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Research Article

EXTRACTION OF CHITIN FROM *PENAEUS MONODON* SHELL WASTES USED AS FOOD PRESERVATIVE AND LIFE ENHANCER

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ABSTRACT

Chitin and its associated chemical compounds are the most potential and functional substrates which have a vital role to perform in various medicinal, agricultural and industrial fields. In this study, *Penaeus monodon* shell wastes and infected prawn samples were collected from the local fish market. From the infected prawn samples, a total of 5 seafood pathogens and shell waste total of 54 associated bacteria were isolated. Then collected bacteria cultures were tested for proteolytic and chitinolytic activities. The proteolytic evaluation was performed in casein hydrolyzing agar and chitinolytic behaviour was detected by the colour change in bromocresol purple agar using stranded methods and techniques. In the present study, bacterial strain SVCAS34 showed maximum activity in all tests. In this experiment, the chemical method shows 34.2 % of chitin but the biological method produces yield around 20.12%. Produced chitin film was tested against fresh tomato to check whether it effectively controls the shelf-life period. It was found to be no damage at room temperature by using chitin film after 10-12 days of incubation. This indicates that chitin increases the life span of post-harvested vegetables and fruits. The prepared chitin was tested for their inhibitory and bacterial effects. The outcome of the study exhibited that *E. coli* showed a maximum zone of inhibition compared to other tested pathogens. Therefore this study proved that these important properties of chitin may have a great scope in several fields of commercial applications with economic interests.

Keywords: Chitin, Antibacterial, Proteolytic, Chitinolytic, Film.

1. INTRODUCTION

The marine and freshwater environment are abundant resources of organic and biochemical diversity. Such marine diversity was rich with novel metabolites and involved in the development of medicines, cosmetics, nourishing supplements, molecular probes, other chemicals, and agriculture materials etc [1]. In the past decade, a good number of novel compounds with strong pharmacological potential were recorded from the marine organisms. In that novel compounds some are released to the market, some are currently in the different phases of clinical trials [2]. While the marine environment offers an enormously rich source for novel metabolites, it represents a great challenge that involves more contributions from various scientific research areas to take the marine compounds diversity up to its therapeutic potential.

Research on renewable resources is one of the greatest significant research areas. In the last decade, the study in the field of renewable resources has greatly increased due to the great commercial value in the utilization of productive feedstock's for the manufacture of important and beneficial compounds and products. Currently, overexploitation of natural resources is a serious issue to the marine environment and generates large amounts of marine waste materials too. In recent years a huge amount of shellfish waste was produced by the fish processing industry. The major reason is the lack of sustainable waste management knowledge in the field to produce alternative valuable products from waste [3]. The environmental consequences of the disposal practices of such waste, combined with the strengthening of environmental laws in several countries, has given rise to an increase in better approaches for the disposal/use of waste and the handling of fish, which would otherwise this waste will cause significant environmental contamination to the country.

On a global basis, more than 700000 million tons of

shell waste was produced by the shellfish processing industry [4]. In the raw material of the shellfish, more than 35-45 % by weight was disposed off as waste when processed into headless and tailless material. The shellfish were rich with protein and polysaccharides with minerals. These shell wastes act as a significant source for many value-added products, including chitin and chitosan, carotenoid pigments and flavourings [5]. Recently many researchers are taking significant steps to invent new techniques to reuse and recovery the beneficial biopolymer from shell wastes. These new inventions procedures are can control the cause of the environmental pollution by shell wastes and they save the expenditure spent by the industry to disposing of the waste materials. Also, these may produce more employment and it may use to develop the country's economy by improving the full utilization of chitin and protein present in the waste [6].

In recent years, the production of new products and the utilization of chitin and chitosan have increased dramatically in various fields [7]. These chitin and chitosan have currently been successfully implemented in the medicinal and pharmaceutical, food and feed, agriculture, materials and textiles sectors [8]. Currently, research on nanoparticles incorporated chitin and chitosan shows tremendous development in their biological properties such as nontoxicity, biocompatibility, biodegradability, and antibacterial ability, they are also used as stimulating drug delivery carriers [9].

This significant chitin and chitosan also perform an important role in the applications of dietary applications as the foremost resource of amino sugar, N-acetyl-D-glucosamine, and D-glucosamine [7]. Chitosan can also act as a thickening component, and recent experiments have shown that chitosan can associate with proteins to alter protein interactions, results in enhanced the food products texture. Chitosan also acts as a food preservative and prevents microbial development, which ensures that chitin/chitosan film can be used to pack fruit and vegetables. This trend could reflect the most important developments in the food industry in the coming decades. In the present research, taking into account the ability of food preservatives, this chitin and chitosan were focused on analyzing the potential for inhibiting microbial growth and functioning as a food enhancer.

