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Antibacterial and Antioxidant Activity of Newly Keratinolytic Bacteria, *Azotobacter chroococcum* B4

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Abstract : Keratinolyticbacteria of *A. chrococum* B4 was evaluated for its potential of antibacterial and antioxidant activity. Kirby-Bauer method was used to know antibacterial potential of B4 againts*Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Proteus* sp., enteropathogenic*Escherichia coli*, *Enterobactersakazakii*, and *Salmonella enterica*. Antioxydant test was done using DPPH radical scavenging activity assay with ascorbic acid as a controll. In this study, hydrolysate of pellet, dialysis, and fraction 25 of B4 keratinase purification of previous study was used for antibacterial and antioxidant test. The result showed that B4 hydrolysatesinhibited Gram positive pathogenic bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes*, and Gram negative *Enterobacter sakazakii*. All hydrolysates showed to have antioxidant properties in which fraction 25 showed higher compared to that of others. This study showed poultry waste-derived keratinase of B4 might be useful as supplementary protein, antibacterial, and antioxidant in the animal feed formulations.
Keywords : Antibacterial, antioxidant, *Azotobacter chrococum*, keratinase.

Introduction

World wide, poultry-processing industries generate more than 4 billion pounds of feathers annually as waste. Feather weight accounts up to 5-7% of mature chicken. Chicken feather is a material that contains about 90% of its protein as keratin[1]. Microbial degradation is an economical method for the conversion of keratin waste into useful products. As previously reported,keratinase can be utilized as biocatalysts in fields of leather, textile, and waste recycling[2]. Keratinous wastes are used as source of amino acids, soluble peptides, and proteins, which are typically utilized as animal feed additives as well as nitrogen source for plants[2,3].

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Antioxidants play an important role in humanhealth and food processing.Many antioxidativepeptides have been identified and characterized as well as antimicrobials[4].Such peptides, encrypted in protein sequences, only demonstrate biological activities after their release from the native protein such as keratin[5]. Recently, we isolateda newly *Azotobacterchroococcum* B4 producing keratinase[6]. The purpose of this study was to examined the antibacterial and antioxidant potential of feather hydrolysates of B4 for its possible use in animal feed formulation.

Materials and Methods

Strain and growth medium

*Azotobacterchroococcum*B4 was grown on Feather Meal Broth (FMB) composed by chicken feather powder (15 g), K_2HPO_4 (0.7 g), KH_2PO_4 (0.4 g),NaCl (0.5 g), $MgSO_4$ (0.1 g) in 1000 mL distilled water[7]. Chicken feather waste was collected from slaughterhouses. Feathers were washed using detergent-water and cut into small pieces (± 2 cm), prior soaking in acetone for 24 h. Washed feathers were dried in oven at 40°C for 72 h to yield chicken feather powder. Growth medium was sterilized at 121°C, 1 atm for 15 min.

Antibacterial activity of keratinase B4

Hydrolysate of pellet, dialysis, and fraction 25 solution of B4 keratinase of previous study were used for antimicrobial and antioxidant test. Antibacterial activity was tested against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Proteus* sp., enteropathogenic*Escherichia coli*, *Enterobactersakazakii*, and *Salmonella enterica*using Kirby-Bauer method. Ten microlitres of sample solutionswas put on sterile disc paper. The discs were placed on top of bacterial lawns grown on nutrient agar (Oxoid UK). The culture was incubated at 28°C. Diameter of clear zones or inhibition zones around the discs was measured after 24 h.

Antioxidant activity of feather hydrolysate

Scavenging of 2,2 diphenyl-1-picrylhydracyl (DPPH) radical assay was used to measure antioxidant activity of supernatant of feather hydrolysates. Ascorbic acid was used as the positive control. Each 20 µL sample was added to 180 µL DPPH in methanol solution, making it a final total volume of 200 µL. The mixture was maintained for 30 min in the dark at 37°C, and then DPPH radical reduction was measured the using UV–Visible microplate reader (Thermo Scientific) at 517 nm. In addition, the following formula was used to determine the DPPH radical (scavenging activity) inhibition percentage:

$$\text{Scavenging activity (\%)} = [1 - (A_{sample} - A_{sample\ blank})/A_{control}] \times 100$$

$A_{control}$ is the absorbance of DPPH solution (without hydrolysate), A_{sample} is the sample absorbance (DPPH solution with hydrolysate) and $A_{sample\ blank}$ is the sample absorbance only (extract without DPPH solution). The test was conducted in triplicate using ascorbic acid as positive control of antioxidant activity. Ascorbic acid was used as a control.

