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Bio-Efficiency of Paediococcus acidilactici ATCC 8042 for Recovery of Chitin and Carotenoids in the Fermentation of Shrimp Biowaste

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Abstract: Fermentation based on Lactic acid bacteria (LAB) hold great promise for the management of a shrimp biowaste. Although shrimp biowaste is usually dried on beaches it encourages not only environmental pollution but also reduces the recoverable components. Experiments were carried out to test the *Paediococcus* bacteria for recovery of chitin and carotenoids. *Paediococcus acidilactici* ATCC 8042 was found to be the efficient among three cultures. Optimized conditions were found to be 5% inoculum, 15% glucose, 2% NaCl and 72 h of incubation time at 37ºC. The pH of 3.04 and total titrable acidity of 2.204 was achieved at the end of 72 h. The effect of fermentation shrimp biowaste by *P. acidilactici* ATCC 8042 on the production of chitin and recovery of carotenoids was studied. Deproteinization of 96.2% and demineralization of 71.9% was achieved by fermentation of shrimp biowaste. The carotenoid recovery in fermented shrimp biowaste was 70.6%. Thus, *P. acidilactici* was found to have a synergistic effect on fermentation of shrimp biowaste

***Keywords*:** Shrimp biowaste, *Paediococcus*, LAB, Deproteinization, Demineralization, Carotenoid.

Introduction

The important waste material in shrimp processing industries is head and shell waste, which comprise about 40- 45% of whole shrimp waste[1]. Shrimp is a high value aqua cultural product and is processed for the meat, leaving the carapace and head as waste products[2]. Making use of such waste has been of interest to researchers for two reasons. First those wastes are highly perishable and create environmental pollution[3, 4]. Second, they are rich sources of chitin and protein[5, 6]. Shrimp biowaste being alkaline (pH 7.5-8.0) supports the growth of undesirable putrefying micro .flora resulting in spoilage[7, 8]. The fermentation of shrimp biowaste results in the removal of protein, minerals in addition to obtaining crude chitin, a liquor fraction rich in protein and astaxanthin. Traditionally, chitin preparation from shrimp biowaste involves the use of alkalies (4% NaOH) for deproteinization and acids (4% HCl) for demineralization, making this process expensive, ecologically aggressive and source of pollution[9, 10]. While the chemical process isolates chitin efficiently, the protein and carotenoid components are rendered useless during protein removal and demineralization stages[11]. Partial

fermentation of this biowaste using microorganisms for the production of chitin has been studied[9]. The usefulness of fermentation is mainly due to its eco-friendly nature compared to more ecologically aggressive and economically unviable methods like acid/ alkali treatment or drying[12].

Fermentation of shrimp biowaste using lactic acid bacteria for the production of chitin[13, 14, 15, 16], carotenoids[17] has been reported. It has successfully optimized processing conditions which include sugar level (carbon source) varying from 5 to 20 %, inoculum levels varying from 5 to 20% and incubation time ranging from 24 to 72 h[15, 16, 18]. A wealth of information is available on biological ensilation using lactic acid bacteria cultures.

Shrimp is a source of carotenoid pigments. These compounds soluble in lipids are the factors that give yellow-red color to plant and animal products. In this group of pigments astaxanthin has important applications in human and animal food industries specifically in pharmaceutical and cosmetic industries. The food and drug administration of United States has permitted it for use in the aqua- cultural industry[19]. The main sources for this pigment can be found in many sea foods. Astaxanthin has effects on body functions like prevention from oxidation of essential unsaturated fatty acids, prevention from effects of ultra violet light, immunological reactions, pigmentation, reproduction, prevention of degenerative diseases such as atherosclerosis, cancer, aging and eye diseases[20]. The objective of this research is to evaluate the bio-efficiency of *Paediococcus acidilactici* ATCC 8042 for recovery of chitin and carotenoids.

Materials and Methods

The shrimp (*Peanus monodon*) biowaste was obtained from shrimp processing industries located at Bhimavaram, West Godavari district and Visakhapatnam, Andhra Pradesh. It was transported to the lab under frozen conditions and kept at -20°C till further use.

Microorganism and preparation of inoculum:

The lactic acid bacteria (LAB) culture *Paediococcus acidilactici* ATCC 8042 was obtained from National collection of industrial microorganisms NCIM (Pune). The culture was maintained on Mann-Rogosa-Sharpe (MRS) agar (Hi-media, India) slants and stored at 4°C and subcultures were made periodically[21]. All the chemicals used for the study were of analytical grade (AR).

