Jurnal Ilmiah Farmako Bahari
Journal Homepage : https://journal.uniga.ac.id/index.php/JFB

EXPLORATION OF CHITINOLYTIC MICROORGANISMS FROM SEAWATER AS ANTI-CANDIDIASIS AGENTS USING FUNCTIONALIZED CHITIN SUBSTRATE DERIVED FROM GREEN MUSSEL WASTE

Rahadhyan Adnyanaschah[1*], Dzava Prawinsyah Fairus Ismail[1], Bintang Satrio Mahardika[1], Yolla Adellia Putri[1], Nafis Shafa[1], Tina Rostinawati[2]

[1]Bachelor of Pharmacy Study Program, Department of Pharmaceutical Biology,Faculty of Pharmacy, Universitas Padjadjaran,Jl. Raya Bandung-Sumedang km 21, Kec. Jatinangor, Kab.Sumedang, West Java, 45363, Indonesia
[2]Department of Pharmaceutical Biology,Faculty of Pharmacy, Universitas Padjadjaran,Jl. Raya Bandung-Sumedang km 21, Kec. Jatinangor, Kab.Sumedang, West Java, 45363, Indonesia

*Corresponding author: Rahadhyan Adnyanaschah (adnyanaschah.rahadhyan@gmail.com)

ARTICLE HISTORY

Received: 25 November 2024 | Revised: 18 January 2025 | Accepted: 22 January 2025

Abstract

Candidiasis is a *Candida* sp. infection (*Candida albicans* in common) that happens in nails, hairs, skin, and oesophagus, with around 20-25% prevalence in Indonesia. Chitin is one of the main components of its cell wall and serves as a target for candidiasis therapy using natural degradant compounds, such as chitinase enzyme. Chitinase enzyme is collected from chitinolytic bacteria found massively in chitin-rich environments like seawater. The screening is needed to identify the varieties of bacteria with chitinolytic activity. The screening was done using green mussel waste *(Perna viridis),* which contains 14-35% chitin and reached 10.776,75 tons of trash in 2020. This research aims to get a chitinolytic bacteria using green mussels as a chitin substrate source and a chitinolytic bacteria with anti-candidiasis activity. This research was conducted by isolating chitin from green mussel shells, chitinolytic bacteria screening, and anti-candidiasis activity test. Results collected from 5.00, 5.08, and 5.18 km isolates were the *Vibrio alginolyticus*, *Vibrio neocaledonicus*, and *Marinobacter persicus* species, which had a chitinolytic index of 1.246, 1.560, 1.492 also 64.29; 61.30; 69.01 U/ml chitinase enzymatic activity. The highest anti-candidiasis activity was found in a sample 5,18 km from the coast of Kejawanan Beach based on the number of chitinase enzyme activity and the chitinolytic index.

Keywords: candidiasis, chitin, chitinolytic, green mussel, microorganism

Introduction

Candidiasis is an infectious condition caused by an opportunistic fungus, *Candida* sp., which includes *Candida albicans*. As many as 20-25% of the Indonesian population suffer from candidiasis, affecting the hair, skin, nails, mucous membranes, mouth, and oesophagus.[1] The fungus that causes this infection has a cell wall composed of chitin compounds. Therefore, chitinolytic activity is needed to inhibit the growth of C. albicans.[2]

ISSN: 2087-0337
E-ISSN: 2715-9949

The chitinolytic activity needed to treat candidiasis can be found in several kinds of microorganisms, mainly bacteria with chitinase enzymes, which are natural degraders of chitin substances. These chitinolytic bacteria are commonly found in seawater due to the high chitin content in other organisms in the sea. Some genera that have chitinolytic abilities are *Bacillus sp., Clostridium sp., Serratia sp., Aeromonas sp., and Trichoderma sp*.[3] Therefore, it is necessary to screen microorganisms to identify a variety of bacteria sourced from seawater isolates that have chitinolytic activity.[4]

