MICROBIAL ENHANCEMENT OF THE PINK SHRIMP (*Penaeus notialis*) WASTE

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Abstract
Shellfish processing generates a large amount of wastes which is rich in chitin and protein. This shrimp waste is indiscriminately disposed resulting in environmental pollution. Biofermentation of this waste could enhance its protein content and improve digestibility, making it a cheap alternative for supplementing fish meal, which is expensive. In this study, bacteria and fungi were isolated from shrimp waste. Two bacteria (Bacillus subtilis, Serratiamercescens) and 2 fungi (Thanatephoruscucumeris,Penicilliumcorylophilum)were selected (based on their ability to degrade chitin) and used for solid state fermentation of shrimp waste and then proximate analyses of the fermented products were carried out. The fermentation of the shrimp waste with the four organisms increased the nutritional quality of the substrates.Bacillus subtilis gave the highest increase in crude protein (28.95%) Hence, the bioconversion of shrimp waste into products with enriched microbial biomass could be achieved through solid state fermentation. In addition, such products could be used to develop simpler components with higher nutritional value. These simpler components could be applied as a feed ingredient in livestock and aquaculture industries.
Keywords: Shrimp waste, biofermentation, chitinases, microbial protein, livestock feed

Introduction

Worldwide fishery production was estimated to be about 144 million tonnes in 2006, 36% of which was from aquaculture, and 33% used for non-food purposes. Shrimp trade represents 16% of the total value of internationally traded fishery products (FAO, 2009) and Nigeria supplying an abundant amount of fin and shell fishes from her continental shelf.

For most shellfish (such as shrimp, crab, and krill), 50 -60% of their total weight are nonedible materials, i.e., "heads" and exoskeletons which are rich in chitin and protein (Jo *et al*., 2011).

Shrimps, whose demand has been on the increase in recent times, are usually processed before their exportation or sale in supermarkets. Shrimp deteriorates quickly within a short period of time as a result of the high protein content. The spoilage of shrimp often starts from the head, thus, they are processed and the head and shell removed disposed indiscriminately.

The uncontrolled dumping of this highly perishable waste in tropical environments results in environmental pollution, with decay starting within an hour of processing. Microbial decomposition results in the production of biogenic amines with a very offensive smell. Consequently, this biomaterial becomes an environmental health concern and sometimes a financial burden (Kandra *et al*., 2012).

Shrimp waste contains about 30 - 40% protein, which is not easily accessible due to the presence of exo-skeletal chitin and ash (Bhuiyan, 1989). This chitin component could be potentially broken down by bioconversion/ biofermentation. Bioconversion of waste is the transformation of complex organic substances into simpler compounds by enzymes and microorganisms through solid state or submerged fermentation. It is a cost-effective and environment friendly procedure for waste utilization (Healy *et al*., 1994). Therefore, microorganisms; which have the ability to break down chitin through the production of chitinase (an enzyme capable of degrading the complex structures of chitin into simpler sugar molecules and other essential minerals needed in animal feeds), could be used as bioconversion agents (Amar, 2001).

The biofermentation of shrimp waste into products with enhanced nutritional quality, is potentially promising in livestock and aquaculture feed development as well as environmental sanitation (Wang *et al*., 2001). Therefore, this study assessed the chitinolytic microorganisms from shrimp waste and the nutritive value of the biofermentation products from shrimp waste that was exposed to selected bacteria and fungi.

Materials and Methods

Isolation, Identification and Selection of Chitinolytic Microorganisms

Fresh shrimp waste of Pink shrimp (*Penaeus notialis)*, was collected from shrimp processors at Ijora market, Lagos, Nigeria. The waste (made up of shrimp heads and shells) was collected in sterile polythene bags, placed on ice in an insulated container and transported to the laboratory for further processing. In the laboratory,

the waste was washed and cultured on Nutrient Agar supplemented with colloidal chitin following the method of Zarei*et al.*,(2010). The cultures were incubated at 30°C for 3 days for bacteria and 7 days for fungi growth. For the isolation of fungi, Rose-Bengal was added to Potato Dextrose Agar supplemented with colloidal chitin to prevent bacteria growth. The time prior to beginning the microbiological analysis did not exceed 3 hours.

The isolates obtained were identified based on their cultural, microscopic and molecular characteristics. Qualitative cup - plate assay was used for the selection of isolates. This qualitative screening was based on the appearance of clear zones around chitinolytic/ chitinase-producing organisms on colloidal chitin agar as a result of the degradation of chitin due to chitinase enzyme production by the microorganisms (Zarei *et al*., 2010; Park *et al*., 2000). Thus, Clear-zone forming bacteria were selected as the chitinase producers.