2. MATERIAL AND METHODS

2.1. Collection of shell samples

The *Penaeus monodon* shell waste (250 dry-wet) was collected from the local fish market, Kanathur beach, East coast of Chennai, Tamil Nadu. Shell waste cleaned properly with tap water to remove adhesive sand, clay, etc.

2.2. Isolation of marine bacteria

The shell waste associated marine bacteria were isolated by using the Zobell marine agar medium. Shell waste homogenized with sterile seawater with the use of mortar and pestle. The resultant (1 mL) was added to the 9 mL of distilled seawater and serial diluted up to 10^{-6} dilutions then applied on Zobell marine agar plates. All the plates were kept in the incubator at 32°C for 48 hrs. Colonies were chosen based on different morphology and pure cultures were preserved in the same medium slants at 4°C for further analysis.

2.3. Proteolytic and chitinolytic activities

The isolated bacteria cultures were mass cultured in the Zobell marine agar medium and tested for the chitinolytic [10] and proteolytic [11] potentials. Casein Hydrolysis agar was used for proteolytic activity and Bromocresol purple agar was used for the chitinolytic activity test.

2.4. Chitin preparation

2.4.1. Chemical method

The production of chitin was followed by the method of Kumari and Rath [12]. To get purified chitin, it must be isolated from proteins, minerals and other ingredients. This separation is achieved mainly by three steps (Fig-1): 1) Demineralization to eliminate the calcium carbonate, 2) Deproteinization, 3) Chitin preparation. The shell waste was demineralized to be treated with a 7% HCl solution (solid ratio of 10 ml/g) at room temperature. The resulting solid fraction washed with distilled water until the fraction was demineralized. The sample fraction was dried and weighed. Then chitin deproteinization was done using 10% NaOH at 60°C. This process was repeated several times until colour changes which indicate the absence of proteins. The resultant was washed with water and hot ethanol (10 mL/g). The collected resultant was dried at room temperature.

2.4.2. Biological method

The chosen bacterial strain was inoculated in the MRS liquid medium (5 mL) and incubated at 30°C for 24 hours. Five grams of shell waste has been added to the glass beaker and sterilized at 121°C. After cooling, 100

mL (20%) of sterile glucose solution (1:20 w/v) was added as a carbon source. The inoculum was then inoculated into the sterilized fermentation flask and kept in the shaking incubator at 30°C at 200 rpm. The fermented mixture was filtered after 7 days. The extracted material was then washed repeatedly with deionized water and finally washed with 70% ethanol. The resultant was eventually dried in the oven at 60°C for 24 hours.

2.5. Preparation of chitin film

The preparation of chitin film was followed by the method of Bhuvaneshwari [13]. Different concentrations (1 & 2 g) of chitin have added to the 100 mL acetic acid solution (1 %, 2 % and 3 %) in separate conical flasks and mixed until the powder gets dissolved. The mixed solution kept for 2 hrs for degassed and poured in the Petri plate. The plate was placed in the oven at a temperature of 72°C for 20 hours to dry and develop a film. Dry film was soaked in 2N NaOH at room temperature for 30 min and rinsed with water. Finally, the film was dried at room temperature for one week [14].

2.6. Chitin film as a food preservative

Fresh tomatoes were taken and properly cleaned with tap water. The experimental set-up of tomatoes was maintained at room temperature (28-30°C). In this experiment, one tomato was coated with chitin film and another tomato was kept under observation (without chitin film coating). These laboratory systems have been maintained for two weeks.

2.7. Microorganisms

The infected prawn was collected from the local fish market, Kanathur beach, East coast of Chennai, Tamil Nadu. The full body of infected fish was homogenized with distilled water. The resultant (1 mL) was added to the 9 mL of distilled seawater and serial diluted up to 10^{-6} dilutions then applied on Zobell marine agar plates. Following seafood pathogens such as *E. coli, Vibrio cholera, Vibrio parahaemolyticus, Salmonella* spp. and *Shigella* spp. were isolated from the infected prawn sample and used for the antibacterial assay. Bacterial cultures were preserved at 4°C on the Zobell marine agar slants.

2.7.1. Antibacterial activity

The minimal inhibiting concentrations values for the chitin-sensitive bacteria were investigated in the agar diffusion method of Nadarajah et al., [15] with slight modification. Bacterial inoculums have been prepared from 12 hours of broth cultures. The chitin was first dissolved in 10% dimethyl sulfoxide (DMSO). MIC values of the chitin were determined based on a well diffusion method. In the solidified agar, wells of 7 mm diameter were prepared using a sterile cork borer. 60 µL of chitin content has been applied to each well. Antibiotics have been used as a standard. Plates were maintained in the incubator for 24 hours at 32°C. The antibacterial activity of the compounds was estimated by measuring the inhibition region across the well.