Results and Discussion

Antibacterial activity of keratinase B4

Hydrolysate samples obtained from precipitation with ammonium sulphate, dialysis, and column fractionation of keratinasewere used in testing antimicrobial activity against Gram-positive and Gram-negative bacteria. Inhibition to bacterial growth were shown as clear zone around paper disc (Figure 1.)

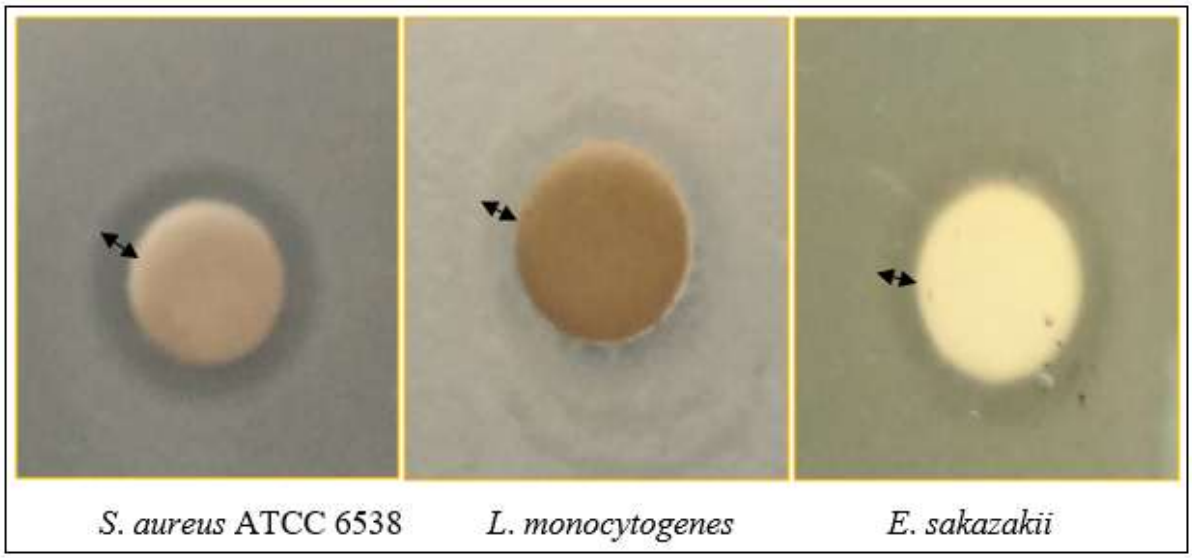

Figure 1. Antibacterial activities of B4 hydrolysate against pathogenic bacteria *S. aureus*ATCC 6538, *L.monocytogenes* and *E. sakazakii* shown as clear zone around paper dics (arrowed)

Table 1. Antibacterial activity of B4 hydrolysate

Bacterial isolates	**Inhibition zone (mm ± SD)**		
	Precipitation	**Dialysis**	**Fraction 25**
*S. aureus*ATCC 6538	0	0	11±0.6
S. epidermidis	0	0	9±0.3
B. cereus	0	0	8±0.3
B. pumilus	0	0	0
B. subtilis	0	0	9.7±0.3
L. monocytogenes	0	8±0.3	10±0.4
Proteus sp.	0	0	8±0
*E. coli (*EPEC*)*	0	9±0.3	9.8±0.3
E. sakazakii	0	9±0.3	10±0.3
*S. enterica*serovar*typhimurium*	0	8±0.3	8±0

Hydrolystae inhibition to pathogenic bacterial isolates varied to some extent (Table 1).

It was observed that higher inhibition zone was found in Gram-positive bacteria *S. aureus*by 11 ± 0.6 mm, *L. monocytogenes* by 10 ± 0.4 mm, and Gram-negative bacteria *E. sakazakii* by 10 ± 0.3 mm.