Preparation of starter culture:

The frozen shrimp waste was thawed and minced in a warring blender (Local made) for 10min to homogenize the mass. The LAB culture was grown in 100 ml of MRS broth (Hi-media, India) for 24 h at 37±1°C in an orbital shaker (Remi-India Ltd.) set at 100 rpm. The cells were harvested by centrifuging (Model C24, Remi-India Ltd.) at 3000rpm for 10 min. The harvested cells were washed twice with physiological saline. The inoculum was assayed by counting colony forming units (cfu) on MRS- agar (Hi-media).

Effect of fermentation of shrimp biowaste for recovery of chitin and carotenoids:

For the initial screening experiments, 100g of shrimp biowaste was mixed with 100ml of distilled water (1:1 v/w) then 10% inoculum (v/w) with carbon source 15% (w/w) o f glucose and 2% (w/w) NaCl was added to 250ml conical flasks. The flasks were sealed with a layer of parafilm (Hi-media, India) to create anaerobic conditions. The mixture was allowed to ferment for 72 h in an orbital shaking incubator at 100 rpm and 37 ±1°C. The pH and Total Titrable Acidity (TTA) were noted at 0, 24, 48 and 72 h. The pH was determined by using a digital pH meter (model: Elico-101, India Ltd). TTA was estimated as per the method described in Sacchindra *et al*., [17]. Each sample was filtered using cheese cloth to collect the fermentation liquor. The residue was washed for three times with distilled water and one gram can be used for estimation of carotenoids remaining residue was dried at 60°C overnight in laboratory oven (Labomed India Ltd.). The dried sample was referred to as crude chitin.

Protein and ash contents in the fresh waste without fermentation were considered as the basis for computing demineralization (DM) and deproteinization (DP) efficiency. Protein content in the sample was estimated by a modified form of *Lowry* method[22] after digesting 100 mg dried material with 10 ml of 0.5 M NaOH for 4 h[11] at 40°C and the data was used for computing DP efficiency. Ash content was determined as per AOAC method[23] and the data was used for DM efficiency. Demineralization (DM) was calculated by using the following equation where A_0 and A_R are ash concentrations (g/g) before and after fermentation. O and R the mass (g) of the original sample and fermented residue respectively[9].

$$\%DM = \frac{[(A_O \times O] - (A_R \times R)]}{A_O \times O} \times 100$$

Deproteinization (DP) was calculated using the same equation where P_o and P_R are the protein concentrations (g/g) before and after fermentation. O and R are the mass (g) of original sample and fermented residue respectively[9].

$$\%DP = \frac{[(P_O \times O] - (P_R \times R)]}{P_O \times O} \times 100$$

The fermentation liquor and wet residue were analyzed for their total carotenoid content described by Sacchindra *et al.,* [24]. The sum of total carotenoids extracted in both fermented liquor and residue was compared to the total carotenoid content in the shrimp biowaste to express recovery percentage.

Table 1: Changes in pH and total titrable acidity (TTA) of shrimp biowaste (*Peanus monodon)* fermented with different strains of *Paediococcus acidilacticic* ATCC 8042

Bacterial strains	Lab load[a]	pH			TTA		
		24 h	48 h	72 h	24 h	48 h	72 h
***Paediococcus acidilactici* (NRRL B-1117)**	8.99±0.01	4.640±0.04	4.33±0.04	4.10±0.04	0.99±0.01	1.49±0.01	1.99±0.01
***Paediococcus acidilactici* (NRRL B-1723)**	8.99±0.01	4.74±0.05	4.597±0.03	3.96±0.08	0.97±0.01	1.49±0.01	1.97±0.01
***Paediococcus acidilactici* (ATCC 8042)**	8.44±0.01	4.69±0.01	4.494±0.01	3.04±0.01	0.99±0.01	1.57±0.01	2.20±0.01

pH and TTA of the original shrimp biowaste were 8.01 and 0.0000 respectively.
[a]Total bacterial strain load in the shrimp waste; cfu g^{-1} calculated from the counts in the inoculum.

Table 2: Effect of *P. acidolactici* ATCC 8042 on the deproteinization (DP) ,demineralization (DM) and chitin recovery of shrimp biowaste (original wet weight of shrimp biowaste –100g).