Chitinolytic bacteria can be screened by utilizing waste from one of the marine commodities, such as green mussels (*Perna viridis*). The high consumption of green mussels aligns with the high amount of waste generated. Based on data from the Cirebon Regency Marine and Fisheries Service, in 2020, there were 10,776.75 tons of green mussel consumption, which showed an increase of 35.33% compared to 2017 data.[5] Green mussel shells contain about 14-35% chitin can be reused as a substrate in screening chitinolytic microorganisms.[6]

The screening results will then be specified related to the variants of the bacteria obtained by Whole Genomic Sequencing (WGS) and Polymerase Chain Reaction (PCR) methods. These two methods were chosen because they have high sensitivity and accuracy and require fewer samples quickly.[7,8] Thus, the problems related to candidiasis and waste accumulation in marine ecosystems can be handled by reusing the sea's resources. Therefore, this research aims to reveal the potential of chitinolytic microorganisms from seawater as anti-candidiasis agents using functionalized chitin substrate derived from green mussel waste.

Method

Equipment

The equipment used were an autoclave, 40 mesh sieve, petri dish, centrifuge (EBA 20 and 1-16 Sigma), chamber, Fourier Transform Infrared (FTIR), grinder, hotplate (Cimarec), incubator (Yenaco), micropipette (BIOHIT), oven (Memmert), pH meter (Peak Instruments), shaking incubator (Labnet International), UV-Vis spectrophotometer, micropipette tips (Onemed), laboratory glassware (Pyrex), analytical balance (Mettler-Toledo AL204), compact electrophoresis (Biometra), thermoblock TB2 (Biometra), 96 standard gradient thermal cycler (Biometra), biodocanalyze (BDA) liveh (Biometra), electric microscope (Telview).

Material

The materials used were sterilized seawater, 70% alcohol, aluminum foil, 32% hydrochloric acid (HCl) (Kimia Market), distilled water (Kimia Market), Fluconazole (PT. Bernofarm), GeneJET™ Genomic DNA Purification Kit, *C. albicans* isolate, potassium hexacyanoferate III ($K_3[Fe(CN)_6]$) (Pudak Scientific), cotton wool, carbol fuchsin (PT. Segara Husada Mandiri), carbolic gentian violet (PT. Segara Husada Mandiri), universal pH paper, pH 7 buffer solution (Kimia Market), green mussel waste, MR-VP media, Simmons citrate media, tryptic soy agar (TSA) media, immersion oil, sodium hydroxide (NaOH) (CV. Sabiya Pelita Scientific), sodium hypochlorite (NaOCl) 4% (CV. Sabiya Pelita Scientific), anhydrous sodium carbonate ($Na_2 CO_3$) (ROFA Laboratorium Center, Indonesia), nutrient agar (NA), nutrient broth (NB), paper disc, potato dextrose agar (PDA), potato dextrose broth (PDB), plastic wrap, primer 27f, primer 1492r, barrit A and barrit B reagents, kovac reagent, lugol iodine reagent, methylene blue reagent (ROFA Laboratorium Center, Indonesia), and methyl red reagent (ROFA Laboratorium Center, Indonesia).

ISSN: 2087-0337
E-ISSN:2715-9949

Procedure

Isolation of Chitin from Green Mussel Shells

The green mussel shells were washed under running water until clean, then dried in an oven at 80ºC. After drying, they were pulverized to a size of 40 mesh. Green mussel shells were deproteinated using 3% NaOH in a ratio of 1:6 (b/v) while stirred and heated at 80ºC-85ºC for 1 hour. The solution and the solids were separated and washed with distilled water. The solid was then dried at 80ºC until dry.

The dried solid was demineralized using 1.25 N HCl solution (1:10 (b/v)) and stirred at 70ºC-75ºC for 1 hour. The solid was filtered and washed with distilled water until neutral, then dried at 80ºC. The result of demineralization was depigmented using 4% NaOCl solution (1:10 (b/v)) and stirred at 80ºC for 1 hour. The solution was washed with distilled water until neutral and filtered to obtain a chitin precipitate. The chitin precipitate was dried in an oven at 80ºC. The chitin obtained was then tested using FTIR to determine the functional groups.