Interestingly, ammonium sulphate precipitation showed no inhibitory activity, dialysis fraction showed low inhibitory activity against *L. monocytogenes*, *E. coli* (EPEC), *E. sakazakii*,and *S. typhimurium*, while high antibacterial activity was observed in fraction 25 to all pathogenic bacterial isolates (Table 2). Sequential purification steps resulted more purified protein/keratinase, which showed to have antibacterial activity by disturbing cell wall or membrane structure at variable degrees. The bacterial damage by proteases like papain is due to a broad proteolytic activity, resulting in the rupture of the cell wall or membrane and subsequent loss of cell integrity[8].

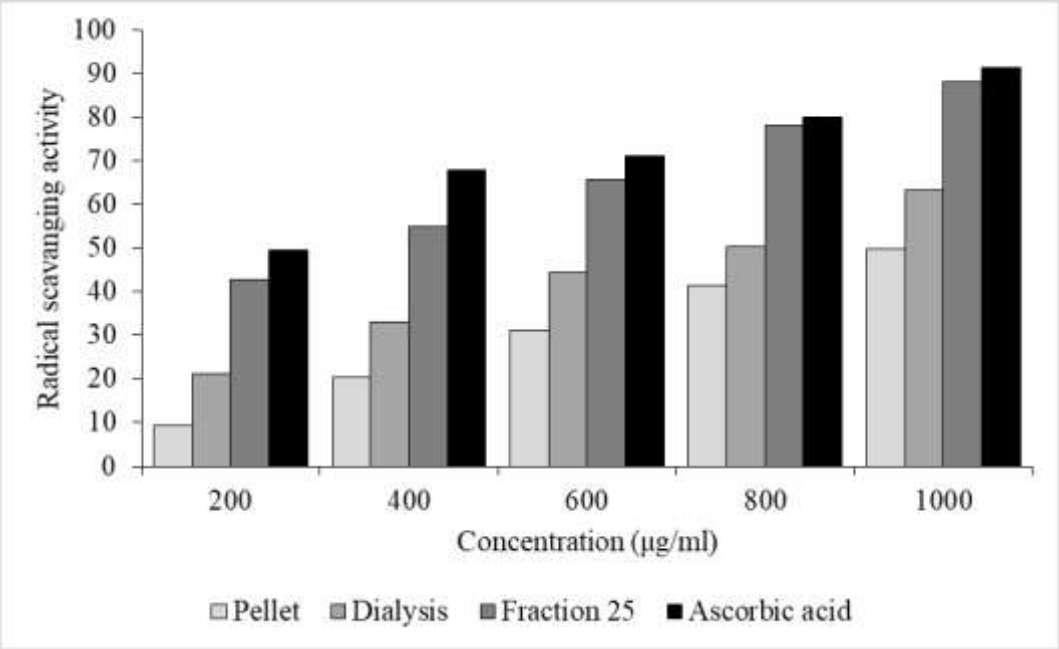

Figure 2. Potential of antioxidant activity of B4 hydrolysate of keratinase B4 purification as measured using DPPH assay

This work indicated that keratinase B4 migh be potentially to inhibit pathogenic bacterial growth as reported previously. An antimicrobial activity of some microbial keratinases, such as those produced by *B. subtilis* CH-1 inhibiting the growth of *S. aureus* and *E. coli*[7], *Nocardiopsis*sp. 28ROR inhibiting *S. aureus*[9], and *B. safensis*LAU 13 inhibiting *E. coli*[10].

Antioxidant activity of keratinase B4

In this study three hydrolysate of keratinase purification of *A. chroococcum* B4 was also used for its antioxidant properties. The results indicated that allhydrolysates presented antioxidant activity as concentration dependent (Figure 2).

B4 hydrolysates showed to have varied in antioxidant activity, in which 200 µg/ml of pellet was lower antioxidant activity compared to others. Fraction 25of 1000 µg/ml was found to have relatively high antioxidant activity. At that concentration, fraction 25 and ascorbic acid have scavenging activity of 88% and 91.27, respectively.

Antioxidant activity is likely to occur through various mechanisms[4]. The ability of hydrolysates might due to composition of bioactive peptides that contribute to antioxidant activity[11]. As previously reported, this study showed similar result in that protein and peptide hydrolysateshave an effective antioxidant activity*in vivo* and in cellular cultures[12],[13]. Several studies on bioactive peptide was reported from feather hydrolysates from *B. pumilus* A1[14], *Chryseobacterium*sp. kr6[15], and *B. subtilis* S1-4[5].

