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Characterization of nano chitin from shrimp shell waste after biosynthesis with *Pseudomonas aeruginosa* as an exterminator of *Ganoderma* sp.

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ABSTRACT

In the process of oil palm cultivation, one of the obstacles that have not been overcome so far is stem rot (BPB) caused by the fungus *Ganoderma* sp. Microorganisms that can inhibit the growth of *Ganoderma* sp. is a group of chitinolic bacteria capable of producing chitinase enzymes such as *Pseudomonas aeruginase*. Abundant shrimp shell waste can be used as a source of chitin, for this reason this research is important in utilizing bacteria in the biosynthesis process of chitin sources into nanochitin. This study aimed to optimize nanochitin as a controlling agent for *Ganoderma* sp. to oil palm plantations. We focused mainly on comparing between Physico-chemical properties of nano chitin before and after biosynthesis. We performed the product's characterization by various tools such as: UV-Visible spectroscopy, particle Size Analyzer (PSA), Fourier Transformed Infra-Red (FTIR), Scanning Electron Microscopy (SEM) analysis. The results of this study indicate that the optimum time for producing chitinase enzyme by *Pseudomonas aeruginosa* is 18 hours with the optimum concentration of sterile shrimp shell powder at 2% (w/v). Characterization of nanochitin based on particle size using Particle Size Analyzer (PSA) analysis showed that *Pseudomonas* was able to biosynthesize nanochitin up to an average size of 14.76 nm. The particle size based on SEM analysis shows that the sample is not homogeneous or not uniform. FTIR analysis showed that there was a wave range of 3200-3500 cm-1, it was known that the presence of N-H and in the wave range of 3200-3650 cm-1 indicated the presence of O-H indicating that the products produced were chitin and chitosan. Therefore, the nanochitin after biosynthesis open new excellent potency for controlling root rot disease caused by the fungus *Ganoderma* sp.

Keywords:

Ganoderma sp., chitinase, shrimp shell, *Pseudomonas aeruginase*, nanochitin

INTRODUCTION

Indonesia is a country with very abundant marine products. Indonesia has a variety of seafood and seafood processing for Cructaceae, but this has not been done optimally. Indonesia's foreign exchange production obtained from the fisheries sector 34% came from shrimp exports amounting to 125,596 tons in 2007 [1]. Shrimp waste generated by the shrimp processing business comes from the head, shell and tail. Shrimp shell waste consists of three main components, namely chitin (15%-20%), protein (25%-44%), and calcium carbonate (45%-50%) [2]. The chitin

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content in shrimp shell waste is about 20%-50% dry weight.

Through the right technological approach, this potential waste can be further processed into polysaccharide compounds which include chitin [(C8H13NO5)n]. This chitin can be further processed into chitosan [(C)] and glucosamine (C6H13NO). These three products are biodegradable and non-toxic, so they are very friendly to the environment. Shrimp shells can be used as a producer of chitin which is then isolated and synthesized into chitin nanoparticles (nanochitin) through an enzymatic process that produces chitinase enzymes from chitinic bacteria.

Chitinolytic bacteria are a group of bacteria capable of producing chitinase enzymes to decompose chitin substances [3]. Chitinolytic activity can be qualitatively determined by the presence of a clear zone around bacterial colonies growing on chitin agar medium [4]. The very prospective role of chitinase in people's lives has encouraged scientists and researchers to explore chitinolytic microorganisms, namely microorganisms that can degrade chitin by using the chitinase enzyme. One of the chitinolic bacteria known to produce the largest chitination enzymes is Pseudomonas aeroginosa.

According to [5], *Pseudomonas aeruginosa* bacteria are motilbacteria that utilize sugar for the oxidase process with oxygen as a terminal acceptor. Pseudomonas aeruginosa uses glucose to form acid, breaks down nitrate into nitrite which is further broken down into nitrogen gas and these bacteria do not produce indole and MR.