Chitin Media Preparation

20 g of chitin was dissolved in 400 mL of concentrated HCl, allowed to stand for 24 hours under closed conditions, and filtered with cotton wool. The filtrate was centrifuged at 6000 rpm for 30 minutes until a brownish-white colloidal precipitate of chitin was formed.

After the chitin colloidal precipitate was formed, 2 g colloidal chitin, 2 g agar, and 1 g nutrient broth were dissolved in 100 ml sterile seawater. The solution was heated on a hotplate until boiling and autoclaved for approximately 15 minutes at 121ºC. The media was then poured into sterile petri dishes, waiting to harden. Then, it was wrapped using plastic wrap and incubated for 24 hours.

Isolation and Purification of Chitinolytic Bacteria

5 ml of seawater samples were obtained from Kejawanan Beach at 5.00, 5.08, and 5.18 km from the shore to the sea. 1 ml of each seawater sample was suspended in NB medium. The suspension was incubated at 37ºC for 18-24 hours. Bacterial suspension was streaked on the chitin medium and incubated at 37ºC for 18-24 hours. The bacteria was then purified into another chitin media and incubated at 37ºC for 24-48 hours.

Determination of the Chitinase Enzyme Activity

Two loops of bacterial isolate were put into 20 ml NB containing 0.5% colloidal chitin and incubated on a shaker at 37ºC and 100 rpm for 24 hours. The isolates were centrifuged at 4,000 rpm for 30 min to obtain crude extract chitinase enzyme in the supernatant. Chitinase enzyme activity was determined by measuring the N-Acetylglucosamine (GlcNAc) produced in a period as a product of chitin hydrolysis.

GlcNAc was measured by adding 0.15 ml of a crude enzyme (cell-free supernatant) in a mixture of 0.15 ml of 1% chitin substrate and 0.3 ml of pH seven buffer solution. The mixture was homogenized, incubated at 37ºC for 60 minutes, and then heated at 100ºC for 3 minutes. The control solution was prepared by adding 0.15 ml of crude enzyme after heating at 100ºC for 3 minutes. The solution was centrifuged at 3000 rpm for 20 minutes. Then, GlcNAc was measured by mixing 0.5 ml supernatant, 0.5 ml distilled water and 1 ml Schale reagent. The absorbance was measured at a wavelength of 420 nm, and the GlcNAc concentration was calculated based on the GlcNAc standard curve to determine the chitinolytic activity of the bacteria using a UV-Vis spectrophotometer using the enzyme activity assay formula as follows:

ISSN: 2087-0337
E-ISSN:2715-9949

$$Enzyme\ activity\ (U/ml)\ = \frac{K\ x\ 1000}{t\ x\ BM}$$

K = concentration (ppm)
t = incubation time (minutes)
BM = molecular weight (GlcNAc = 221, 21 g/mol)

Characterization of Chitinolytic Bacteria Isolates

Macroscopic and microscopic observations carried out characterization. Macroscopic observation included the colony's colour, edges, elevation and shape. Microscopic observation included cell shape and cell arrangement using an electric microscope. Gram staining and biochemical tests were also performed. Finally, molecular characterization was carried out by PCR using forward primer 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492r (5′-TACGGYTACCTTGTTACGACTT-3′). Before the bacteria cells go through the PCR steps, the chromosome was isolated first using iNtRON G-spinTM for bacteria. The sample cells were harvested for around 2 ml by centrifuging at 13,000 rpm for 1 minute. The sample filtrate was mixed with 300 µl G-buffer solution and incubated at 65°C for 15 minutes. Binding buffer was added, and the sample was centrifuged at 13,000 rpm for 1 minute. After that, the sample was washed with washing buffer and was centrifuged at 13,000 rpm for 1 minute. Then, the solution was removed, and the sample was centrifuged at 13,000 rpm for 1 minute. The G-spin™ column was placed, and the sample was added to 200 µl elution buffer. Then, the sample was incubated at RT for 1 minute and centrifuged at 13,000 rpm for 1 minute. To prove the genetic material on the sample, the Genetic DNA Extraction Kit and electrophoresis are conducted on the isolated sample. After the genetic material was to exist on the sample, GoTaq® Green Master Mix was added to the sample. The sample solution is then frozen and sent for the DNA sequencing method. The sequence result is then used for phylogenetic analysis.

