a serious disposal problem leading to environmental pollution. Insoluble feather keratin can be converted to feedstuffs, fertilizers, glues and films or used for the production of the enzymes [2–5]. Currently, feathers are converted to feather meal through chemical processes that requires high energy input and results in loss of essential amino acids [6–8]. Microbiological methods for hydrolysis of feather to soluble protein and amino acids represents an environment friendly approach which offers a cheap, and mild reaction condition that prevent loss of valuable amino acids and thus results in nutritionally upgraded feather meal.

Keratinolytic activity has been reported in many actinomycetes [9–11], bacteria [12–15], dermatophytes and saprophytic fungi [16–20] which secrete keratinolytic enzymes. A keratinolytic bacterium *Elizabethkingia meningoseptica* KB042 was isolated from dropped off feathers. This strain completely degrade feather at room temperature showing its potential application in processing of poultry wastes. In this report the production of feather hydrolysate by this bacterium has been described.

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Materials and methods

Substrate

Feathers were obtained from local poultry market (Sagar, India). Raw feathers were washed with mild detergent and tap water several times before washing with sterile distilled water and dried at 45°C for 6 h in hot air oven. After drying feathers were cut into small pieces of 2–3 cm.

Inoculum preparation

The bacterium used for production of feather hydrolysate was *Elizabethkingia meningoseptica* KB042 (MTCC 8360). One milliliter of 24-h old bacterial culture, grown on nutrient broth at 37°C and 150 rpm was used as an inoculum (2% v/v).

Growth conditions

The experiment was carried out in 150 ml Erlenmeyer flask containing 50 ml of basal medium (K_2HPO_4 0.4 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, NaCl 0.05 g and $FeCl_3$ 0.01 g/l distilled water, pH 7.0) with 0.5 g raw feather. The cultures were incubated at 37°C at 150 rpm for 6 days for the study of feather degradation during different incubation periods.

To test the effect of substrate concentration the concentration of raw feather in cultivation medium was varied from 0.5 to 2.0% (w/v) and a set of uninoculated flask in each case were kept as control. To test the effect of initial pH on feather degradation the bacterium was grown on feather containing basal medium (1% w/v) and pH value of media was adjusted to 6–10 with dilute HCl or NaOH solution prior to sterilization.

Analysis of cultivation medium

The content of test flasks (residual feather along with bacterial biomass) was harvested by filtration with Whatman No. 1 filter paper, washed with distilled water and dried at 65°C to constant weight. The percentage of feather degradation was calculated from the difference in dry weight of residual feather between control (feather without bacterial inoculation) and treated samples.

The culture filtrate was analyzed for concentration of soluble protein by Folin phenol method [21]. Concentration of amino acid was determined by ninhydrin method [22]. For determination of the concentration of cysteine residue method of savielle was followed [23].

Amino acids in feather hydrolysate were detected by thin layer chromatography using N-butanol:acetic acid:water (4:1:1) as solvent system and 0.2% ninhydrin in ethanol as spraying reagent [24].

Results and discussion

A gram-negative non-endospore forming rod shaped bacterium was isolated from partially decomposed feathers. Strain KB042 was identified as *E. meningoseptica* KB042 (MTCC 8360) on the basis of cell morphology, colony morphology, physiological and several biochemical tests. The results of taxonomical studies on the isolated strain KB042 are summarized in Table 1. The isolate showed higher level of hydrolysis than previously described keratinolytic bacteria that have optimum growth and feather degradation at mesophilic [25].

Elizabethkingia meningoseptica KB042 showed $82.50 \pm 0.3\%$ feather degradation with increase of pH from 7.0 to 10.02 ± 0.10 in 6 days of incubation. The release of soluble protein and amino acids was monitored and their concentration increased till the end of incubation period. The final concentration of soluble protein and amino acids reached the value $1346.11 \pm 3.8 \mu\text{g/ml}$ and $583.98 \pm 3.7 \mu\text{g/ml}$, respectively (Table 2).

The maximum concentration 29.54 $\mu\text{g/ml}$ of free cysteine was observed on the 3rd day which later decreases. Ignatova and coworkers [26] reported liberation of 2 mmol/ml thiol residues by *Thermoactinomyces candidus* at the end of 72 h in the broth supplemented with 6 g/l wool. Growth of *Vibrio* species kr2 on feather yielded 0.35 mmol/ml of sulphhydryl in 72 h [27].

The effect of variable feather concentration on production of feather lysate is shown in Table 3. Maximum ratio of soluble protein per gram feather was achieved when 1% feather was used as substrate; however maximum productivity of soluble protein was nearly similar when 1%, 1.5% and 2% feathers were used as substrate. The amount of amino acid reached a maximum concentration of $937.85 \pm 11.9 \mu\text{g/ml}$ on 6th day in the medium containing 2% feathers; however the maximum ratio of amino acids per gram feather was found when 0.5% concentration of feather was used as substrate. The strain appears to result in higher amount of hydrolysis product than other feather degrading organism studied earlier. The concentration of amino acid released from feathers under anaerobic condition by *Bacillus licheniformis* is found to be 0.29 mg/ml on 9th day [28]. Grazziotin et al. [29] reported production of 0.15 mg/ml of amino acids when *Vibrio* species kr2 was grown on a medium containing 2% feathers in 5 days.