Hence, four isolates with larger clear zones, showing higher rate of chitin utilization were selected.

Preparation of substrates

This was done according to the modified method of Amar (2001). The waste was washed, oven-dried at 60°C for six hours, milled and preserved in air-tight container for further analysis. Milled sample (30g) was weighed into conical flasks and 45 ml of 50% sterile seawater was mixed with it to obtain a ratio of 1:1.5 (w/v) and covered with aluminium foil to avoid loss of moisture. The substrates were sterilized by autoclaving at 121°C and 760mmHg for 15 minutes and then kept at room temperature, ready for solid state fermentation.

Test Organisms

Four microorganisms were used for the fermentation process. The bacterium (*Bacillus subtilis*) and fungi (*Thanatephoruscucumeris* and *Penicillium corylophilum*) were isolated from shrimp wastes, while *Serratiamerces cens*isolate was obtained from the culture collection of the Environmental Microbiology Unit of the School of Biological and Environmental Studies, University College Dublin (UCD), Dublin, Ireland.

Preparation of inoculum

Selected bacteria isolates were inoculated in 50 ml of pre-culture medium (Zarei *et al*., 2010) for 24 hours at 30°C while fungi isolates were cultured on potato dextrose agar for seven days at 30°C and the spores harvested into 10ml sterile distilled water.

Fungal Spore count

Cell suspension preparations were done by harvesting 7 day old cultures into 10 ml sterile distilled water using a sterile wire loop. About 0.01 ml of the spore suspension was dispensed into the Neubauer Chamber Haemocytometer and covered with a cover slip. The Haemocytometer was then placed under the x40 objective lens of the microscope until the grids containing the cells came into focus. The grids with cells were counted diagonally in two directions and recorded. The number of cells per ml was then calculated using the Neubauer chamber calculation.

Fermentation and shrimp wastes analysis

The prepared samples were inoculated with 6 ml of the bacteria inoculum (1:5 (v/w)), while 1.2 ml of *Thanatephoruscucumeris* spores and 0.5 ml *Penicilliumcorylophilum* spores (about 1 x 10^6 spores/ml) were used for the solid state fermentation. Each treatment was done in duplicate. The experimental units were incubated at 30°C and analysed after 7 and 14 days. Similarly, prepared substrate without bacteria and fungi inoculum was used as control.

Proximate Analysis of Fermented Shrimp Waste

The proximate analysis of shrimp waste was done before and after fermentation. The proximate analysis of fermented wastes was done on day 7 and 14. The moisture, crude protein, crude fibre and ash content were determined following the method of AOAC (1990).

The percentage change in crude protein after fermentation was estimated using Equation 1:

$$\%\ Change\ in\ crude\ protein = \frac{FCP-ICP}{ICP}\ X\ 100 \quad \ldots\ldots (1)$$

Where; FCP = Final crude protein of the samples, ICP = Initial crude protein at day zero.

Results

Six bacterial and 4 fungal isolates were obtained. They belonged to the genera *Bacillus* (5),*Aeromonas* (1), *Aspergillus* (2), *Thanetophorus* (1) and *Penicillium* (1). They all showed clear zone on colloidal chitin agar.

There was an appreciable increase in the crude protein of all the fermented samples after 7 and 14 days (Table 1), except that of the control. The crude protein ranged from 26.89% and 31.28% on day 7, and 23.24% and 41.45% on day 14. The sample fermented with *Bacillus subtilis* had the highest crude protein (41.45%) and the least ash content (8.19%) after 14 days. The crude fibre ranged between 0.22% and 1.11% (Fig. 2), the ash was between 8.19% and 12.44% (Fig. 3), fat was between 2.14% and 6.41%, while the moisture content was between 4.24% and 7.41%. Similarly, *Bacillus subtilis* fermented products recorded the highest increase in crude protein (54.15%) after 14 days, while *Penicilliumcorylophilum* gave the least increase in crude protein (Fig. 1). The *Thanatephoruscucumeris* resulted in the highest

reduction of crude fibre content (79.63%) after 14 days of fermentation, while the *Bacillus subtilis* caused the least reduction (60.19%) (Fig. 2).

The ash content of the fermented products also decreased with the highest observed for products fermented using *Bacillus subtilis* isolate (34.16%), while the least reduction was obtained with *Penicilliumcorylophilum*(Fig. 3).

