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Production and characterization of a keratinolytic enzyme from a feather-degrading *Bacillus thuringiensis* CH3

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1. Introduction

Feathers, which are almost pure keratin protein, are produced in large amounts as a waste byproduct of poultry processing plants, reaching millions of tons per year worldwide. At present, feathers are converted to feather meal, a digestible dietary protein for animals, using physical and chemical treatments. Processing of feather meal destroys certain amino acids influencing protein quality and digestibility. Feather meal, consequently, is a poorly digestible feed ingredient. Keratinolytic enzymes may have important uses in biotechnological processes involving keratin-containing wastes from poultry and leather industries through the development of non-polluting processes. Keratinolytic activity has been observed in microorganisms isolated from the poultry industry. Recently, feather-degrading activity was associated with a Vibrio sp. strain isolated from decomposing feathers. The aim of this study was to identify keratinolytic bacteria showing high feather degradation, with potential application in biotechnological processes.

2. Meterials & Methods

2.1. Isolation and selection of feather-degrading microorganism

Feather wastes were collected from several sites at a local poultry farm (Kimhae, Korea). Feather wastes were flooded in phosphate-buffered saline (PBS) and the suspension used to streak on skim milk agar plate (10g l-1 tryptone, 5g l-1 yeast extract, 50g l-1 skim milk, 5g l-1 NaCl, 20g l-1 agar), which were incubated at 37℃. Single colonies were isolated and screened for their proteolytic ability. Colonies producing the clear zones in this medium were selected. A repeated streak was used to obtain pure isolates. Feather-degrading capacity was determined on whole feather medium (0.3g l-1 K2HPO4, 0.4g l-1 KH2PO4, 0.1g l-1 MgCl2, 0.5g l-1 NaCl, 0.5g l-1 Urea)

2.2. Taxonomic studies

Bacteria was identified based on morphological and biochemical tests, comparing the data with standard species and using API kit (50CHB/E, 20E). The sequence of the 16S rDNA gene was determined after genomic DNA extraction, PCR amplification, and sequencing.

2.3. Enzyme assays

Keratinolytic activity assay described previously (Friedrich et al. 1999) was slightly modified and performed as follows: 4ml of 0.028M Tris/HCl buffer (pH 7.5) and 1.0ml of the enzyme preparation were incubated with 4mg of keratin powder for 60min at 45℃, with constant agitation at 170rpm in a water bath. The enzyme reaction was stopped by adding 2.0ml of 10% trichloroacetic acid (TCA), and the samples were left in a refrigerator at 4℃. After 30min, they were centrifuged for 15min at 14000rpm in a cooled centrifuge. The absorption of the supernatant was measured spectrophotometrically at 280nm against a control. The control was treated in the same way, except that TCA was added before incubation. One unit (U) of keratinolytic activity was defined as an increase of corrected A280 for 0.01 under the conditions described. The data presented are mean values of two parallel determinations. Protein concentration was measured by the absorbance at 280nm or by the method of Bradford using bovine serum albumin as a standard.

3. Results and conclusions

3.1. Identification of feather-degrading strain

The identification of this bacterial isolate was based on morphological and biochemical tests, API kit (50CHB/E, 20E), and 16S rDNA sequence data. Together with physiological and API kit profiling, these characteristics suggest the genus Bacillus. In the genus *Bacillus*, the isolate was similar to *Bacillus thuringiensis*. The isolate was named as *Bacillus thuringiensis* CH3.

3.2. Optimal growth conditions

Growth of the isolate occurred between pH 6.0 and 8.0 and between 25 and 45℃, with an optimum at pH8.0 and 37℃. Optimal nutrient source for enzyme production was 1.5% glycerol, 0.3% casein. The maximum enzyme activities were showed in the late logarithmic growth phase or the beginning of stationary phase, and the production of soluble protein showed the same tendency as that of keratinolytic protease.

3.3. Characterization of keratinolytic enzyme

Optimal pH and temperature for the enzyme were pH 9.0 and 45℃, respectively. The enzyme activity was significantly inhibited by EDTA, Zn^{2+} and Hg^{2+}. The feather keratin was degraded to a higher degree than gelatin, BSA and elastin.

References

Alessandro Riffel, Francoise Lucas, Philipp Heeb, Adriano Brandelli, 2003 Characterization of a new keratinolytic bacterium that completely degrades native feather keratin. Arch Microbiol 179, 258-265

A Riffel and A Brandelli, 2002. Isolation and characterization of a feather-degrading bacterium from the poultry procession industry. Journal of Industrial Microbiology & Biotechnology 29, 255-258

J.M. Kim, W.J. Lim, H.J. Suh, 2001. Feather-degrading Bacillus species from poultry waste. Process Biochemistry 37, 287-291

Yoon-Jeong Lee, Jae-Ho Kim, Ha-Kun Kim, and Jong-Soo Lee, 2004. Production and Characterization of Keratinase from *Paracoccus* sp. WJ-98. Biotechnology and Bioprocess Engineering 9, 17-22

R.C.S Thys, F.S. Lucas, A. Riffel, P. Heeb and A. Brandelli, 2004. Characterization of a protease of a feather-degrading *Microbacterium* species. Letters in Applied Microbiology 39, 181-186

S. Sangali and A. Brandelli, 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. Journal of Applied Microbiology 89, 735-743

Shogei Yamamura, Yasutaka Morita, Quamrul Hasan, Kenji Yokoyama, and Eiichi Tamiya, 2002. Keratin degradation: a cooperative action of two enzymes from *Stenotrophomonas* sp. Biochemical and Biophysical Research Communications 294, 1138-1143