Sample	Chitin [a](g)	Protein[b] (g)	Ash[b] (g)	DP%	DM%
0 h	12.50±0.08	0.90±0.01	2.48±0.04	27.4±4.4	33.0±1.5
24 h	10.65±0.15	0.72±0.02	1.96±0.04	62.1±4.2	68.4±0.8
48 h	6.40±0.04	0.18±0.01	1.28±0.04	81.2±1.4	71.5±1.4
72 h	6.28±0.08	0.09±0.00	1.06±0.04	96.2±0.4	71.9±1.8

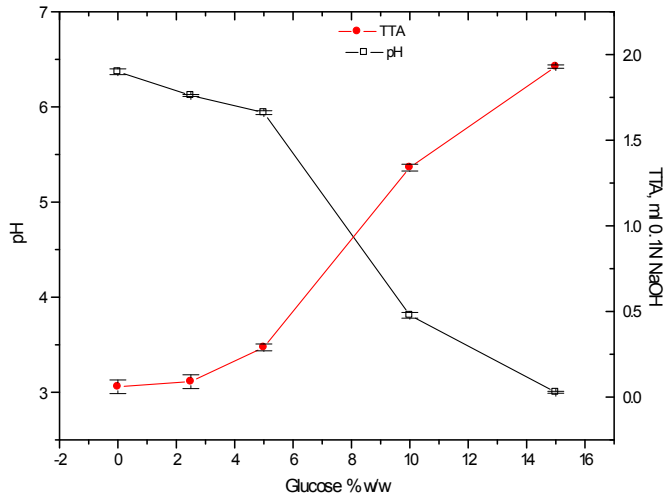
[a] All the values on dry weight basis.
[b] Mass of the content.

Table 3: Effect of *P. acidolactici* ATCC 8042 on the recovery of carotenoid content (µg) of shrimp biowaste (original wet weight of biowaste – 100g)

Sample	Residue wet weight (g)	Carotenoid content(µg) X	Filtrate volume (ml)	Carotenoid content(µg) Y	Total carotenoid content (µg) (X+Y)	carotenoid (% of original)
0 h	35.4±1.0[a]	2985±150	106±2	1571±45	4556±189[a]	77.4 ±1.8
24 h	32.2±1.2[b]	1528±50	100±3	2245±25	4273±70[b]	71.6±1.0
48 h	25.2±0.8[c]	1773±45	120±3	2355±40	4128±83[c]	70.3±1.2
72 h	25.1±0.6[c]	1783±50	150±4	2480±110	4240±160[b]	70.6±2.1

Values with similar letters in the same column are not significantly different ($P \geq 0.05$). Carotenoid content in original was taken as 100%.

Figure 1: Changes in pH and TTA of shrimp biowaste during fermentation with 10% *Paediococcus acidilactici* at different glucose concentrations (% w/w).



Results and Discussion

In the present investigation the shrimp waste used had a moisture content of 81.5%, the protein 28.3%, ash 18% and chitin of 12.85% on dry weight basis. Shrimp had an alkaline pH of (8.01) of wet waste. The results of screening experiments and information on *Paediococcus acidilactici* load per gram of shrimp biowaste after addition of inoculum during fermentation are presented in Table 1. As can be seen from the table *P. acidilactici ATCC 8042* was found to be the best culture for obtaining pH 3.04 Also, the pH obtained after 72 h was significantly different from all other cultures.

The effect of optimized fermentation conditions on the pH and TTA of the fermented mass at different glucose concentration were presented in Fig.1. It clearly indicates the significant reduction in pH as a result of production of acids by the *Paediococcus acidilactici* and the same was indicated by increasing TTA values during fermentation.

The effect of fermentation on demineralization (DM) and deproteinization (DP) of shrimp biowaste and chitin recovered were presented in Table 2. At the end of 72 h of fermentation 71.9% demineralization and 96.2% deproteinization was observed. The recovery of chitin and carotenoids during course of fermentation was presented in Table 3. After 72 h of fermentation the recovery of chitin and carotenoids were 6.28% and 70.6%.

This clearly indicates the usefulness of fermentation by using *Paediococcus acidilactici* as a tool to produce chitin and carotenoids biologically instead of the conventional chemical methods that employ strong mineral acids for demineralization and strong alkali for deproteinization.

