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Full Length Research Paper

Feather wastes digestion by new isolated strains *Bacillus* sp. in Morocco

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Eight strains of *Bacillus* were isolated from non treated soil, characterized and used for the digestion of feather wastes in the laboratory. Non-protein nitrogen (NPN) and total protein (TP) were determined during the incubation time and the microbial counts of the different strains during feather hydrolysis were also monitored. Results of the screening test showed that the solid pieces of feather were completely digested by all the strains. The most efficient isolated strain selected was compared with *Bacillus subtilis* ATCC 6633. Results showed that the total protein decreased from 13.6% to 1.92 % with the isolated strain, and from 12.25 % to 2.99% with the standard strain. The NPN reached a concentration of 43.2mg/100g and 20.5 mg/100g with the isolated and standard strains, respectively.

Key words: Feather, keratin, *Bacillus*, fermentation, process.

INTRODUCTION

Waste feathers from poultry were not a major concern for the environment or for the plants and poultry farms. But with the increasing production of poultry which might be accompanied with an increase in feather wastes. Feathers contain β-keratin as a major component, which occur as pleated shots twisted into microfibrils and are resistant to biological degradation by enzymes. Keratin hydrolysis by microbial enzymes was studied (Shih, 1993). The same author reported keratinolytic bacteria are present in soil and poultry compost. Burtt and Ichida (1999a) showed that the keratin hydrolyzing bacteria could also occur in the plumage of living birds. The presence of feather degrading bacteria on the feathers would be very interesting for the treatment of these wastes. These authors (Burtt and Ichida, 1999b) also demonstrated that inoculation may enhance keratin degradation in poultry compost, and such controlled system is very tangible for an accelerated process for feather digestion.

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Prior to 1990, a few species of fungi (Pugh, 1965; Hubalek, 1976; Hubalek, 1978) and a single bacterium *Streptomyces fradiae* (Noval and Nickerson, 1959) were known to degrade feathers. A feather degrading *Bacillus licheniformis* PWD61 was isolated from the aerobic portion of a poultry waste digester (Wiliams and al., 1990) and bioengineered by Lin et al. (1995) to enhance degradation of β-keratine. In the present investigation, feather digestion process was accelerated by inoculating with keratinolytic species of *Bacillus* isolated from soil.

METHODS

Chemical determinations

The pH was checked by the use of a pH-meter type crison MicropH 2000. Dry matter was determined by oven drying a weighed amount of the product at 105°C until constant weight. Ash was determined by inceniration at 550°C for 6 h. Total protein (TP) was determined by the method described by Lowry (1951). Non-protein nitrogen (NPN) was determined by the Kjeldhal method described by the APHA (1989) on the filtrate after precipitation with a 10% trichloracetic acid solution.

Strains isolation and characterization

Samples were taken from the natural composting wastes in the city of Casablanca (Morocco). All the samples (500 g each) were transported in plastic bags to the laboratory. Serial dilution from each sample was prepared by adding 10 g of the compost to 90 ml of saline water. This initial dilution was heat activated at 70°C during 15 min and dilution up 10^6 in saline water were prepared in tubes. All the dilutions were plated on trypticase soy agar (TSA) and incubated at 30°C for 24 h. The appeared colonies were checked for spore presence and streaked on agar slants for further characterization.

Characterization

All the collected strains were grown on TSA for fresh cultures. Spore production and localization were examined by microscopic observations. The identification was done according to the method described by Larpent and Larpent (1985). Spore shape, growth at different temperatures, growth in anaerobic conditions, lecithinase, gelatinase, caseinase, amylase, indol formation, nitrate and carbohydrates utilization were checked.

Screening test

Eight strains were chosen from the 10 isolates to be screened for the degradation of feather wastes. Growth was evaluated by the DO method (optical density). The strains were grown on a minimal medium containing 0.1 g/L Mg SO_4, 2 g/L KH_2 PO_4 and 2 g/L glucose. 8 g of clean feather cut in slices of 2 cm was distributed into 250 mL Erlenmeyer flasks containing 100 mL of distilled water. The flasks were sterilized at 121°C for 25 min and inoculated. The inoculum was prepared by picking a loop from cultures on slants and suspending in saline water to have an OD_{600nm} of about 0.4.

Batch assays

One of the isolated strains identified as *Bacillus licheniformis* and the reference strain, *Bacillus subtilis* ATCC 6633 were compared for degradation of feather waste. Total protein, non-protein nitrogen and growth were monitored for 16 days.

Table 1. Chemical composition of feather waste used in experiment.

Component	%
Protein	81
Fat	1.2
Dry matter	86
Ash	1.3

RESULTS AND DISCUSSION

Feather wastes were first characterized for their chemical composition (Table 1). Major compounds including proteins, fat and ash were determined. The protein content is high compared to other animal wastes, with an average value of 81%. Fat content and ash represented 1.2 and 1.3%, respectively. The chemical composition of feather wastes indicates a very balanced medium, which may not need other nutrients for culturing the degrading microorganisms.

Eight isolates identified as strains of *Bacillus* were first screened on the feather waste. Growth and protein digestion were determined to compare the activity of these isolates with the reference strain, ATCC 6633. Results showed that two of the isolated strains, *B. licheniformis* and *B. subtilis*, were the most efficient in feather hydrolysis. The final amount of protein was 2.02% and 2.1%, respectively, while the medium with the reference strain had 4.75% final protein content. Also OD_{600nm} reached 5.08 and 4.98 for *B. licheniformis* and *B. subtilis*, respectively, and the strain ATCC 6633 reached only 3.48. These two strains were selected for further uses in a controlled system for both hydrolysis and growth.