The utilization of nanoparticles is developing in various fields. Nanoparticles are particles with a size of 10-1000 nm consisting of natural or synthetic polymer materials, which can be used as drug carriers by dissolving, trapping, encapsulating, absorbing or attaching the active substance [6]. Nanoparticles are the ability of particles to penetrate the intercellular spaces that can only be penetrated by colloidal particle size [7], the ability to penetrate higher cell walls, either through opsonification or diffusion, and their flexibility to be combined with various other technologies thus opening up vast potential to be developed for various purposes and targets. Another advantage of nanoparticles is the increased affinity of the system due to an increase in the contact surface area by the same amount [8]. One of the methods used for the biosynthesis of shrimp shell waste nanoparticles through the enzymatic process of the chitinolic bacterium *Pseudomonas aeroginosa* is UV-Vis Spectrophotometry, Particle Size Analyzer (PSA), SEM (Scanning Electron Microscope), and FTIR (Fourier Transform Infrared Spectrometer).

METHODS

This research was carried out in the laboratorium terpadu dan halal center Universitas Islam Malang, the laboratory of LSIH of Brawijaya University, the laboratory of Ma Chung University of Malang, the Laboratory of Minerals and Advanced Materials, FMIPA State University of Malang and the Pharmacy laboratory of UIN Maliki Malang.

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Preparation of Shrimp Shell

Shrimp shell waste is washed and then dried under direct sunlight and then crushed by grinding using a grinder. The Shrimp Shell Powder was then sieved using an 80 mesh sieve. Then it was weighed using an analytical balance and then put a falcon tube wrapped in brown paper and tied with a rope. Furthermore, the Shrimp Shell powder was sterilized using an autoclave with a pressure of 1 atm for 15 minutes at 121ºC.

Rejuvenation of *Pseudomonas aeruginosa* isolates

Rejuvenation and manufacture of Pseudomonas aeruginosa isolates were carried out to make *Pseudomonas aeruginosa* stock. Pseudomonas aeruginosa isolates as much as 100 ml were obtained from the collection of the microbiology laboratory of the Faculty of Science and Technology, Airlangga University. Furthermore, 5 ml of bacterial isolate was inoculated on 50 ml of LB media and incubated in a shaker incubator at a speed of 180 rpm and a temperature of 30ºC.

Chitinase Enzyme Production (Biosynthesis)

LB medium (0.1% K2HPO4; 0.01% MgSO4.7H2O; 0.05% Yeast extract; 0.1% Tryptone) together with sterile shrimp shell powder [2% (w/v)] and *Pseudomonas aeruginosa* bacteria were incubated in a shaker incubator for 18 hours. The optimum time in the production of chitinase enzyme (biosynthesis) was 18 hours with an optimum concentration of 2% (w/v) (data not shown). Measurement of chitinase activity using the Schale method. The chitinase enzyme was then centrifuged at 10.000 rpm at 4ºC for 10 minutes. The resulting supernatant was separated and then the freeze-drying process was carried out.

Characterization of Nanochitin

Characterization using Fourier Transform InfraRed (FTIR) analysis and Scanning Electron Microscope (SEM) analysis using results that have undergone freeze drying treatment for 48 hours. Meanwhile, the resulting sample solution was used for UV-Vis Spectrophotometry and Particle Size Analyzer (PSA) analysis.

RESULT AND DISCUSSION

1.1 UV-Vis Spectrophotometry

The sample treatment after the biosynthesis process obtained a maximum wavelength at 248 nm with an absorbance of 8.9166. The peak wavelength produced is more than one which indicates that the size of the chitin nanoparticles in the sample includes polydispers, that is, the particle size is included in a wide range.

The maximum wavelength range indicating the presence of chitin nanoparticles is in the range of 217 nm-250 nm [9]. According to [10] which show that the maximum wavelength in chitin nanoparticles is located at 226 nm. In the results of this study, the sample treatment after the biosynthesis process obtained a maximum wavelength at 248 nm with an absorbance of 8.9166.

