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Biodegradation Of Jungle Fowl Feathers By Indigenious Isolatesof *Bacillus* Sp.

KEYWORDS

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ABSTRACT *Keratinases produced from the keratin degrading bacterial isolates were isolated from feather compost soil. Bacillus sp. was isolated poultry compost in the poultryfarm. Among 6 isolates, 2 Bacillus isolates showed notable keartinolyticactivity which the highest was observed by Bacillus sp. KB-4 and KB-5, which wereidentified as Bacillus licheniformisKB-4 and Bacillus subtilis KB-5 respectively.Both isolates, the maximum keratinase production occurred at 1% concentration offeather meal with starch as carbon source at pH-7, 35°C to 40°C for 6 days. Innitrogen source, the maximum amount of enzyme production was achieved withpeptone for B. licheniformisKB-4 and with yeast extract for B. subtilis KB-5. B.licheniformisKB-4 isolate rapidly utilized feather as nutrient source and multipliedmaximum when compared with B. subtilis KB-5. B. licheniformisKB-4 isolatedegraded the feather up to 43.72% on 5 days incubation and B. subtilis KB-5was36.26%. Based on all experiment B. licheniformisKB-4 isolate showed better resultand it was considered as an efficient isolate for feather degradation.*

INTRODUCTION

Feather is a by-product in the poultry industry. Degradation of the feather needs large amount of energy to degrade. Traditional ways of degrading consumes more time so the alternative method of degrading feathers by microorganism i.e. biodegrdation. A number of keratinolytic microorganisms have been reported including some species of *Bacillus*, *Actinomycetes* and *Fungi*. *Bacillus* sp. shows potential in keratinase production in industry level (Madan *et al.*, 2000; Yamamura *et al.*, 2002; Suntornsuk *et al.*, 2003). The present study deals with the identification of Bacillus sp. In the feather compost soil and characterized their capacity of keratinase production.

MATERIALS AND METHODS

Samples (soil and feather) were taken from the poultry compost in the poultry farm, Namakkal. Bacillus sp is isolated from the sample. By microscopic examination and biochemical tests as described in the Bergey's manual of systematic bacteriology strain was identified (Sneath *et al.*, 1986). *Bacillus sp* screened for keratinolytic activity (Agrahari and Wadhwa, 2010)

Optimization of enzyme production at different concentrations of feather from 1 to 5% , different pH in the range of 4-9, temperatures in the range of 25-40°C, different carbon sources of 1% strength (glucose, sucrose, lactose, starch and maltose), nitrogen sources of 0.1% strength (beef extract, yeast extract, malt extract, peptone and urea).

Assay for keratinolytic activity

Keratinnolytic activity was monitored as describe previously (Sangali and Brandelli, 2000). One unit (U/ml) of keratinolytic activity was defined as an increase of collected adsorption of 595nm (A_{595}) with the control for 0.01/ml under the condition described above and calculated by the following equation.

$$U/ml=4 \times n \times A_{595}/0.01 \times 30$$

Were n is the dilution rate 4 is the final reaction volume (ml) and 30 is the incubation time (minutes).

Degradation of feather

The capacity of degradation of feather was tested on the basal medium with 1% raw feather. Two selected efficient isolates were inoculated and incubated at 37°C for 5 days. After degradation of substrate was visually inspected against control (without organism) and aliquots were removed for monitor the growth of isolates by spectrophotometrically 600nm for each day (Zerdani *et al.*, 2004).

RESULTS

Bacillus sp. was isolated from the sample. *Bacillus* isolates showed notable keartinolytic activity in which the highest was observed by KB-4 and KB- 5 were selected for further studies. KB-4 and KB-5 were identified and designated as *Bacillus licheniformis* KB-4 and *Bacillus subtilis* KB-5.

Maximum keratinase production occurred at 1% concentration of feather meal. The highest keratinase production

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was observed at (288.34± 1.5199 U/ml) by *B. licheniformis* KB-4 and by *B. subtilis* KB-5 (208.83± 1.9204 U/ml) (Figure 1). When compared with both isolates, *B. licheniformis* KB-4 had more keratinase activity than *B. subtilis* KB-5. The highest keratinase production was observed in pH 7 at 40°C by *B. licheniformis* KB-4 and *B. subtilis* at 35°C KB-5 (Figure 2 & 3).