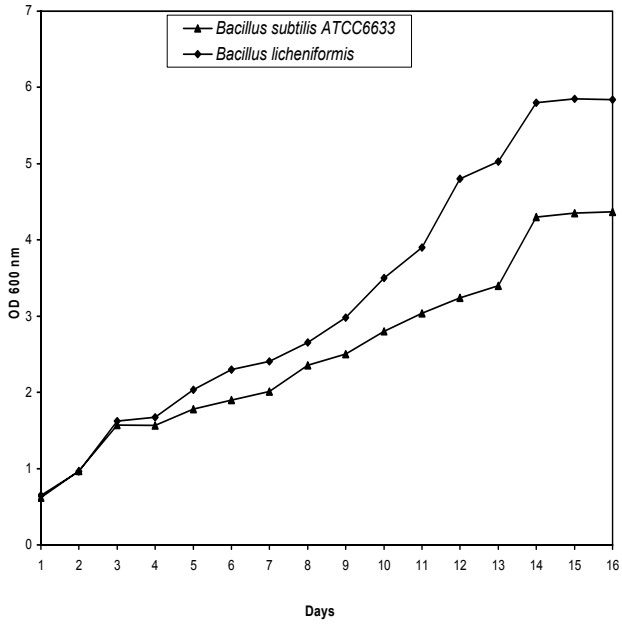

Figure 1. Growth pattern of *B. licheniformis* and *B. subtilis* ATCC 6633 on feather waste (20 g/l).

The growth pattern of the isolated *B. licheniformis* and the reference strain is depicted in Figure 1. The isolated strain showed a growth pattern higher than the reference strain. Growth was determined to access the nutritional need for the strains and their capacities to use the nitrogen and other nutrients from the feather from the protein hydrolysis. Feather waste hydrolysis by the two strains are reported in Figure 2, which showed the decrease of the total protein from 13.6% to 1.92 % for *B.*

Table 2. Final amount of protein when 8g/l of feather were hydrolyzed by strains of *Bacillus*.

Bacterial strain	11	12	15	8	1	13	X	6	*B. subtilis* ATCC6633
Proteins g/100g	2.016	2.1	2.35	2.51	2.7	3.63	2.68	2.42	4.75

Table 3. The initial and final values of pH and non-protein nitrogen (NPN).

Parameters	*B. subtilis* isolated		*B. subtilis* ATCC6633	
	Initial values	Final values	Initial values	Final values
pH	7	8.8	6.9	8.1
NPN mg/100g	1.9	43.2	2.3	20.5

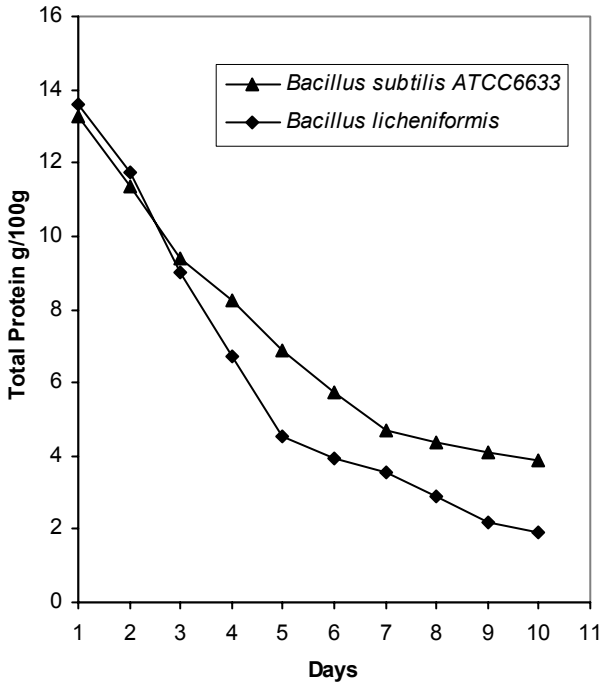

Figure 2. Total protein nitrogen profiles in the assay inoculated with the isolated strain and *B. subtilis* ATCC 6633.

licheniformis and from 12.25% to 2.99% for the reference strain. The hydrolysis most likely occur through the proteolytic enzymes produced by the strains, which degrades the β-keratin and other proteins found in feathers producing usable carbon, sulfur and energy for growth and maintenance of the bacteria (Hansen et al., 1993, Burtt and Ichida, 1999b).

Bacillus strains are ubiquitous microorganisms, which can grow on natural media without any special requirements. These properties can be exploited in the degrading feathers, which are produced in huge amounts throughout the world. Moreover, *Bacillus* strains are thermophile microorganisms and this property can be used in controlled process for efficient and fast degeneration of feathers. Other bacterial strains known by their keratinolytic activity include *B. pumilis* (Burtt and Ichida, 1999a), *Streptomyces pactum* (Bokle et al., 1995) and *Streptomyces fradiae* (Kunert, 1989; Sinha et al., 1991), *Bacillus* spp. (Kao Ming-Muh and Hsing-Yao, 1995). *B. licheniformis* is, however, very convenient for the feathers degradation because of its thermophylic properties, their ability grow at high temperatures (50 - 65°C). Results of the NPN analysis (Table 3) showed that the hydrolyzing activity of the isolated strain is higher (43.2 mg/100g) than the reference strain (20.5 mg/100g).

Keratinasases are enzymes that can hydrolyze both native and denatured keratin. The enzymes are widely used not only in chemical and medical industries, but also in animal feed industry and basic biological science (Ichida, 2001). Several studies on *B. licheniformis* (Ichida, 2001; Evans, 2000; Zhao et al., 1998) confirmed the high activity of kertinase produced. Therefore enzymatic hydrolysis of feather wastes or dead chickens 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.

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