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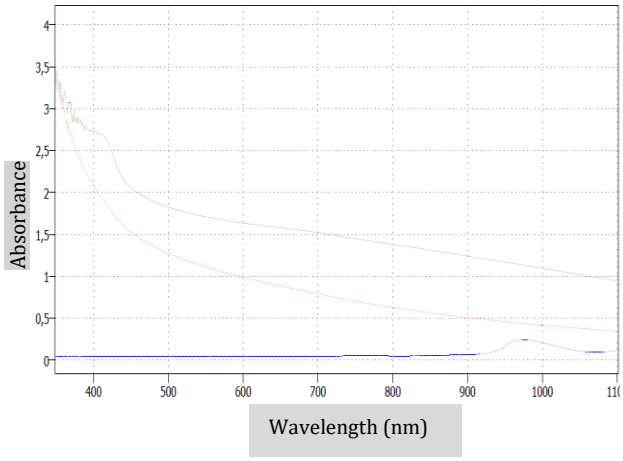

Figure 1. The Uv-Visible spectrum of Absorbance. = blanko ; = treatment (after biosynthesis); = control

1.2 Particle Size Analyzer (PSA)

The results of the PSA test showed that the average size of nanochitin in the sample formed was 14.76 nm with a PDI (Polydispersity Index) value of 0.621. The PDI value is a particle size distribution. If the PDI value is greater than 1.0, the particle size tends to be non-uniform. The results of this research indicate that the PDI value is < 1.0 which means that the nanochitin size is homogeneous. Based on the results of the comparison of samples and controls, it can be seen that the chitinase enzyme produced by the bacterium Pseudomonas aeruginosa is capable of degrading chitin and biosynthesis of chitin nanoparticles. By looking at the distribution of samples characterized using PSA, it can be concluded that the sample has a good level of uniformity. As for the average size in the control as much as 60.1% measuring 653 nm and 39.9% measuring 3530 nm with a PDI (Polydispersity Index) value of 0.709.

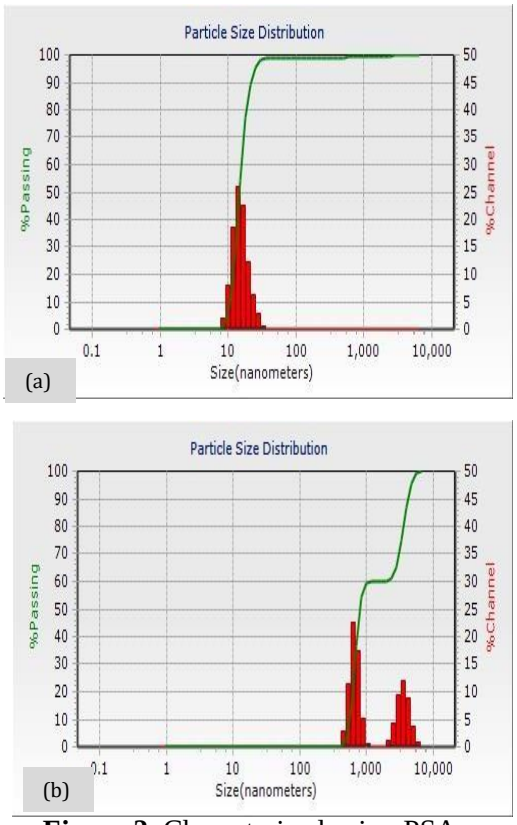

Figure 2. Characterized using PSA

1.3 Fourier Transport Infra Red (FTIR)

The presence of a wide and strong absorption peak in the wave range of 3200-3500 cm-1 indicates the presence of N-H and in the wave range of 3200-3650 cm-1 indicates the presence of O-H, indicating that the products produced are chitin and chitosan. The FTIR spectra of the formation of chitin compounds in this study can be seen in Figure and Table and compared with the literature.