3. RESULT AND DISCUSSION

In the present study, a total of 54 morphologically different marine bacterial colonies were isolated from crustacean shell waste. Table 1 shows chitinolytic and proteolytic activity of isolated marine bacterial colonies. Among these isolates, SVCAS34 exhibited high inhibition zones on nutrient agar medium, this particular bacterial strain has high chitinolytic and proteolytic potential than other strains. Other strains such as SVCAS04, SVCAS09, SVCAS20 showed an average level of proteolytic and chitinolytic activity. In this experiment, the chemical method shows 34.2 % of chitin but the biological method produces yield around 20.12% only. Fig. 1 expresses the formation of chitin.

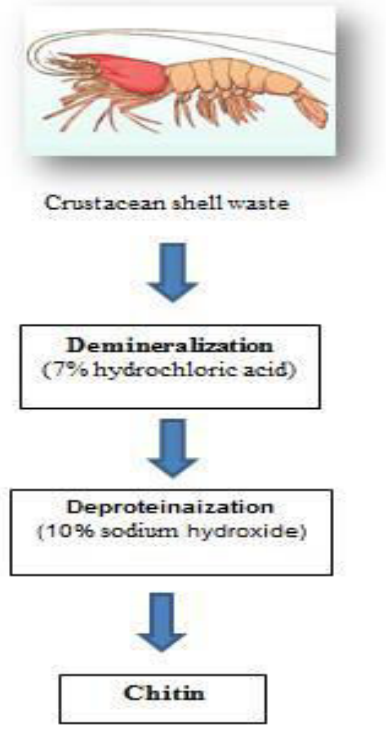

Fig 1: Preparation of chitin

Table 1: Proteolytic and Chitinolytic activity of isolated marine bacterial colonies

Strain name	Proteolytic activity	Chitinolytic activity
SVCAS01	++	-
SVCAS02	+	-
SVCAS03	-	-
SVCAS04	++	++
SVCAS05	+	-
SVCAS06	-	-
SVCAS07	+	-
SVCAS08	-	-
SVCAS09	++	++
SVCAS10	+	-
SVCAS11	++	-
SVCAS12	-	+
SVCAS13	-	+
SVCAS14	-	+
SVCAS15	+	+
SVCAS16	+	+
SVCAS17	-	-
SVCAS18	-	-
SVCAS19	-	-
SVCAS20	++	++
SVCAS21	-	-
SVCAS22	-	+
SVCAS23	-	-
SVCAS24	-	-
SVCAS25	++	-
SVCAS26	+	-
SVCAS27	+	+
SVCAS28	+	-
SVCAS29	++	+
SVCAS30	+	-
SVCAS31	-	+
SVCAS32	-	-
SVCAS33	-	-
SVCAS34	+++	+++
SVCAS35	-	-
SVCAS36	-	+
SVCAS37	-	-
SVCAS38	-	-
SVCAS39	+	-
SVCAS40	+	+
SVCAS41	+	-
SVCAS42	-	+
SVCAS43	-	+
SVCAS44	-	+
SVCAS45	++	-
SVCAS46	-	-
SVCAS47	+	-
SVCAS48	-	-
SVCAS49	++	++
SVCAS50	+	-
SVCAS51	-	-
SVCAS52	-	-
SVCAS53	-	-
SVCAS54	++	-

− = no zone, + = zone up to 4 mm, ++ = zone up to 8 mm; +++ = zone up to 12 mm

Proteases are digestive enzymes that interact with the protein-peptide bond and the polypeptide or free amino acid hydrolysis protein [16]. Such property was tested with skimmed milk agar containing casein in compliance with its purpose [17]. This mechanism has shown us to observe the rupture of the enzymatic potential as a halo of the colony. Chitinase is one of the digestive enzymes that has been found in a wide range of species performing various roles, specifically in fungi where it plays an autolytic, nutritional and morphogenetic role [18]. Visualization of the N-acetyl glucosamine rupture of chitin is the most common techniques for determining Chitinolytic enzymes activity. The bromocresol purple in the medium with colloidal chitin was used in this analysis to detect colouration from yellow to purple by adjusting the pH of acid to basic [19-20].

Now a day's few new reports bring forward that chitin coating mostly applies to the varieties of fruit and vegetables or compound coating [22]. After covering with chitin, the antioxidant enzyme of fresh fruits and vegetables normally retains a high degree of activity, and natural antioxidants such as reactive oxygen in the fruit and vegetable cells may be quickly removed. Therefore the ageing of fruit and vegetables was postponed [23]. Based on this ability the present study was conducted to test with tomato coated with Chitin film. In the study, chitin film was coated in the outer layer of tomato under the sterile condition at room temperature up to 2 weeks. After two weeks, normal tomatoes that were not covered with chitin were rotten and infected with the fungus, but chitin film coated with tomatoes appeared fresh without any signs of spoilage even after 10 to 12 days at room temperature. Figure 2 showed prepared chitin film and Fig 3 showed tomato covered chitin film. This result proves that prepared chitin based films were controlled the food spoilage activity and enhancing shelf life.