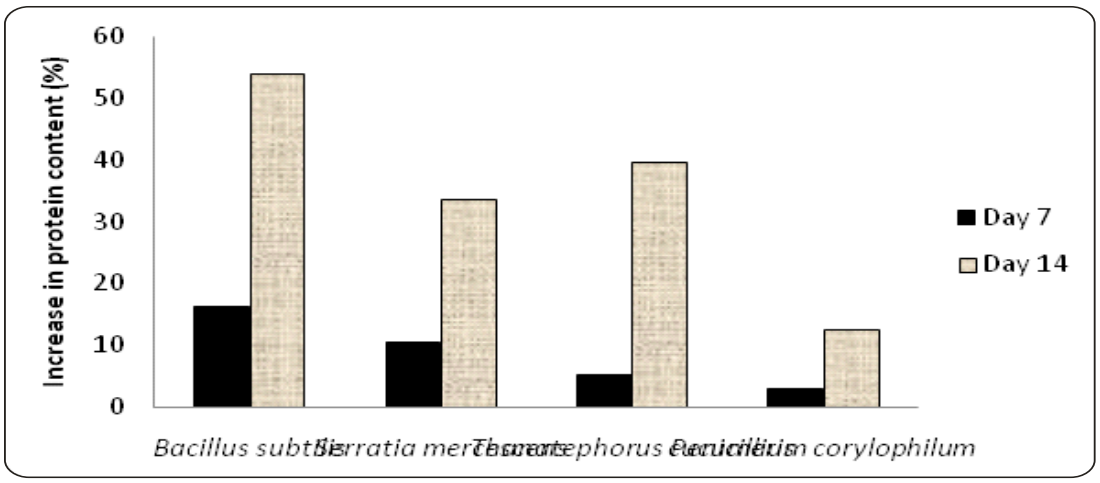

Fig. 1: Change in crude protein Content of Shrimp Waste fermented with different microorganisms

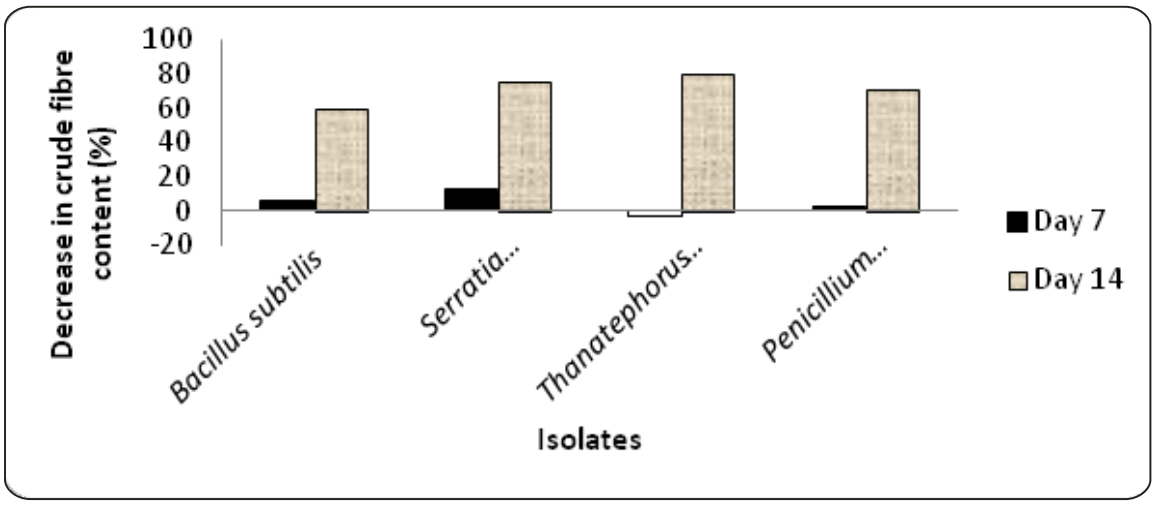

Fig. 2: **Change in crude fibre content of Shrimp Waste fermented with different microorganisms**

