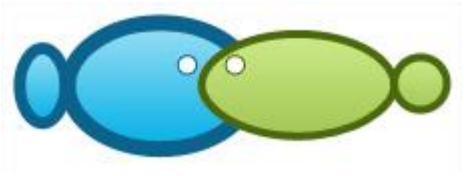

Identification of seaweed-associated chitinolytic bacteria capable in forming chitosan from Tongkaina waters, North Sulawesi

[1]Elvy L. Ginting, [1]Deiske A. Sumilat, [1]Natalie D. C. Rumampuk, [1]Riorifki Kabense, [2]Emma M. Moko, [1]Mayse S. Siby

[1] Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Manado 95115, Indonesia; [2] Faculty of Mathematics and Natural Science, Biology Department, Manado State University, North Sulawesi 95618, Indonesia. Corresponding author: E. L. Ginting, like.ginting@unsrat.ac.id

Abstract. The Tongkaina waters in North Sulawesi Indonesia are home to various seaweed species, such as *Gracilaria* sp. Seaweed-associated bacteria had the ability to degrade chitin and form chitosan. Transformation of chitin to chitosan by bacteria is environmentally friendly and economical. This study detailed seaweed-associated bacteria potentials in chitin degradation and chitosan formation. The bacteria were isolated from seaweed *Gracilaria* sp. from Tongkaina waters, North Sulawesi Indonesia. Chitin media was utilized to screen the chitinase activity of the bacteria. Chitin to chitosan transformation was evaluated using chitinase degrading activity media. 16S rRNA gene sequences were the basis for the molecular identification. This research discovered four seaweed-associated chtinolytic bacteria, and from those four, SG3 and SG4 contained the highest chitinolytic index values with the range of 3.04-3.52. The bacteria have the ability to form chitosan based on the existence of chitosan in the precipitation formation in the bacteria fermented broth. SG3 has been identified as *Staphylococcus equorum* and the SG4 as *Bacillus tropicus*. The bacteria can be utilized to exploit the biotransformation process of chitin into chitosan, which is an environmentally friendly and economical process.
Key Words: *B. tropicus*, eco-friendly, economical, *Gracilaria* sp., *S. equorum*.

Introduction. Seaweeds, or macroalgae, are aquatic organisms that carry out photosynthesis. They belong to the domain Eukaryota, kingdoms Plantae (red and green algae), and Chromista (brown algae) (Pereira 2017), and mostly live in the intertidal zone. Therefore, they are exposed to diverse chronic stresses such as intense irradiance, salinity, radiation of ultraviolet, desiccation, and submergence caused by periodic tidal rhythms (Kumar & Ralph 2017). Seaweeds thrive on rocky shores, seabeds, and various substrates. This represents multilayered, perennial vegetation that grows photosynthetically (Singh et al 2015).

Various kinds of microorganisms live in association with seaweed. The surface of the seaweed allows for an appropriate substrate that microorganisms need to survive and to release varying organic substances which may be used as sustenance for the multiplication of microbes and the creation of microbial biofilms (Singh & Reddy 2014).

The microbial community that lives on the superficies of seaweed is very complex, and contains a pool of microorganisms, which include bacteria, diatoms, fungi, protozoa, marine invertebrates' larvae, and spores (Burke et al 2011; Lachnit et al 2011). The existence of this consortium of microorganisms, such as bacteria, greatly determines the various stages of life cycle states of the host eukaryotic organisms, including seaweed. Seaweed-associated bacteria can be considered as a potential source for active compounds and specific enzymes (Satheesh et al 2016), like chitinase.

Chitinases (EC 3.2.1.14) are enzymes that hydrolyze the β-1,4-glycoside bond of chitin and other compounds of N-acetylglucosamine. The majority appertain to families 18 of glycosyl hydrolases (classes III and V) and 19 (classes I, II, and IV), with molecular size of 20 to 90 kDa. The enzymes are constructed by a variety of organisms,

ranging from plants to microorganisms, to fungi and bacteria. They are involved in processes of symbiosis, pathogenicity, morphogenesis, and nutrition (Kumar et al 2018). Microbial chitinases, such as chitinolytic bacteria, have garnered worldwide attention in fundamental research and industrial applications. These enzymes offer a wide spectrum of applications in human and plant health, biochemistry, agriculture, food industries, and biofuel production (Hartl et al 2012).

