Original Article

Utilization of beaks as alternative Growth medium for Fungi

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ABSTRACT

Fowl beak is a solid waste generated from poultry farms between age 1 to 5th days of hatching, and also between 8th and 12th weeks. If sealing is not properly done, the beak can grow again. Degradation of beak by biological methods has been increasingly interesting because of environmental awareness. The study was done by isolation of soil fungi on enriched media using dilution techniques, spread and pour plate method. Beak powder was used to prepare beak both using mineral salts components and the selected isolates were inoculated into the beak medium. From the fermented beak media, parameters such as pH, protein, sugar, total viable and optical density were determined. In this study, different species of fungi were isolated from poultry soil which includes *Aspergillus niger, A. flavus, Penicillium spp, Rhizopus spp, Fusarium spp, Saccharomyces cerevisiae* and *Mucor*. Two isolates were selected after growth on solid medium with beak meal as sole carbon and energy source. *A. niger* and *Saccharomyces cerevisiae* were chosen due to their occurrence and prevalence on the medium used for degradation during fermentation of a basal medium containing 1% beak. This was done at room temperature in an orbital shaker set at 120rpm for 12 days. The fermented wort was screened for protein activity, sugar activity, total viable count, optical density and pH. It was observed that there was a decreased in sugar content, decreased in the pH, increased in the protein content, increased in total viable count and increased in optical density as the days of fermentation increases. As a result, it was concluded that the beak can serve as a cheap growth medium for fungi.

Key word: Beak, Poultry, Fermentation, Microfungi, Soil.

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INTRODUCTION

The soils represent the main reservoir of microfungi, some of which are potential pathogen to both human and animals. Soils that are rich in keratinous materials are the most conducive for the growth and occurrence of keratinophilic fungi (Mohammed, *et al.,* 2000). The poultry farm yard, park soils, as well as sediments of the rivers and oceans contained humus and organic material which are the best source for growth of keratinolytic fungi (Mohammed *et al*., 2000; Moallaei *et al*., 2006).

Beak is the hard pointed or curved outer part of a bird's mouth. It is used for eating, grooming, manipulating objects, killing preys, probing for food, courtship and feeding the young birds. In poultry farm, de-beaking is commonly practiced to blunt the beaks enough so that pecking cannot do any great damage. Re-trimming can also be carried out if a bird's beak grows back enough to cause pecking damage. Beak itself is commonly used as animal food stuffs or meal. Beak degradation by biological methods has been increasingly interesting because of environmental awareness. Beaks vary significantly in size and shape from species to species. The beak is composed of an upper jaw called the maxilla and a lower jaw called mandible. The jaw is made of bone, typically hollow or porous to conserve weight for flying. The outside surface of the beak is covered by a thin horny sheath of keratin called rhamphotheca (Moallaei *et al.,* 2006).

Keratin is also an insoluble macromolecule requiring the secretion of extra cellular enzymes for biodegradation to occur. Keratin comprises long polypeptide chains which are resistant to the activity of non-substrate specific proteases. Adjacent chains are linked by disulphide bonds though responsible for the stability and resistance to degradation of keratin (Safranek and Goos, 1982; Tamilmani *et al*.,_2008). However, despite their rigidity, they are efficiently degraded by some bacteria, actinomycetes and fungi. (Ramnani *et al*., 1997).

The degradation of keratins material is important medically and agriculturally. Secretion of keratinolytic enzymes is associated with dermatophytid fungi, for which keratin is the major substrate (Matsumoto, 1996). However, the production of such geophilic species have demonstrated keratinase production (Kushwaha and Vigam, 1996; Nigam, 1996).

The expansion of biotechnology has produced an increasing demand for high-quality inexpensive microbial growth media. Utilization of beaks from the poultry processing industry as a substrate for fermentation might offer an inexpensive alternative for a microbial method of metabolite production, such as sugar enzymes and unicellular protein. These metabolites production can be achieved provided an efficient utilization of beak media (other poultry by-product are feathers that are rich in protein mainly keratin) and generated in very large quantities as a waste product from poultry processing industry. For many years, they have been the object of nutritional studies in order to incorporate them as nitrogen

supplement in animal food stuff (Xiang Lin *et al*., 1992; Nereida Coello and Luis Vidal, 2001).

In this present investigation, utilization of beak as alternative source of growth media for fungi was evaluated.

MATERIALS AND METHODS

Collection of Materials

Soil samples were collected randomly from poultry farm at various sites in Kwara State Polytechnic Quarters Ilorin. The samples were collected from horizon A (2–4cm) with sterilized spatuler. The samples were transferred immediately to the laboratory in sterile, tightly closed polythene bags and kept for some weeks.