The Optimization of keratinase production at different incubation period showed higher production occurred at 6th days for *B. licheniformis* KB-4 (256.58± 2.5593 U/ml) and *B. subtilis* KB-5 (212.28± 1.8028 U/ml).

Starch was found to be sole carbon source for the production of keratinase by both *B. licheniformis* KB-4 (202.80 ± 1.7422U/ml) and *B. subtilis* KB-5 (208.53 ± 1.7192U/ml). The lowest keratinase production was observed at lactose for *B.licheniformis* and maltose for *B. subtilis*.

The maximum amount of enzyme production in peptone for *B. licheniformis* KB-4 (281.24± 1.3101 U/ml) and in yeast extract for *B. subtilis* KB-5 (222.8 ± 1.6504 U/ml). The lowest keratinase production was observed in urea for both isolates.

The growth pattern was gradually increased with increase the growth period. *B. licheniformis* KB-4 isolate rapidly utilized feather as nutrient source and multiplied maximum when compared with *B. subtilis* KB-5 (Figure 4).

B. licheniformis KB-4 isolate degraded the feather up to 43.72 % on 5 days incubation and *B. subtilis* KB-5 was 36.26%. Based on all experiment *B. licheniformis* KB-4 isolate showed better result and it was considered as an efficient isolate for feather degradation.

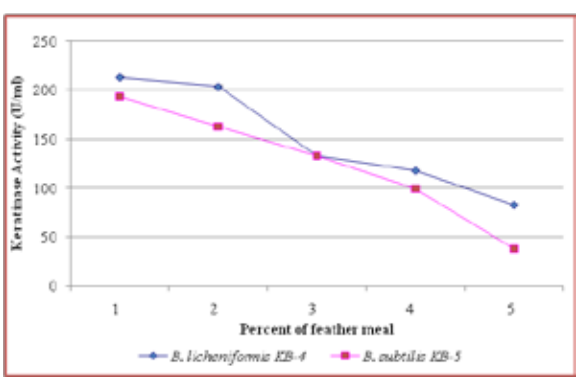

Figure 1. Optimization of keratinase production at different concentrations of feather meal