Table 1 Cultural, physiological and biochemical characteristics of *E. meningoseptica* KB042 (MTCC 8360)

Colonial characteristics	
Configuration	Circular
Margin	Entire
Elevation	Low convex
Surface	Smooth and shiny
Opacity	Translucent
Morphological characteristics	
Gram's reaction	Gram-negative
Cell shape	Thin rods
Arrangement	Single and pairs
Spore(s)	Non-sporulating
Physiological characteristics	
Growth temperature	25–42°C
Growth on NaCl (%)	Inhibited
Grow under anaerobic	Weak
Biochemical characteristics	
Casein, gelatin, esculin, tween 20, tween 40, tween 60, tween 80, citrate utilization, phosphate solubilization	+
Amylase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, urease	–
H ₂ S production, gas production, NO ₃ reduction, ONPG test, O/F test, tyrosine degradation, hippurate hydrolysis	–

Table 2 Feather degradation and hydrolysate production by *E. meningoseptica* KB042 (MTCC 8360)

Incubation period days	Weight loss %	Final pH	Soluble protein µg/ml	Free amino acids µg/ml	Cysteine release µg/ml
1	38.48 ± 0.7	7.51 ± 0.01	652.30 ± 1.1	81.01 ± 1.0	8.615 ± 0.0
2	45.43 ± 1.2	7.90 ± 0.02	721.53 ± 1.9	121.37 ± 14.8	13.718 ± 0.3
3	69.80 ± 0.9	8.30 ± 0.12	1153.46 ± 3.4	222.88 ± 3.5	29.540 ± 0.6
4	72.13 ± 0.2	9.21 ± 0.31	1238.07 ± 5.7	366.10 ± 1.2	22.451 ± 1.7
5	79.21 ± 1.0	9.83 ± 0.24	1291.53 ± 3.0	448.37 ± 4.7	15.807 ± 0.29
6	82.50 ± 0.3	10.02 ± 0.10	1346.11 ± 3.8	583.98 ± 3.7	16.561 ± 0.5

Each value represents mean ± SE of three independent determinations.

Table 3 Effect of substrate concentration on feather hydrolysis by *E. meningoseptica* KB042 (MTCC 8360)

Feather concentration (%)	Weight loss (%)	Soluble protein µg/ml	Yield of protein/g feather	Amino acid µg/ml	Yield of amino acids/g feather	Cysteine released µg/ml	Yield of cysteine/g feather
0.5	72.35 ± 0.4	729.23 ± 17.6	1458.46	369.49 ± 1.5	738.98	18.92 ± 1.2	37.848
1.0	81.38 ± 0.1	1814.42 ± 1.3	1814.42	606.90 ± 13.3	606.90	25.12 ± 2.0	25.12
1.5	84.81 ± 0.4	1954.61 ± 7.3	1303.30	889.60 ± 8.7	593.06	26.73 ± 0.7	17.82
2.0	64.9 ± 0.1	1871.02 ± 18.0	935.51	937.85 ± 11.9	468.92	26.01 ± 0.5	13.00

Cultures grown at 37°C, 150 rpm for 6 days.

Each value represents mean ± SE of three independent determinations.

Table 4 Effect of initial pH on feather hydrolysis by *E. meningoseptica* KB042 (MTCC 8360)

Initial pH	Weight loss %	Soluble protein µg/ml	Free amino acid µg/ml	Cysteine released µg/ml
6.0	46.085 ± 0.3	523.01 ± 14.2	122.42 ± 1.3	8.96 ± 0.2
7.0	80.96 ± 0.6	1346.11 ± 5.0	689.41 ± 1.7	22.78 ± 0.02
8.0	83.29 ± 0.2	2155.38 ± 4.9	739.52 ± 10.7	25.42 ± 1.5
9.0	87.41 ± 0.7	2761.53 ± 18.4	956.72 ± 5.1	27.86 ± 1.5
10.0	93.63 ± 0.02	2884.61 ± 1.3	1030.25 ± 2.6	30.49 ± 1.0

Cultures grown at 37°C, 150 rpm for 6 days.

Each value represents mean ± SE of three independent determinations.

As shown in Table 4 the feather degradation was enhanced at alkaline pH with highest yield of soluble protein and amino acid in 6 days coinciding with maximum feather degradation (93.63%) in the culture broth at initial pH 10.0 at 37°C. The low level of cysteine release at pH 6.0 indicates reduced level of feather hydrolysis. This also suggests effective break down of disulfide linkages at neutral and alkaline pH making the keratin more accessible for degradation [30].

The strain KB042 produced essential amino acids in submerged fermentation. Results of thin layer chromatography confirmed the presence of threonine (R_f 0.49), valine (R_f 0.4) and tryptophan (R_f 0.52), leucine (R_f 0.46), cysteine (R_f 0.32) and minor amount of arginine (R_f 0.16) and methionine (R_f 0.14) when compared with standard amino acids. Sangali and Brandelli [25] also reported that feather hydrolysate by *Vibrio* sp. kr2 had higher concentration of valine and threonine.

In the light of our results, we would like to conclude that the *E. meningoseptica* KB042 is a keratinolytic bacterium, which degrades feather efficiently. Its fermentation broth is an excellent source of soluble protein and essential amino acids. The strain has shown its potential for exploitation as a novel source for poultry waste management and to get value added products including feed grade protein from such waste.

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