Determination of Antifungal Activity against *C. albicans*

C. albicans was given a rejuvenation treatment using PDA media for 1x24 hours. After that, the fungi were equated for turbidity value with 0.5 McFarland standard. Bacteria isolates in PDB were added by 0.5% chitin colloid. The isolates were then incubated for 14 days under 6 pH and 30ºC temperature conditions using a shaking incubator. Paper discs were inoculated by 10, 15, and 20 µl of each isolate in physiologic NaCl and placed on the plates with PDA media already planted by *C. albicans*. Test media were incubated for 2x24 hours at 25ºC temperature and observed every 1x24 hours. The inhibition zone was observed around the paper discs and then documented and measured. The Diameter of inhibition was then calculated for the chitinolytic index using a formula:

$$Chitinolytic\ Index = \frac{Clear\ Zone\ Diameter}{Colony\ Diameter}$$

The same treatment was also done for the standard test using 20 µg/100 µl fluconazole.

Result

The FTIR spectrum Figure 1, UV interpretation Table 2, colony growth Figure 2, biochemistry test data interpretation Table 3, Gram staining result Table 4, chitinolytic index Table 5, antifungal activity Table 6, and phylogenetic analysis result Figure 4-7 can be seen below:

ISSN: 2087-0337
E-ISSN:2715-9949

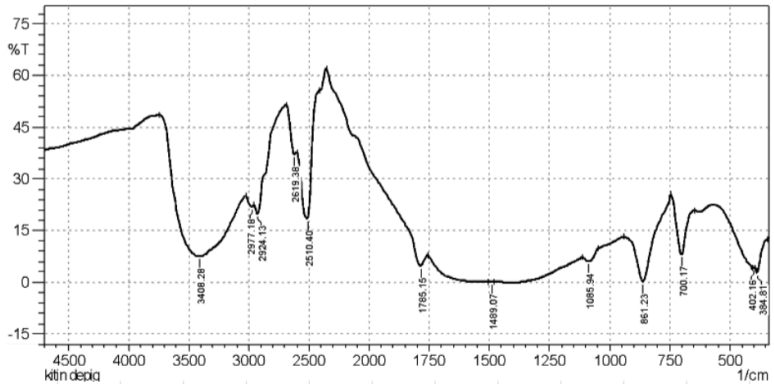


Figure 1. FTIR spectrum of isolated chitin from green mussel shells

Table 1. FTIR Spectrum Interpretation of Isolated Chitin from Green Mussel Shells

Functional Groups	Wave Number (cm^{-1})
O-H stretching	3408.28
CH_3 symmetrical stretching and CH_2 asymmetrical stretching	2924.13
Amide I	1785.15
Amide II	1489.07
C-O stretching	1085.94
O-H out of plane	700.17

Table 2. GlcNAc Absorbance Data, GlcNAc Concentration, and Chitinase Enzyme Activity

Sample	Absorbance	Concentration (ppm)	Chitinase Enzyme Activity (U/ml)
5.00 km	0.131 ± 0.0008	426.67	64,29
5.08 km	0.167 ± 0.0004	406.83	61,30
5.18 km	0.079 ± 0.0004	458.02	69,01

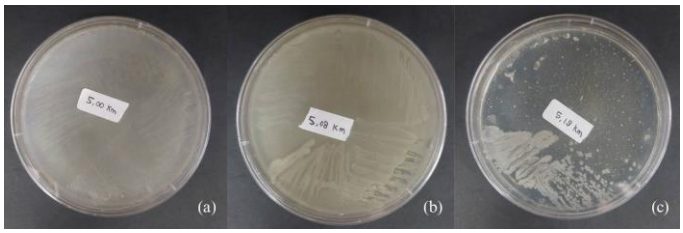


Figure 2. (a) Sample 5.00 km; (b) Sample 5.08 km; and (c) Sample 5.18 km colony

ISSN: 2087-0337
E-ISSN:2715-9949

Table 3. IMViC Biochemistry Interpretation Data

Sample	Tests			
	Indole	**MR**	**VP**	**Citrate**
5.00 km	+	-	-	-
5.08 km	+	-	-	-
5.18 km	+	-	-	-