Isolation of Organisms from Soil

This was carried out by employing the method of Naroida and Luis, 2001) which involved preparation of enriched media to isolate soil microbes using serial dilution techniques, spread method and pour plate method were inoculated with 0.1 mililitre of inoculum on PDA. All the plates were incubated at room temperature for 48 to 72 hours. After the incubation, subcultured were carried out to obtain pure cultures.

Identification of Fungal Isolates

This was carried out using the method of Moallael *et al.,* 2006; Sparrow, 1960, Alexopoulous and Mims 1974; and Alabi, 1994 based on the criteria such as colour, structure of the spores and conidia, and nature of hyphae.

Degradation and Fermentation of Beak by Microbial Isolates

Beaks were collected from a nearby poultry farm in Kwara State Ilorin during the de-beaking or trimming of the fowls mouth. They were ground into a powdery form using mortal and blender.

Cultivation of Fungal Isolates

Out of many isolates obtained, two fungi were chosen (*Aspergillus niger* and *yeast*) and these were inoculated into PD broth tubes separately.

Beak broth was prepared by using 0.5g of NaCl, 0.1g $MaSO_4$, 0.4g KH_2PO_4 and 10g of beak powder. These were dissolved in 100ml of distilled water, stirred properly, distributed into 250ml conical flask and then autoclaved. After cooling, from PD broth containing A. niger and *Saccharomyces cerevisiae* were taken, 10% of spores of *A. niger* and 10% of *Saccharomyces cerevisiae* of 0.5 MacFarland Standard ($1.5xd10^6$cfu/ml) of *Saccharomyces cerevisiae* extract was inoculated into the beak broth separately and in mixed culture. All the inoculated flasks were incubated on orbital shaker set at 120rpm for 12 days at 28 ± 2^0C. The control experiment contained only beak medium without inoculum.

Determination of Some Parameters

The following parameters were determined from the fermented beak media at different days of fermentation from 0 hour to 12 days: The following parameters: pH, protein, sugar, total viable count and optical density were determined using the methods below under each parameter.

(i) **Determination of Protein Content:** Total protein was determined by the method described by Lowry (1951) and by Kjedhal method described by the APHA (1989) used by Iham *et al.,* (2004). This was carried out by measuring 2ml of fermented beak medium and transferred into a kjeldahl digestion flask. One Kjeldahl tablet was added as catalyst to the digestion flask. A Kjeldahl tablet is made up of 1g $MgSO_4$; 1g of $CuSO_4.5H_2O$ and 2ml of concentrated sulphuric acid was added. The flask was heated gently in an inclined position initial, but when the trothing ceased, strong eat was applied for 1 hour and the distillation was carried out to determine the protein content.

(ii) **Sugar Content** was determined by colorimetric method b modifying the Ofuya and Nwajiuba method (1990) and Iham *et al*., (2004) . The beak wort or medium of varying amounts (0.1ml – 0.5ml) were mixed with concentrated sulphuric acid of specific gravity 1.84 and phenol 5%. The mixtures were put in separate clean tubes and each was made up to 1ml by adding distilled water. The content of each tube was mixed together by shaking and 1ml of phenol was added. This was followed by the addition of 5ml of concentrated sulphuric acid rapidly but carefully by directing the acid against the side of the tube and allowed to stand for 10 minutes, shaken together again and placed in water bath for 15 minutes. The optical density of the mixture was read at 490nm wavelength in a spectrophotometer.
The blank was used for zeroing and was prepared by substituting distilled water for fermented beak medium.

(iii) The pH of the fermented broth was determined using a pH meter (electronic). pH meter (model = Hanna pH209 model HW 268).

RESULTS

The isolated fungi from the poultry soil samples were identified as *Aspergillus niger, A. flavus, Rhizopus species, Penicillium species, Fusarium species, Saccharomyces cerevisiae* and *mucor.* Out of the isolates, only *A. niger* and *Saccharomyces cerevisiae* were selected to grow on medium containing beak meal as sole carbon and nitrogen sources. The two fungi acted on beak during the fermentation to produce protein and enzyme when the fermented beak media were tested due to the production of proteolytic enzyme by

the isolates during the incubation on orbital shaker. The results are summarized in table 1 – 3. At initial hour (i.e. zero hour) of fermentation, there was no protein or enzyme production because the organisms inoculated have not acted on the beak media. As the length of fermentation increases major compounds detected was protein.