Chitin deacetylase (CDA) can be produced by chitinolytic bacteria, which is an enzyme that causes the chitin to chitosan conversion by deacetylating residues of N-acetyl-D-glucosamine (Harmsen et al 2019). Nowadays, chitosan is created from chitin using the NaOH chemical method. Drawbacks of this method include environmental pollution, the chitosan poor quality, and high prices. The presence of CDA can theoretically overcome this problem by using it to produce chitosan (Schmitz et al 2019).

Chitosan has great potential in biotechnology, pharmaceutical industries and biomedical applications such as in drug delivery, cancer treatment, and wound healing (Baharlouei & Rahman 2022). Its non-toxic properties, biocompatibility, and biodegradability fared well in medical applications (Yang 2011). Chitosan may also be applied to plants as protection from bacteria, viruses, and fungi, and may be further used as plant growth regulator and fertilizer additive (Malerba & Cerana 2018). In wastewater treatment, the chelating potential and fat-binding of chitosan functions as fat, dye, and heavy metals removal in the wastewater (Badawi et al 2017). However, current chitin to chitosan conversion uses chitin deacetylase, which is produced by fungi through complicated fermentation. With several improvements, the chitin-chitosan biotransformation from bacteria can be used as an ecological, and economical alternative for chitosan production (Schmitz et al 2019). The seaweed-associated bacteria possess the potential in producing chitinase and forming chitosan through the function of the chitin deacetylase.

The Sulawesi Sea located in Indonesia, specifically the Tongkaina waters in North Sulawesi, is home to a variety of seaweed. There were 15 species of seaweed identified in this habitat, consisting of 7 green algae species, 4 brown algae species, and 4 red algae species (Kepel et al 2018). One of the red algae is *Gracilaria* sp. and there are not a lot of studies regarding bacteria associated with *Gracilaria* sp. Their abilities to produce chitinase and form chitosan are not studied yet. The study of seaweed associated bacteria may help in finding new sources of bacteria that produce chitin deacetylase which could be developed in a chitosan production through a more economical and eco-friendly process. This current study screens and identifies seaweed-associated chitinolytic bacteria that sourced from Tongkaina waters and their ability to form chitosan. This finding could be useful to build a coastal protection program in relation with conserving the seagrass ecosystem as an important habitat of the microbial hosts.

Materials and Methods

Preparation of colloidal chitin. The study was conducted from April to October 2023, starting with the preparation of materials. To screen chitin-active enzymes (chitinases and CDA), we used colloidal chitin as a substrate. The preparation of substrate was done by mixing about 5 g of chitin in 30 mL of concentrated hydrochloric acid (35.5% - MERCK, Germany) and the mix was incubated for 24 hours at 4°C. A volume of 250 mL of chilled ethanol (50% - Merck, Germany) was added slowly, with constant stirring at 4°C, and left overnight for precipitated the colloidal chitin. The colloidal chitin was then centrifuged at 10,000 g for 20 minutes, and the pellets were rinsed with sterile distilled water to adjust the pH to 7 (Subramanian et al 2020). The colloidal chitin achieved was then autoclaved and stored at 4°C until further use as a substrate.

Seaweed collection. *Gracilaria* sp. was collected from Tongkaina water, North Sulawesi (Figure 1). The sample is presented in Figure 2. The identification of the sample was referred to AlgaeBase (https://www.algaebase.org/). Approximately 10 g of alive and healthy seaweed thalli, still submerged in seawater during low tide, were picked up

aseptically and put in aseptic poly bags containing 1/4 seawater. Then, the poly bags were placed in a cool box and immediately brought to the laboratory for bacterial isolation.