Table 4. Gram Staining Result Interpretation

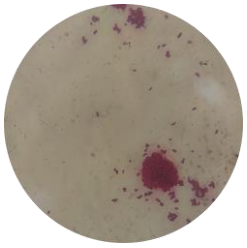
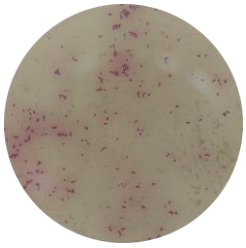
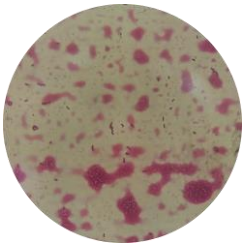
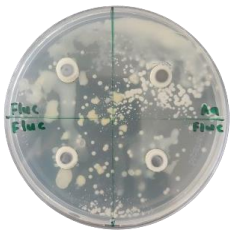
	5.00 km	**5.08 km**	**5.18 km**
Microscopic Observation Result			
Interpretation	negative Gram	negative Gram	negative Gram

Table 5. Bacteria Chitinolytic Index

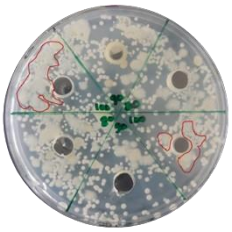
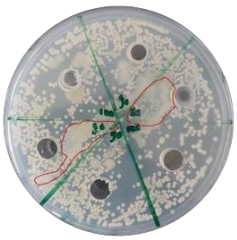
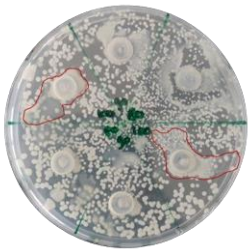
Sample	Average Diameter (cm)	STD	%RSD	Chitinolytic Index
5.00 km	0.748	0.012	1.732	1.246
5.08 km	0.936	0.018	1.966	1.560
5.18 km	0.895	0.017	1.974	1.492

Table 6. Antifungal Activity Result and Interpretation

Sample	Documentation	Interpretation
Fluconazole (Positive Standard)		*C. albicans* cell ruptured show a cloudy form (lysis)

ISSN: 2087-0337
E-ISSN:2715-9949

Table 6. (Extension)

Sample	Documentation	Interpretation
5.00 km		*C. albicans* cell ruptured show a cloudy form (lysis)
5.08 km		*C. albicans* cell ruptured show a cloudy form (lysis)
5.18 km		*C. albicans* cell ruptured show a cloudy form (lysis)