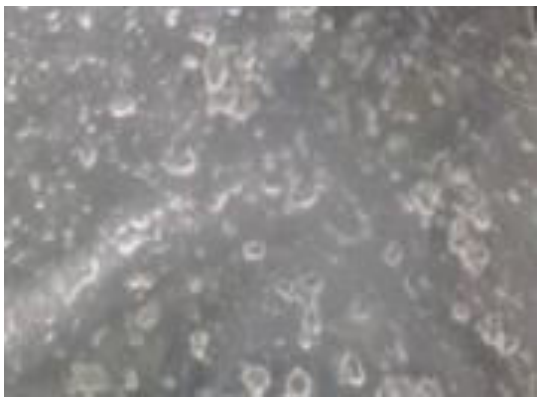

Fig. 2: Thin film prepared from the chitin

Fig. 3: shows tomato covered chitin film

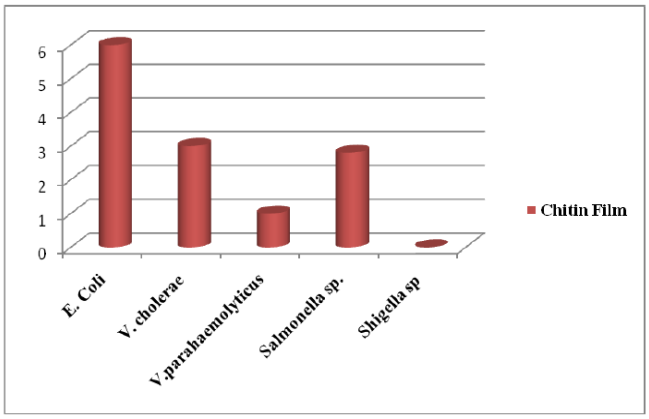

Fig 4: Antibacterial activity against the tested seafood pathogens

A previously similar study was conducted by Bangyekan, et al., [24] and they believed that chitin film had the potential to use it for packing, especially as a food packing. This is because of its excellent barrier capabilities for oxygen and carbon dioxide and fascinating antimicrobial properties. For example, degradable and nutritious chitosan films have been developed to protect food from fungal attack and to improve fresh fruit atmospheres. Kim et al., [25] specified that the application of antimicrobial properties of chitosan film was used in food packaging products, medical supplies, and so on, or as covered film on objects for which surface colonization is undesirable. Kumar et al., [26] have investigated the fact that antifungal and antibacterial action of chitosan can be used in the manufacture of commercially available biopesticides, and the anti-oxidant behaviour of chitosan is of great significance to the food industry and its possible use as natural ingredients has led to a strong interest in the replacement of synthetic additives [27].

To test this assertion, this analysis was performed to assess the inhibitory activity of chitin in terms of MIC. The chitin properties were tested against seafood pathogens isolated from infected shrimp. The outcomes showed that the maximum zone of inhibition was recorded in *E. coli* and minimum inhibitions were recorded in *Vibrio cholera, Salmonella* spp, *V. parahaemolyticus*, no inhibition was recorded *Shigella* spp, (fig. 4). The precise mechanism of antimicrobial activity of chitin, chitosan and its derivatives is still unclear, although various mechanisms have been suggested. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes results in the leakage of proteinaceous and other intracellular constituents [28].

There are several experiments which have been performed to investigate the many opportunities of using the different properties of chitin, and research is still ongoing on these perspectives. Chitin has a promising variety of uses as commercial material. Chitin has had an appreciable potential as a most valuable biomaterial and can be obtained from a very readily available source [29]. Given certain challenges and limited means of commercial use of Chitin, it is still a significant material with potential. Chitin has been used as several successful therapeutic applications in laboratory condition. There are still several opportunities for commercial applications and several efforts have been made to boost the results of such applications. Chitin is a very versatile compound for a wide variety of manufacturing uses in a wide range of fields.

4. CONCLUSION

Marine crustaceans are a rich source of bioactive materials with antibacterial effects that can be useful for application in biotechnological, pharmaceutical, and other fields. In the present research, chitin, which demonstrates therapeutic potential and food preservatives from crustaceans, is the right option to solve many commercial challenges. These essential properties of chitin are believed to have many industrial benefits with high economic importance.

5. ACKNOWLEDGEMENT

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