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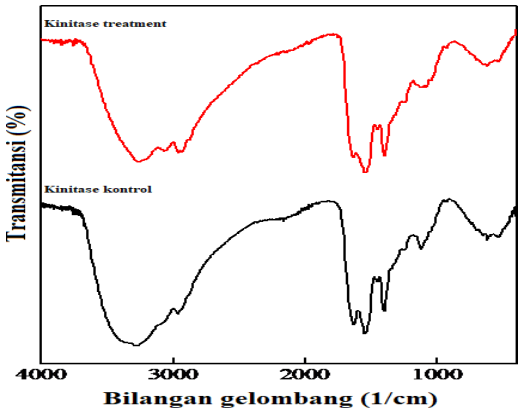

Figure 3. The FTIR spectrums

Table 1. Functional groups and wavelength from FTIR

Functional groups	Wavelength (cm^{-1}) (Stuart, 2003)	Wavelength (cm^{-1}) Control	Wavelength (c Treatment
OH (3200-3500 cm^{-1})	3448	3279.36	3257.18
N – H strecth (3200-3650 cm^{-1})	3300 – 3250	3279.36	3257
C – H strecth (2850-2965 cm^{-1})	2891,1	2963.09	2959
C = O strecth (1600-1650 cm^{-1})	1680 – 1640	1629.55	1637
N–H wag (1560-1650 cm^{-1})	1560 – 1530	1629.55	1637.37
CH3 (1365-1405 cm^{-1})	1419,5	1400.07	1396.21
C – O – C	1072,3	1120.44	1116.58

1.4 Scanning Elctron Microcope (SEM)

Measurements on both samples, both control samples and biosynthetic treatments used a magnification of 10000x. in the picture, chitin and also nanochitin have the shape of a particle in the form of a sphere resembling a ball. It is known that the nanochitin is filled with ketoprofen which has a completely spherical shape. As for the size distribution of the sample is not homogeneous or not uniform. The non-uniform particle size is thought to be because ketoprofen does not only enter the nanochitin matrix, but sticks to the surface [11].

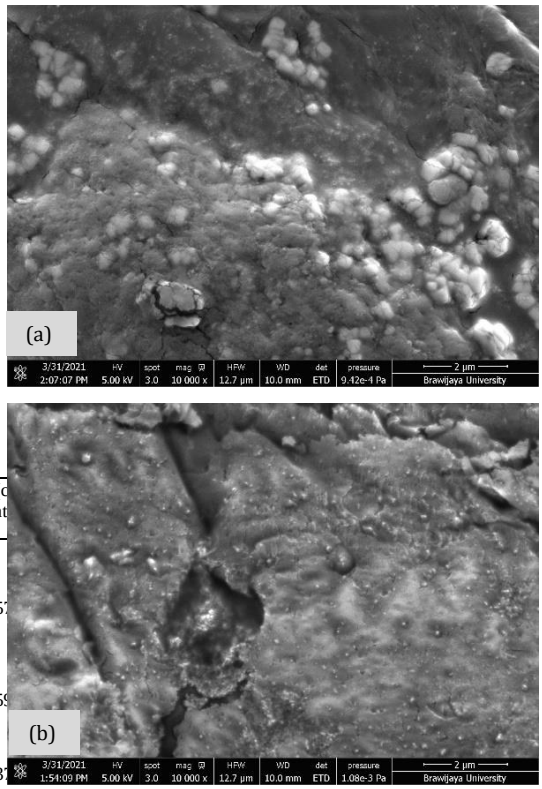

Figure 4. Scanning Electron Microscope (SEM) analysis with magnification 10.000x (a) control ; (b) After biosynthesis.

CONCLUSION

We concluded that particle size of nanochitin showed that *Pseudomonas aeruginosa* was able to biosynthesis up to an average size of 14.76 nm. The results of the SEM show that the sample is not homogeneous or not uniform. The results of the FTIR, namely the presence of a wave range of 3200-3500 cm-1 indicates the presence of N-H and in the wave range of 3200-3650 cm-1 indicates the presence of O-H indicating as a marker the products produced are chitin and chitosan. Based on these results, the biosynthetic nanochitin can be recommended as a control for

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root rot disease caused by the fungus *Ganoderma* sp.

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