3A 3B

Figure 3. (3A) Living *C. albicans* cells; (3B) Ruptured *C.albicans* cells

ISSN: 2087-0337
E-ISSN:2715-9949

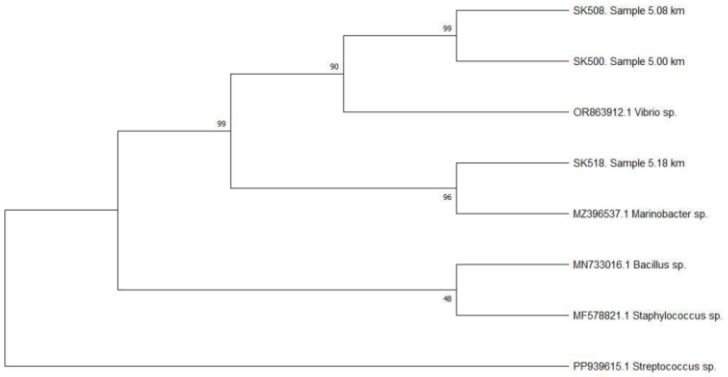


Figure 4. General phylogeny tree

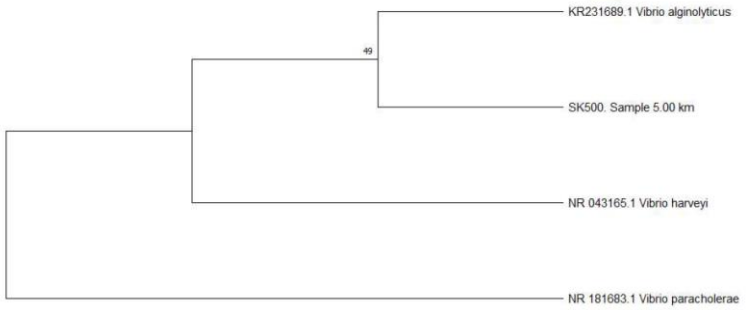


Figure 5. 5.00 km isolate phylogeny tree

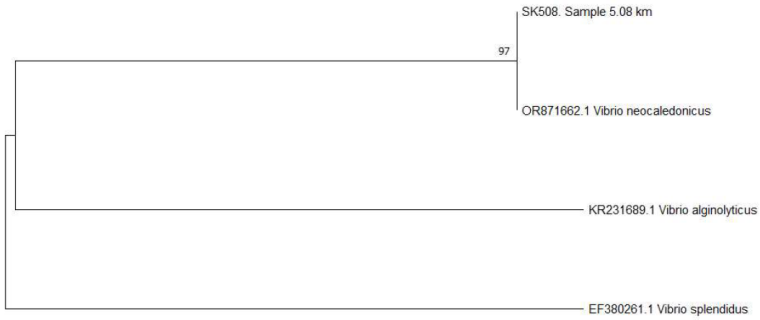


Figure 6. 5.08 km isolate phylogeny tree.

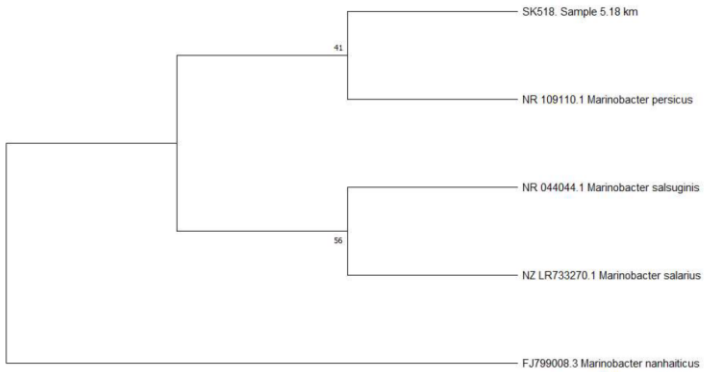


Figure 7. 5.18 km isolate phylogeny tree

ISSN: 2087-0337
E-ISSN:2715-9949

Discussion

Chitin Isolation

The green shell was isolated in three steps (deproteinization, demineralization, and depigmentation) and characterized using the FTIR instrument. Deproteinization was done to eliminate the protein's covalent bond with chitin, and the proteins bonded physically from the rest of the mussels' meat on the shells. Protein elimination happens due to the interaction with NaOH, so the proteins are loose and form a soluble proteinate called sodium.[9]

In addition, demineralization steps were done to eliminate mineral content (calcium with $CaCO_3$ form) on the 'mussel's shell. In this step, HCl was used to destroy the mineral due to its 10% more effective in dissolving calcium than H_2SO_4. The last chitin isolation step, depigmentation, was done to clean up the iso\'ated chitin from impurities in the previous steps to collect the pure chitin.[9]

The chitin structure contains some functional groups that can be analyzed using the FTIR instrument. Those functional groups are O-H, CH_3, CH_2, amide, and C-O. The FTIR analysis result composed of 198 mg of KBr and 22 mg of depigmented chitin gives a spectrum result like in Figure 1, with functional groups wave number interpretation presented in Table 1 that confirmed the compound is chitin.

Chitinolytic Bacteria Screening

The chitinase enzyme activity was observed from GlcNAc, which was produced from chitin degradation by the enzyme. In other words, one chitinase enzyme activity unit is defined as the number of enzymes which produce 1 µmol N-acetylglucosamine (as a standard) per minute.[10] The absorbance, concentration of GlcNAc, and chitinase enzyme activity of each isolate are provided in Table 2. The ability to produce GlcNAc can be one of the parameters used to measure the chitinase enzyme activity through this reaction see Figure 8.