Table 1: Shows the sugar content, protein content, total viable count, optical density and pH *(Aspergillus niger).*

Days	Sugar Content (mg/ml)	pH	TVC (cfu/ml)	Optical Density	Protein Content (mg/ml)
0	0.0	0.0	0.0	1.101	0.0
4	5.5	6.8	3.0×10^4	1.236	1.203
8	8.0	7.0	4.5×10^5	1.831	3.321
12	4.2	4.0	6.3×10^6	2.563	3.732

Table 2: The sugar content, protein-content, total viable count, pH and optical density (*Yeast*).

Days	Sugar content (mg/ml)	pH	TVC (cfu/ml)	Optical density	Protein Content (mg/ml)
0	0	6	0	0	0
4	5.8	5.5	4.0×10^5	1.101	1.013
8	8.0	6.3	6.2×10^6	2.100	3.532
12	4.0	4.5	2.1×10^8	2.652	3.821

Table 3: Shows sugar content, protein content, pH, total viable and optical density in fermented wort with *A. niger* and *Saccharomyces cerevisiae* cultures.

Days	Sugar content (mg/ml)	pH	TVC (cfu/ml)	Optical density	Protein content (mg/ml)
0	—	—	—	—	—
4	5.8	6.7	6×10^6	1.403	1.036
8	9.3	7.3	3.1×10^7	2.861	4.017
12	4.2	5.1	2.3×10^8	3.103	4.852

From table 1 – 3, the decreased in sugar production was observed after 8 days of degradation of beak by *A. niger* and *yeast;* while there was increased in the protein content from 8th days to 12th date of fermentation due to rapid production of enzyme into the medium by the organisms. The pH value also reduced as the length of fermentation increased. Total viable count and optical density increased in the fermented wort with *A. niger*; *Saccharomyces cerevisiae* and in the fermented worth with *A. niger, Saccharomyces*

cerevisiae and in the mixed culture. Moreover, there were changes in the pH values, during the 4th – 8th days of fermentation, it increased from acidic level to neutral level. In the control experiment, no changes in the beak media.

DISCUSSION

This investigation indicated that beak meal promoted cell biomass production concomitant to beak degradation. The increased in the protein value during the beak fermentation was an evidence that the microorganisms produced enzyme(s) capable of digesting keratin of beaks to produce peptides and amino acids that can be used as carbon and nitrogen sources for the growth of the organisms and this was similar to the observation of Nereida and Luis, (2001) during the fermentation of feathers using bacteria. The microorganisms used in this work present different characteristics because the optimal activities were detected between 28 ± 30^0C, whereas, as discovered by other authors that keratinolytic organisms such as bacteria mostly have feather - degrading activity at elevated temperatures (Mohammedin, 1999).

An increased in pH values was observed during the early period of beak fermentation but decreased toward the end which was a trend similar to other microorganisms with large keratinolytic activities. This trend may be associated with proteolytic activity, consequent deamination reactions and the release of excess nitrogen as ammonium ions (metabolites). This increased in pH was an important indication of the ability of the organisms used in degradation of beak meal. This is in line with the observation of Moallaei *et al.* (2006). The fungi employed in this study were both active since the maximum protein production was observed during the incubation period due to increase in enzyme production. The protein content of beak is high compared to other animal wastes and the chemical composition of beak generally indicates a very balanced medium which may not need other nutrients for culturing the degrading organisms. This observation was corroborated with the findings of Iiham *et al.* (2004) during the degradation of feather waste.

Growth inform of total viable count was determined to access the nutritional need for fungi strains and their capacities to use the nitrogen and other nutrients from the beak from the protein hydrolysis. The sugar content decreased as the length of degradation increased. The ability of the soil isolates to produce enzyme in the fermented medium was in line with the report of Ofuya and Nwajiuba 1990; Adebiyi and Adeyanju, 1998; Iyayi and Losel, 2004; Agboola, 2009) to mention a few. The presence of sugar during the degradation of beak did not affect the production of protein in this study. This was deviated from the findings of some authors who reported that proteolytic activity is frequently suppressed by sugar (e.g. glucose) (Brasch *et al.,* 1991). Also from the study, there was an increased in optical density value. This was because as the fermentation days increased, there was increased in microorganisms body component; since optical density is to determine how the

component were closely or loosely packed to light intensity.

CONCLUSION

It can be concluded that enzymatic hydrolysis of beak wastes could be a safe method of recycling these organic materials and alternative source of fungal growth medium. Beak wastes management should be encouraged and the isolated fungi may also be evaluated in the treatment of other kind of wastes. This would be beneficial for the utilization of these residues. This study present potential biotechnological use in processes involving keratin hydrolysis. Finally, to my knowledge this is the first report concerning beak degradation. Further research concerning qualitative and quantitative keratin content and factors influencing keratin production from beak must be done as some authors had done in feather degradation.

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