This study of antioxydant property of feather hydrolysate of *A. chroooccum* B4 was firstly reported. The insight of antioxidant assay of bacterial hydrolisateof enzyme purification is an important and necessary step before being applied in humans and animals, so it is very important to evaluate peptide activity of *A. chroococcum* B4 antioxidant usigvarious methods in the future.

Conclusion

Hydrolysates of B4 keratinase purification showed antibacterial and antioxidant activities.Relatively high antimicrobial activity was observed against pathogenic Gram positive *S. aureus*and *L. monocytogenes*,and Gram negative *E. sakazakii* by fraction 25. DPPH radical scavenging of fraction 25 of 1.000 µg/ml activity reached 88%. This was closed to ascorbic acid which was it radical scavenging activity reached 91%. Thus,

biodegradation of feathers by *A. chroococcum* B4 keratinase represented a beneficial biotechnological process for handling feather waste by having proteolytic/keratinolytic, antimicrobial, and antioxidant activities used for animal feed formulation.

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References

1. AL M-H, CV S. Keratin Fibers from Chicken Feathers: Structure and Advances in Polymer Composites. Nova Science Publishers; 2012.
2. Mamangkey J, Suryanto D, Munir E, Mustopa AZ. Isolation, Molecular Identification and Verification of Gene Encoding Bacterial Keratinase from Crocodile (Crocodylus porosus) Feces. IOP Conf. Ser. Earth Environ. Sci. 2019;
3. Syed DG, Lee JC, Li WJ, Kim CJ, Agasar D. Production, characterization and application of keratinase from Streptomyces gulbargensis. Bioresource Technol., 2009, 100; 1868–71.
4. Wan MY, Dong G, Yang BQ, Feng H. Identification and characterization of a novel antioxidant peptide from feather keratin hydrolysate. Biotechnol Lett., 2016, 38; 643–9.
5. Fontoura R, Daroit DJ, Correa APF, Meira SMM, Mosquera M, Brandelli A. Production of feather hydrolysates with antioxidant, angiotensin-I converting enzyme- and dipeptidyl peptidase-IV-inhibitory activities. N Biotechnol., 2014, 31; 506–13.
6. Bhange K, Chaturvedi V, Bhatt R. Feather degradation potential of Stenotrophomonas maltophilia KB13 and feather protein hydrolysate (FPH) mediated reduction of hexavalent chromium. 3 Biotech., 2016, 6; 42.
7. Cai CG, Chen JS, Qi JJ, Yin Y, Zheng XD. Purification and characterization of keratinase from a new Bacillus subtilis strain. J Zhejiang Univ Sci B., 2008, 9; 713–20.
8. Gurav RG, Tang J, Jadhav JP. Sulfitolytic and keratinolytic potential of Chryseobacterium sp. RBT revealed hydrolysis of melanin containing feathers. 3 Biotech., 2016, 6; 145.
9. Gartika M, Sasmita IS, Satari MH, Chairulfattah A, Hilmanto D. Antibacterial activity of papain against Streptococcus mutans ATCC 25175. Int J Dev Res., 2014, 4; 2075–7.
10. Omran R., Production of Antimicrobial and Anticancer From Feather-Keratinolytic Nocardiopsis Sp. 28ROR as a Novel strain using Feather meal medium, nt J Pharm Pharm Sci., 2017, 9; 175–9.
11. Lateef A, Adelere IA, Gueguim-Kana EB, Asafa TB, Beukes LS. Green synthesis of silver nanoparticles using keratinase obtained from a strain of Bacillus safensis LAU 13. Int Nano Lett., 2015, 5; 29–35.
12. Gupta S, Nigam A, Singh R. Purification and characterization of a Bacillus subtilis keratinase and its prospective application in feed industry. Acta Biol Szeged., 2015, 59; 1–8.
13. Nwachukwu ID, Aluko RE. Structural and functional properties of food protein-derived antioxidant peptides. J Food Biochem., 2019, 43; 12761.
14. Callegaro K, Brandelli A, Daroit DJ. Beyond plucking: Feathers bioprocessing into valuable protein hydrolysates. Waste Manag., 2019, 95; 399–415.
15. Fakhfakh-Zouari N, Haddar A, Hmidet N, Frikha F, Nasri M. Application of statistical experimental design for optimization of keratinases production by Bacillus pumilus A1 grown on chicken feather and some biochemical properties. Process Biochem., 2010, 45; 617–26.

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