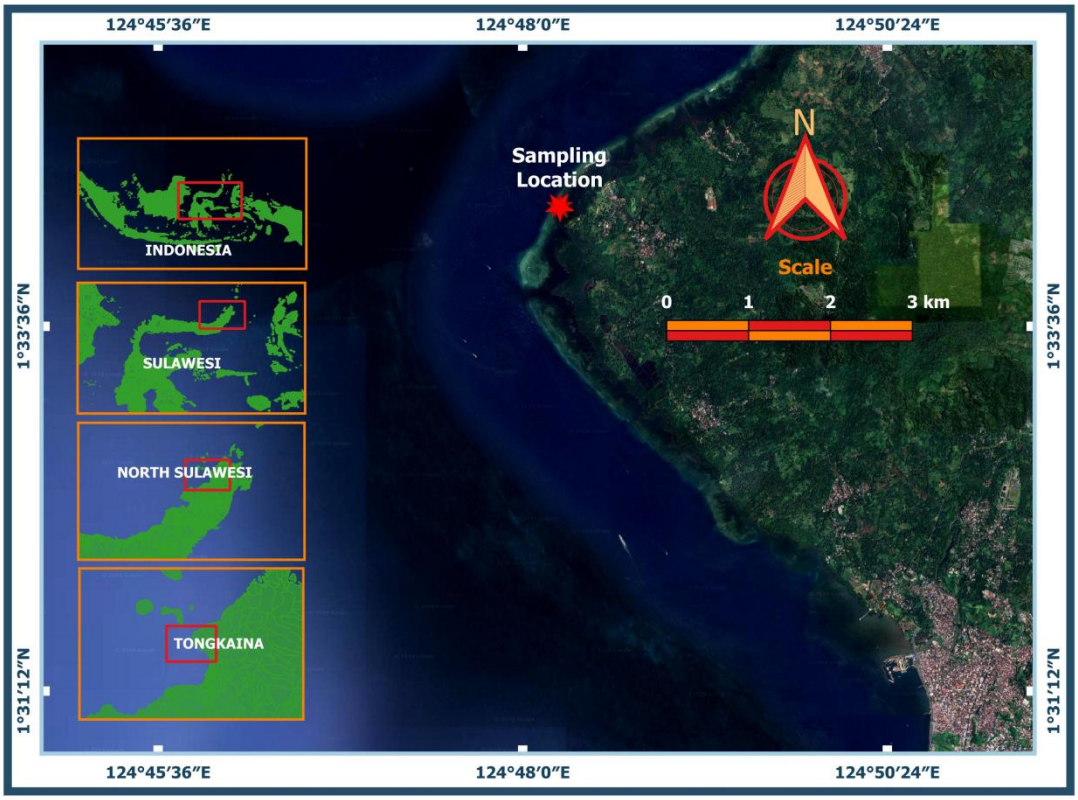

Figure 1. Sampling location of *Gracilaria* sp., Tongkaina waters, North Sulawesi, Indonesia.

Figure 2. The sample of *Gracilaria* sp.

Isolation and screening of chitin degrading bacteria. Serial tube dilution and plating methods were used in isolating the bacteria. A fresh thallus of seaweed weighing 1.0 g was homogenized, and the volume was arranged to 10 mL using sterile seawater under aseptic conditions. Furthermore, the homogenate thallus of seaweed was diluted serially using sterile seawater and plated on nutrient agar (Merck, Germany), which was then incubated at 28°C for 24 hours to obtain colonies. The individual colonies were elected based on their morphological characteristics, such as color, shape, edge, and elevation. The colonies were subjected to repeated streaking on nutrient agar plates. The seaweed-associated bacterial isolates obtained were stored at 4°C for further studies.

To select chitinolytic isolates, each single colony of bacterium was picked up and subsequently spotted on medium of chitin (nutrient agar containing 2% of colloidal chitin), followed by 24 hours of incubation at 28°C. After the incubation period, clear

zones forming around the colony showed the bacteria ability to produce chitinase and may be identified as a chitinolytic index. The chitinolytic index was identified by dividing the clear zone's diameter by the bacterial colony's diameter (Korany et al 2019).

