Bioprocessing of shrimp shell for extracting chitin using Bacillus licheniformis and Lactobacillus fermentum

Anupama T.K., Lekshmi R. Kumar, Elavarasan K. and Toms C. Joseph*

ICAR-Central Institute of Fisheries Technology, Kochi-29
**anupamatkcift@gmail.com*

Waste generated during the processing of shellfish has been increasing worldwide over the years. Shrimp waste generally comprises, the head, shell and tail portions accounting for 40-50% of the total weight. Some of the shrimp waste is utilized as feed for aquaculture but the majority is dumped openly on landfills or in the ocean which seriously pollutes the environment. The shrimp waste is heterogeneous in composition and contain 20–40% protein, 30–60% minerals (calcium carbonate), 20–30% chitin, and 0–14% lipids (muscle residues and carotenoids) (Shahidi and Synowiecki,1991; Kaur and Dhillon, 2015). However, the presence of rich sources of calcium, protein, chitin, and pigments in the shrimp shell waste paved the way for the researchers to utilize shrimp shells for manufacturing expensive medicines and nutritional food, which creates significant importance in the shrimp processing industry (Zhang et al., 2022).

Chitin, an insoluble linear homopolymer of β-(1→4)-linked-N-acetyl-D-glucosamine, is the second-largest carbohydrate polymer in nature, next to cellulose (Duan et al., 2012; Sharp, 2013). This natural biopolymer is abundant in crustaceans, insects, and microbes and mostly utilized as a base material for making chitin derived materials like chitosan produced by deacetylation. This biopolymer is well known for its uses in many fields such as pharmaceuticals, cosmetics, food, agriculture, paper and packaging industry, wastewater treatment and textile industry (Akar, San, and Akar, 2016; Choo et al., 2016). Traditionally, chitin has been extracted chemically from shrimp shells, but this process produces toxic wastewater produced that is incompatible with environment protection. In chemical method, two harsh chemical treatments are used for deproteinization (DP) and demineralization (DM). Hydrochloric acid is used for removing minerals and strong alkali is used to remove protein and other organic compounds under high temperature (Hongkulsup, Khutoryanskiy, and Niranjan, 2016). HCl used for DM can cause adverse effect on the intrinsic property of chitin which decreases the final quality (Percot, Viton, and Domard, 2003). Therefore, one of the alternative methods used to replace these harsh chemicals is the use of a biotechnological method which is environmentally friendly and has higher degree of acetylation (DA) compared to the

other methods (Mao, Guo, Sun, and Xue, 2017; Zhang et al., 2022:).

The chitin extraction through microbial fermentation has emerged as a promising method because it is environmentally safe, technically adaptable, and commercially viable. Microbial fermentation can be performed in 4 ways: One-step, Two-step, Successive and Co-fermentation. In one-step, only one bacterial strain is used for the process. The chitin extraction using single strain is simple and inexpensive but it has relatively low DM and DP efficiency. In two-step fermentation, one protease and one acid producing bacterium (Zhang et al., 2012 ; Liu et al., 2020) are employed and highly purified chitin can be obtained in this method compared to one-step fermentation. *Bacillus pumilus, B. subtilis, Pseudomonas aeruginosa* (Sini, Santhosh, and Mathew, 2007; Ghorbel-Bellaaj et al., 2013; Sedaghat, Yousefzadi, Toiserkani, and Najafipour, 2017) etc., are used for DP process and *Lactobacillus plantarum, B. coagulans, Streptococcus thermophilus and Gluconobacter oxydans* (Mao et al., 2013; Liu et al., 2014, Zhang, et al., 2017: Dun et al., 2019) were commonly used for DM process. One of the disadvantages of two-step fermentation is the protease-producing bacteria must be removed before the DM process, due to the competitive inhibition and interference existing between protease and acid producing microorganisms (Zhang et al., 2021). The requirement of re-sterilization, change of medium and collecting residue between the DM and DP process extends the processing time and also affects the efficiency.

Successive fermentation is sequential execution of DP and DM and Co-fermentation is simultaneous execution of DP and DM process. In this method, resterilization process can be omitted and thereby reducing the cost. Zhang et al., (2012) performed successive fermentation of shrimp shell powder using *L. plantarum* ATCC 8014 and *Serratia marcescens* B742 to extract chitin and results of two-step fermentation showed higher efficiency than one-step fermentation. Liu et al., (2021) studied both one-step and successive co-fermentation and found that successive co-fermentation with *B. licheniformis* and *G. oxydans* have better DM and DP process than individual fermentation. However, many factors such as inoculum volume, pH, type and concentration of carbon source, temperature and reaction time affects the efficiency of fermentation. Table 1 provided the summary of different methods used for chitin extraction using microbial fermentation.

A study was conducted by utilizing microbial fermentation approach to obtain chitin from *Penaeus vannamei* shell waste. In the present study, successive co-fermentation technique was employed for chitin extraction from shell using *Bacillus licheniformis* and *Lactobacillus fermentum*. 5g of shrimp shell waste was added to 100ml

of water supplemented with 5% glucose and inoculated with 5% *B. licheniformis* at concentration of 7-8 log cfu/g. after incubation in shaker incubator at 30°C for 72 hours, 5% *L. fermentum* was added with 5% of glucose and further incubated under similar conditions for 144hrs. The DP and DM value reached to 90.21±0.07% and 87.47±0.33% respectively after 144 hrs. of fermentation. The study also found that *B. licheniformis* and *L. fermentum* can be utilized to develop a fermentation system that will extract chitin from shrimp shells and produce chitin that is of better-quality. Therefore, it was found that the microbial fermentation approach for chitin extraction from shrimp shells is a simple and feasible technology which can serve as a substitute for traditional chitin extraction.

Table 1. Chitin extraction by different microbial fermentation methods

Shrimp shell source	Fermentation methods	Incubation Conditions	DP	DM	Yield of Chitin (%)	References
Penaeus merguiensis	Fermentation using *Pseudomonas aeruginosa*	Incubation 30 °C for 6 days	92%	82%	47%	Sedaghat et al., 2017
Metapenaeopsis dobsoni	Fermentation by *B. subtilis*	30 °C for 15 days	84%	72%	ND	Sini et al., 2007
P. vannamei	Fermentation using *Halobacterium salinarum* and *Halococcusdom browskii*	37 °C for 16 days	95%	ND	ND	Dayakar et al., 2021
Litopenaeus sp.	Successive fermentation using *Lactobacillus brevis* and *Rhizopus oligosporus*	30 °C for 8 days	96%	66.45%	ND	Aranday et al., 2017
Shrimp shells	Successive two-Step fermentation using *Exiguobacterium profundum* and *Lactobacillus acidophilus*	Room temperature for 5 days	85.9%	95%	16.32%	Xie et al., 2021

P. vannamei	Successive fermentation using *B. licheniformis* followed by *Gluconobacter oxydans*	30 °C for 4 days	87%	93.5%	ND	Liu et al., 2014
Shrimp shells	Successive co-fermentation using *Bacillus subtilis* and *Lactobacillus plantarum*	37 °C for 6 days	94.1%	96.3%	21.2%	Zhang et al., 2022

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