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References

1. Subasinghe S., Chitin from shellfish waste-health benefits over- shadowing industrial uses, *Info fish International*, 1999, 3, 58-65.
2. Omum J.V., Shrimp waste-must it be wasted? Info fish International, 1992, 6, 48.
3. Bataille M.P and Batallie P.F., Extraction of proteins from shrimp processing waste, *Journal of Chemical Technology and Biotechnology*, 1983, 33B, 273-284.
4. Tan E.W.Y and Lee V.R., Enzymatic hydrolysis of prawn shell waste for purification of chitin. Final report R & D project, Superwised by Hall GM. Department of Chemical Engineering, Loughborough University, UK, 2002.
5. Ferrer J., Paez G., Marmol Z., Ramonee E., Garcia H and Forster C.F., Acid hydrolysis of shrimp shell wastes and the production of single cell protein from the hydrolysates, *Bioresource Technology*, 1996, 57, 55-60.

6. Synowiecki J and Al- Khateeb N.A.A.Q., The recovery of protein hydrosylate during enzymatic isolation of chitinnfrom shrimp cragon cragon processing discards, *Journal of food chemistry*, 2000, 68, 147-152.
7. Dapkevicius MDLE, Batista I, Nout MJR, Rambouts FM, Houben JH., Lipid and protein changes during ensilage of blue whiting (micromesistius Pooutassou Risso) by acid and biological methods, *Food Chem*, 1998, 63, 147-152.
8. Rao MS, Stevens W F., Quality parameters of chitosan derived from fermentation of shrimp biomaterial using a drum reactor, *J chem technol Biotechnol*, 2005, 80, 1080-1087.
9. Rao M.S, Munoz J, Stevens WF., Critical factors in chitin production by fermentation of shrimp bio wastes, *Appl Microbiol Biotechnol*, 2000, 54, 808-813.
10. Zakaria Z, Hall GH, Shama G., Lacticacid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery, *Process Biochem*,1998, 33, 1-6
11. Shirai K, Guerrero I, huerta S, Saucedo G, Castillo A, Gonzalez R *et al.,* Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation, *Enz Microbiol Biotechnol,* 2001, 28, 446-452.
12. Simpson BK, Haard NF., The use of proteolytic enzymes to extract caratenoproteins from shrimp wastes, *J Appl Biochem*, 1985, 7, 212-222.
13. Fagbenro OA, Bello-olersojji OA., Preparation, nutrient composition and digestibility of fermented shrimp head silage, *Food Chem,* 1997, 60, 489-493.
14. Cira LA, Huerta S, Hall GM, Shirai K., Pilot Scale lactic acid production of shrimp wastes for chitin recovery, *Process Biochem*, 2002, 32, 1359-1366.
15. Bautista J, Jover M, Gutierrez JF, Corpas R, Cremadas O, Fontiveros E, et al., Preparation of craw fish chitin by insitu lactic acid production, *Process Biochem,* 2001, 37, 229-234.
16. Rao MS, Stevens WF., Fermentation of shrimp biowaste under different salt concentrations with amylolytic and non-amylolytic *Lactobacillus Strains* for chitin production, *Food Technol Biotechnol,* 2006, 44, 83-87.
17. Sacchindra NM, Bhaskar N, Siddegoeda GS, Sathisha AD, Suresh PV., Recovery of carotenoids from ensilaged shrimp waste, *Biores Technol,* 2007, 98, 1642-1646.
18. Healy, M., M. Green, and Healy A., Bioprocessing of marine crustacean shell waste, *Acta Biotechnol,* 2003, 23, 151-160.
19. Golkhoo, Sh., Barantalab, F., Ahmad, A., Zuhair, M.H., Purification of Astaxanthin from mutant of phaffiarhodozyma JH-82 which isolated forest trees of Iran. Pakistan, *J. Biol. Sci*, 2007, 10, 5, 802-805.
20. Guerin, M., Huntley mark, E., Olaizola, M., Haematococcus astaxanthin: applications for human health and nutrition, *Trends in Biochem*, 2003, 21, 5, 210-216.
21. DeMan, J.C., M. Rogosa, and Sharpe M.E., A medium for the cultivation of lactobacilli, *J. Appl. Bacteriol.* , 1960, 23, 130-135.
22. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the *Folin* Phenol reagent, *J. Biol chem*, 1951, 193, 265-275.
23. AOAC., Official methods of analysis. In: Helirich K (eds.) *Association of official analytical chemists*, 1995, 16th ed. Arlington, VA: AOAC.
24. Sacchindra, N.M., N. Bhaskar and Mahendrakar N.S., Carotenoids in different body components of Indian Shrimps, *J. Sci. Food Agric*, 2005, 85, 167-172.

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