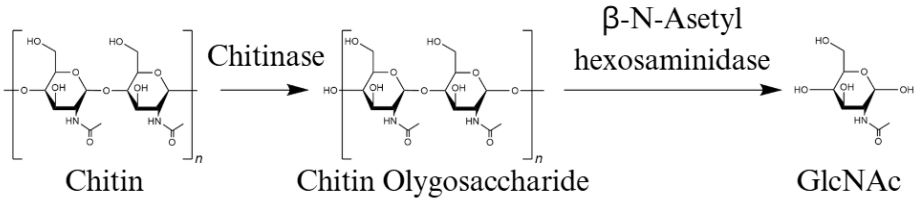


Figure 8. GlcNAc synthesis reaction

The enzymatic activity of chitinase was defined as the number of enzymes that produced one µmol/minute GlcNAc from the substrate. The high chitinase enzyme activity results in implementing the higher bacteria potential on lysing chitin. Therefore, it can be concluded that the sample from the 5.18 km station has the highest chitinase enzyme activity.

Characterization of Bacteria Isolates

The macroscopic observation on chitin media showed only one kind of bacteria based on the colonies' appearance. This can be concluded due to an absence of difference in the visible colony's colour or shape on the media see Figure 2.

The microscopic observation and cell colouring method was also run. The result showed that samples from the 5.00 and 5.08 km stations are comma-shaped bacteria, while a sample from the 5.18 km station is rod-shaped. The Gram colouring method was also conducted. Gram colouring method was used to differentiate bacteria into

positive and negative Gram. The result showed that these three bacteria were Gram-negative due to the red colour of each bacteria cell.

The bacteria have also undergone a biochemical test called IMViC (Indole, Methyl Red, Voges Proskauer, and Citrate). The interpretation of the 'tests' results is provided in Table 3. Biochemical testing should uncover the bacteria's ability by observing the reaction between the bacteria and the added reagents. Those reactions can be associated with the physiological type of bacteria. The living bacteria on chitin media show that they have chitinase enzyme. These bacteria also showed positive results on the Indole test, so these approved bacteria also contained a tryptophanase enzyme.

Antifungal Activity Against *C. albicans*

The antifungal activity was determined using the chitinolytic index results from each of the bacteria isolates. The chitinolytic index can be known by observing inhibiting zones formed around the well in the media filled with the bacteria—the results are provided in Table 5.

Observing those 'method's results, the C. 'albicans' cells were circle or oval under the microscope at the first stage. The colonies were also visible in white colour and rounded shape. On the other hand, adding bacteria to the well made the *C. 'albicans'* cells rupture under the microscope. The colonies are also "destroyed" as cloudy shapes are shown on the media in Table 6 and Figure 3. Media with fluconazole on its well as a standard shows the same result as the bacteria. It is concluded that *C. albicans* experienced lysis due to its interaction with chitinolytic bacteria. These bacteria are also approved for having antifungal activity against *C. albicans*.

On this test, all samples showed a similar characteristic with fluconazole as the positive standard. The samples also have a cloudy area but not as wide as the standard. It can be concluded from this condition that either samples or standards have an anti-candidiasis activity. The cloudy area on the 5.18 km sample is wider than the other two samples, making this sample have the highest anti-candidiasis activity.

Phylogenetic Analysis

Phylogenetic analysis was the last step for this research. It is done by using MEGA11 software. The DNA sequences of sample bacteria were compared to a few bacterial DNA sequences published on NCBI. The results of this analysis are phylogenetic trees that show the genetic relation between species compared to Figure 4-7.