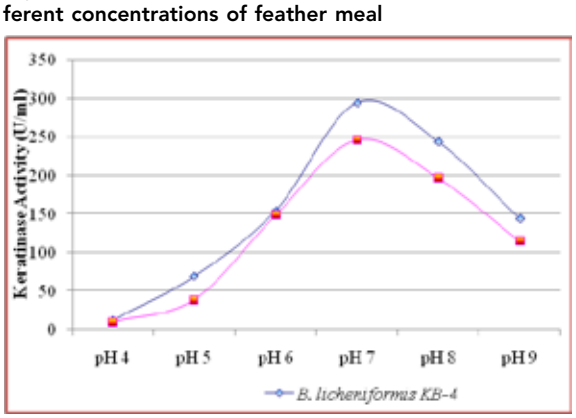

Figure 2. Optimization of keratinase production at different pH

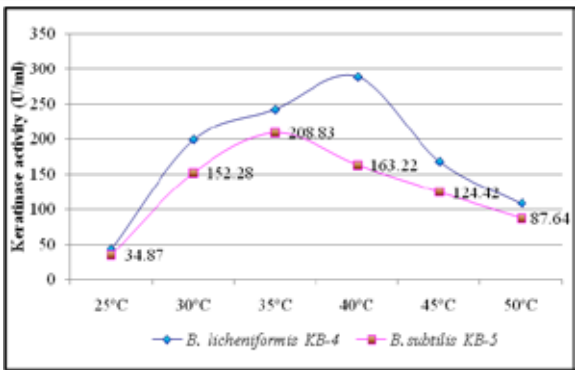

Figure 3. Optimization of keratinase production at different temperature

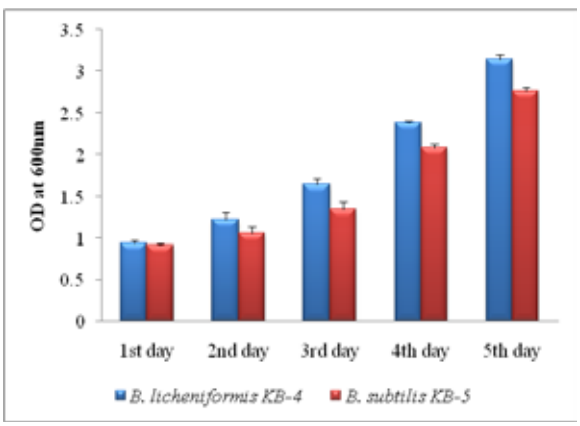

Figure 4. Growth pattern of B. licheniformis KB-4 and B. subtilis KB-5 and during feather degradation

DISCUSSION

Feather has 90% of keratin; in the form of β- keratin (Fraser *et al.*, 1971). Keratins are present in feathers, wool, hooves, scales, hair, nails and stratum cornea. Keratin based materials are hard to degrade because if it's mechanical stability (Friedrich and Antranikian, 1996).

Several studies showed that *Bacillus* sp. has potential sources of producing keratinases ((Lin *et al.*, 1992; Deivasigamani and Alagappan, 2008; Mazotto *et al.*, 2010; Prakash *et al.* 2010). From our study 2 strains has keartinolytic activityKB-4 followed by KB-5. The keratinolytic organisms were observed for producing clear zone on feather meal agar (Kim *et al.*, 2001). *Bacillus* species identified according to cell and colony morphology, growth characteristics, several biochemical tests based on Bergey's Manual of Systematic Bacteriology (Sneath, 1986). The isolates were identified as *Bacillus licheniformis* KB-4 and *Bacillus subtilis* –KB-9.

The highest keratinase production was observed at pH 7 by *B. licheniformis* KB-4 *B. licheniformis* exhibited best keratinase activity under neutral conditions (Wang and Shih, 1999). The highest keratinase production was observed at 40°C in *B. licheniformis* KB-4. No keratinase production was observed at 50°C and 65°C because of an absence of bacterial cell growth at such high temperatures (Pissuwan and Suntornsuk, 2001).

The maximum keratinase production was occurred at 6thday for *B. licheniformis* KB-4 and *B. subtilis* KB-5. *B. thurengensis* showed maximum production on 5th day (Agrahari and Wadhwa, 2010) and *Bacillus* strain on 7th day (Srivastava *et*

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al., 2011; Bhatnagar *et al.*, 2013).

Starch was found to be sole carbon source for the production of keratinase by both *B. licheniformis* KB-4 and *B. subtilis* KB-5. Efficient feather degradation in the absence of a simpler carbon source suggests the inducible characteristic of the keratinase. A study by Laba *et al*. (2010) explained in which addition of simpler carbon sources have led to decreased feather degradation and reduced enzyme production.

The growth pattern of the isolated *B. licheniformis* strain showed a growth pattern higher than the strain *B. subtilis* identified by Zerdani *et al.*, (2004). Many bacteria are involved in keratinolytic activity are *B. licheniformis* (Hossain *et al*. 2007), *Streptomyces albidoflavus* and *S. pactum* (Bockle *et al.*, 1995; Bressollier *et al.*, 1999). In the present study, *B. licheniformis* KB-4 isolate degraded the feather up to 43.72% on 5 days incubation and *B. subtilis* KB-5 was 36.26%. Based on all experiment *B. licheniformis* KB-4 isolate showed better result and it was considered as an efficient isolate for feather degradation.

CONCLUSION

Enzymatic hydrolysis of feather wastes could be a safe method of recycling these organic materials. These encouraging results in feather wastes management should be continued and the isolated Bacilli may also be evaluated in the treatment of other kind of wastes. The present study suggested that *B. licheniformis* KB-4 is found as efficient isolates for degradation of chicken feather.

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