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Keratinolytic activity of *Bacillus megaterium* F7-1, a feather-degrading mesophilic bacterium

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Feather degradation;
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Summary

The aim of this study was to investigate environmental conditions affecting chicken feather degradation and keratinolytic enzyme production by *Bacillus megaterium* F7-1, a feather-degrading mesophilic bacterium. *B. megaterium* F7-1 degraded whole chicken feather completely within 7 days. The bacterium grew with an optimum at pH 7.0–11.0 and 25–40 °C, where maximum keratinolytic activity was also observed. The production of keratinolytic enzyme by *B. megaterium* F7-1 was inducible with feather. Keratinolytic enzyme production by *B. megaterium* F7-1 at 0.6% (w/v) skim milk was 468 U/ml, which was about 9.4-fold higher than that without skim milk. The amount of keratinolytic enzyme production depended on feather concentrations. The degradation rate of autoclaved chicken feathers by cell-free culture supernatant was 26% after 24 h of incubation, but the degradation of untreated chicken feathers was unsuccessful. *B. megaterium* F7-1 effectively degraded feather meal, duck feather and human nail, whereas human hair and sheep wool showed relatively low degradation rates. *B. megaterium* F7-1 presented high keratinolytic activity and was very effective in feather degradation, providing potential use for biotechnological processes of keratin hydrolysis.

Introduction

Environmental wastes are found in large quantities in many countries. Although some of them contain a considerable amount of protein and various carbon compounds, little attention is given

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to utilizing or recycling these wastes in a technological way. Additionally, the accumulation of some of these wastes in nature is considered to be a serious source of pollution and health hazards. Therefore, their proper disposal may be considered as a means of avoiding environmental pollution.

Recently, we have focused on the utilization of some polymeric wastes, mainly feather waste. Feathers are generated in large amounts as a waste byproduct at commercial poultry-processing plants, reaching millions of tons per year worldwide (Manczinger et al., 2003). Since feathers are almost pure keratin protein, feather wastes represent a potential alternative to more expensive dietary ingredients for animal feedstuffs. Generally, they become feather meal used as animal feed after undergoing physical and chemical treatments. These processes require significant energy and also destroy certain amino acids (Papadoulos and Ketelaars, 1986). Therefore, biodegradation of feather keratin by microorganisms represents an alternative method to improve the nutritional value of feather waste and to prevent environment contamination.

Because of a high degree of cross-linking by cysteine disulfide bonds, hydrogen bonding, and hydrophobic interactions, keratin is insoluble and not degradable by proteases such as trypsin, pepsin, and papain (Williams et al., 1990). Nevertheless, feathers do not accumulate in nature, since feather keratin can be degraded by keratinolytic enzyme of some microorganisms (Onifade et al., 1998). In this regard, keratinolytic enzymes may have important uses in biotechnological processes involving keratin-containing wastes from poultry and leather industries through the development of non-pollution processes. So far, only some species of saprophytic and parasitic fungi, thermophilic actinomycetes, and *Bacillus* strains have been reported to be able to degrade feather keratin (Onifade et al., 1998). Most of these strains have been isolated from poultry waste using nutrient-rich medium and have been shown to degrade feathers at 50–60 °C. More recently, it was reported that some bacterial strains, such as *Bacillus* sp. strain kr16 (Werlang and Brandelli, 2005), *Chryseobacterium* sp. strain kr6 (Riffel et al., 2003) and *Vibrio* sp. strain kr2 (Sangali and Brandelli, 2000), degrade feathers at 30–37 °C. However, there are few reports on the nutritional conditions (carbon and nitrogen source requirements) that improve the feather degradation and keratinolytic enzymes production by mesophilic feather-degrading microorganisms.

Recently, we have reported on the isolation of *Bacillus megaterium* F7-1, which is able to produce skim milk hydrolyzing protease (Son et al., 2004a). Following this, we attempted to improve the ability of this strain to produce protease using casein as a enzyme substrate (Son et al., 2004b; Son, 2005). Here, for chicken feather recycling, we report some substances that lead to a high keratinolytic enzyme production, using soluble keratin as a testing enzyme substrate, and describe the optimal condition for chicken feather degradation by *B. megaterium* F7-1.

Materials and methods

Microorganism and culture conditions

B. megaterium F7-1 used in this study was recently isolated from a poultry waste in Korea (Son et al., 2004a). In order to examine the feather degradation and keratinolytic enzyme production, the cells were cultivated in the basal salts medium containing 0.1% (w/v) chicken feathers unless otherwise indicated. For shaken culture in flasks, 50 ml of the medium was dispensed into each of 250-ml Erlenmeyer flasks followed by inoculation with 1 ml of *B. megaterium* F7-1 culture (2×10^7 cells/ml) grown in nutrient broth (Difco) at 30 °C for 24 h. Cultivations were performed at 30 °C and 200 rpm for 5 days in a rotary shaker unless otherwise stated. The cultures were centrifuged at 12,000 rpm for 5 min, and the supernatant was used as a crude enzyme preparation.

The basal salts medium used contained the following (g/l): 0.5 NH_4Cl, 0.5 NaCl, 0.1 K_2HPO_4, 0.2 KH_2PO_4, 0.1 $MgCl_2 \cdot 6H_2O$, and 0.1 yeast extract (Williams et al., 1990). The pH was adjusted to 7.5 prior sterilization. Chicken feathers were obtained from a poultry-processing plant. They were washed extensively with tap water and dried at 60 °C for 72 h, and then kept at 4 °C until used.

Dimethyl sulfoxide soluble feather keratin preparation

Soluble keratin was prepared from chicken feathers by the method of Wawrzkiewicz et al. (1987). Native chicken feathers (10 g) suspended in 500 ml of dimethyl sulfoxide were solubilized by heat treatment in a reflux condenser at 100 °C for 1 h. Soluble keratin was then precipitated by addition of cold acetone (1 l) at −70 °C for 2 h followed by centrifugation at 12,000 rpm for 10 min. The precipitate was washed twice with distilled water, and then suspended in 0.1 M phosphate buffer (pH 7.0) up to obtain a keratin suspension 0.06% (w/v) of protein concentration.