The general analysis compared all the 'samples' DNA with *Vibrio sp.* and *Marinobacter sp.* see Figure 4. This analysis scheme was carried out to narrow down the possible genetic relation between the sample and the comparator. The results showed that the samples from the 5.00 and 5.08 km stations are related to *Vibrio sp.,* while the sample from the 5.18 km station was associated with *Marinobacter sp.* After the genera were known, we ran another analysis for each sample. The sample from the 5.00 km station showed a relation to *Vibrio alginolyticus* with about 49% similarities, which is moderate see Figure 5. The sample from the 5.08 km station showed a relation to *Vibrio neocaledonicus* with about 97% similarities, which is on the high level in Figure 6. The sample from the 5.18 km station showed a relation to *Marinobacter persicus* with about 41% similarities, nearly reaching the moderate level see Figure 7. These similarities have not significantly impacted the result, as the correct genus of each isolate has already been stated. Still, further analysis will be needed to confirm the correct species identity. The lower percentage shown before can be found because natural genetic variability can happen in the 'sample's gene compared to the standard, even within the same species.

The phylogenetic analysis results match its identity and characterization from biochemical testing, Gram colouring, and chitinase activity results. These three bacteria are to show suitable results for each test.

Conclusion

Based on the results, this research showed the potential of seawater microorganisms to cure candidiasis. Sea water samples were identified using chitin from green mussel waste, and it was found that three different bacteria were caught from three different spots on the Kejawanan Beach coast, Cirebon—*Vibrio alginolyticus* species found on 5.00 km with a 1.246 chitinolytic index and 64.29 U/ml chitinase activity. *Vibrio neocaledonicus* species were found on 5.08 km with a 1.560 chitinolytic index and 61.30 U/ml chitinase activity. Lastly, *Marinobacter persicus* species was found on 5.18 km with a 1.492 chitinolytic index and 69.01 U/ml chitinase activity. These three bacteria can lysis the *C. albicans* cell wall under microscopic observation, with the best activity shown on a sample from 5.18 km.

Acknowledgement

The authors thank the Ministry of Education, Culture, Research, and Technology and Universitas Padjadjaran for the funds and facilities through the 'Students' Creativity Program in 2024.

Reference

1. Sophia A, Suraini. Efektivitas perasan daun meniran Phyllanthus niruri L. sebagai antifungi terhadap pertumbuhan jamur Candida albicans. Bioma J Biol Makassar. 2024;9(1):128–34.
2. Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N. The fungal cell wall: candida, cryptococcus, and aspergillus species. Front Microbiol. 2020;10.
3. Sudin S, Sulistijowati R, Harmain RM. Penapisan dan pola pertumbuhan bakteri kitinolitik dari cangkang rajungan (Portunus pelagicus). Jambura Fish Process J. 2020;2(1):36–45.
4. Pentekhina I, Nedashkovskaya O, Seitkalieva A, Gorbach V, Slepchenko L, Kirichuk N, et al. Chitinolytic and fungicidal potential of the marine bacterial strains habituating pacific ocean regions. Microorganisms. 2023;11(9):2255.
5. Safitri N, Wahana S, Hudaya AR. Pengaruh kualitas produk, harga dan lokasi terhadap keputusan pembelian Kerang Hijau (Perna viridis L.). Paspalum J Ilm Pertan. 2023;11(2):395–405.
6. Arsyi NZ, Nurjannah E, Ahlina DN, Budiyati E. Karakterisasi nano kitosan dari cangkang kerang hijau dengan metode gelasi ionik. J Teknol Bahan Alam. 2018;2(2).
7. Peykov S, Strateva T. Whole-genome sequencing-based resistome analysis of nosocomial multidrug-resistant non-fermenting gram-negative pathogens from the Balkans. Microorganisms. 2023;11(3).
8. Setyawati R, Zubaidah S. Optimasi konsentrasi primer dan suhu annealing dalam mendeteksi gen leptin pada Sapi Peranakan Ongole (PO) menggunakan Polymerase Chain Reaction (PCR). Indones J Lab. 2021;4(1):36–40.
9. HS S, Ramayana R, Ramadani K. Pemanfaatan limbah cangkang kerang hijau (Perna Viridis) menjadi kitin sebagai biokoagulan air sungai. Al-Kimia. 2017;5(1):89–99.
10. Nurmalinda A, Rachmania Mubarik N, Sudirman L. Seleksi dan karakterisasi bakteri penghasil kitinase penghambat pertumbuhan cendawan patogen tanaman. J Ilmu Pertan Indones. 2020;25(1):35–42.