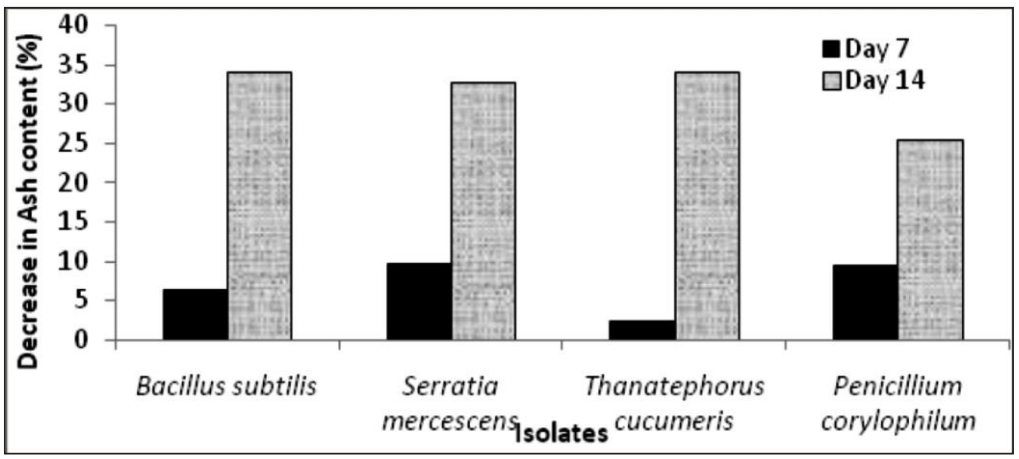

Fig. 3: **Change in ash content of shrimp waste fermented with different microorganisms**

Discussion

The screening of the microorganism for chitinase production is essential when assessing their chitinolytic activities. This becomes pertinent with the recent advances in biotechnology, which encourage the use of microbial enzymes as alternatives to artificially synthesized ones . Invariably, screening of large numbers of microorganisms in order to discover the enzymatic activities of the organisms is becoming important (Aminzadeh *et al*., 2006).

Cody *et al.*, (1990), also observed the chitinolytic activities of some bacteria genera such as;*Achromobacter, Aeromonas, Arthrobacter, Bacillus, Clostridium, Corynebacterium, Cytophaga, Enterobacter, Flavobacterium, Haloanaerobacter, Micrococcus, Pseudomonas, Serratia and Vibrio.* These organisms synthesized chitinases in order to effectively break down chitin and use it as a source of carbon and energy (Shubakov and Kucheryavkh, 2004).

The fermentation of the shrimp waste with microorganisms resulted in an increase in its nutritional quality. The observed increase in crude protein content; could be due to the production of extracellular enzymes such as chitinases and cellulases by the fermenting organisms. This might also be due to the hydrolysis/transformation of the shrimp waste, the formation of extracellular metabolites by microorganisms and biomass build up (Amar *et al*., 2006; Oboh *et al*., 2002; Iyayi and Aderolu 2004). It has been suggested that the increase in the protein content of a fermented products is consequent to the increased growth and proliferation of microbial complexes such as single cell proteins alongside the bioconversion of complex sugar into mycelia protein (Iyayi and Aderolu, 2004).

The potentials of microorganisms for protein enrichment of shell waste have been promoted in the past. For instance, Rhishipal and Philip (1998) researched the use of marine yeasts for biotransformation of prawn shell waste to single cell protein and they observed a substantial increase in the crude protein content of the fermented product. Also Amar (2001) used *Bacillus sp.* for the biofermentation of prawn shell wastes, and reported a 53-56% increase in the crude protein content. The fermented products were then used for shrimp feed. Fermented products produced from *Bacillus sp.* fermented shrimp wastes have also been used as replacements for fish meal in broiler feed (Hardini and Djunaidi, 2010).

Furthermore, the increasing crude protein is intricately linked to microbial biomass, with increased growth rate of the microorganisms as the substrate was being degraded. Hence, the variation in crude protein among the fermented products could be linked to the rate of isolates' colonization and biodegradative ability. *Bacillus subtilis* which was isolated from the waste had a higher rate of biodegradation.

The variations observed among the crude fibre and ash content of the different fermented products has been attributed to different microorganisms utilizing different complex sugars at different rate (Theodrorou *et al*., 1989). Onilude (1999) had reported that some microorganisms have the capacity to convert crude fibre into protein, during a study that monitored solid state fermentation of poultry litter. An observed loss of crude fibre content might probably be due to the activities of microbial enzymes.

Bacillus subtilis also reduced the ash content of the waste substantially. This is a significant finding because high ash content of shrimp waste hinders the use of shrimp waste as a substitute for fishmeal as it reduces accessibility to the protein stored in the waste and thus results in limited bioconversion (Bhuiyan, 1989).

Conclusion

Fermentation of shrimp waste is a viable way of enhancing its protein content to produce a value-added product which can be useful in the aquaculture industry as a substitute for fish meal. It is also an excellent way of preventing pollution.

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