Formation of chitosan by bacteria. Chitinase degrading activity media (CDA media) consist of 1 g of yeast extract, 0.15 g of potassium dihydrogen phosphate, 0.4 g of ammonium sulfate, and 50 mg of substrate (colloidal chitin). This was used to examine the chitin-chitosan degradation ability of the bacteria. Fifty (50) mL of CDA media were sterilized and filled into flasks with 250 mL capacity. Suspensions containing 1 mL of each bacterial isolate (0.1 O.D.600) were inoculated onto the CDA medium. The CDA medium that was not inoculated with bacteria may act as a control. The flasks were put on a rotary shaker at 28°C for 48 hours for incubation of the bacteria. The cultured CDA medium broth was then centrifuged for 15 minutes at 12,000 rpm. The pellets, comprising a mixture of chitin, chitosan, and bacteria, were obtained, and subsequently transferred to a tube.

Ten (10) mL of 0.1 N NaOH were added, mixed, and autoclaved at 121°C for 60 minutes. The centrifuge process was repeated at 12,000 rpm for 15 minutes, and then the pellets were taken and combined with 10 mL of 2% acetic acid in a new tube. Incubation on a rotary shaker at 28°C for 12 hours was applied to the tubes. Afterward, the tubes were centrifuged at 12,000 rpm for a period of 15 minutes. The pellet was then removed, and 10 mL of the supernatant was nullified using 1 N NaOH. After adding 1 N NaOH, a white precipitate formed, indicating the creation of chitosan (Kaur et al 2012).

Qualitive chitosan estimation. The tubes containing the white precipitates were centrifuged for 15 minutes at 5000 rpm. The white precipitates were collected and neutralized to pH 7 using distilled water. The neutral precipitates were re-suspended in 0.5 mL of distilled water and dried at 55°C for 2-4 hours. Around twenty-tree drops of potassium iodide were mixed to the dry precipitate. Three to four drops of solution containing 1% H_2SO_4 were then dropped in the mix. After the addition of iodine/potassium iodide, the color of the precipitate turned to dark brown, and after the addition of 1% H_2SO_4, the precipitate changed color to dark purple. The coloring indicated the formation of chitosan (Kaur et al 2012; Korany et al 2019).

Identification of bacteria. Identification of the bacteria was performed using a molecular method. The genomic DNA of the bacterium was isolated using the Miniprep kit from QIAprep (Qiagen Hilden, Germany). The DNA genomic was used as the template for PCR amplification of the 16S rRNA gene region employing the pair of universal primer (Integrated DNA Technologies-IDT, Singapore) 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACCTTGTTACGACT-3'). The PCR conditions started at initial denaturation at 95°C for 6 minutes, followed by 35 cycles of 95°C for 30 seconds, 52°C for 30 seconds, 72°C for 30 seconds, and a last extension at 72°C for 10 minutes. Electrophoresis on an agarose gel (1%) with a marker (1 kb DNA ladder - Solis BioDyne, Estonia) was used to observe the size of the PCR products. After that, it was sent to First-Base Co., Selangor, Malaysia for the sequencing of the DNA. The quality assessment of the 16S rRNA gene DNA sequences was performed using Sequence Scanner version 2.0 Software from Applied Biosystems, USA. Furthermore, the traces of sequence were trimmed, constructed, and manually adjusted using Geneious Prime version 2020 (http://www.geneious.com) before being subjected to BLAST analysis for the 16S rRNA sequence at The National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). Finally, the phylogenetic tree was constructed in Geneious using the method of neighbor-joining in MEGA 11.

Results and Discussion. Thirteen different bacterial colonies were isolated from seaweed samples based on the colony's morphological characteristics on nutrient agar medium. All thirteen bacterial isolates showed different morphological characteristics for their colonies.

Bacteria living in association with seaweed will compete to obtain space and nutrients from the seaweed as their host (Suvega & Arunkumar 2019). Therefore, these bacteria will synthesize various enzymes/compounds to absorb nutrients from the seaweed/host. Moreover, bacteria living as endophytes can reside inside the seaweed/host at every phase of growth, supplying additional resources to the host through the production of nutrients, secondary metabolites, and enzymes. These compounds can be utilized by the host to shield itself from abiotic and biotic stresses. Many of the enzymes produced by the bacteria are extracellular enzymes, which means their activity occurs thereout cell of the bacteria and has direct implications for the surrounding environment, including the host (Khan et al 2017).

The results of this research show that four isolates produced extracellular enzymes, specifically chitinase. We referred to these isolates as SG1, SG2, SG3, and SG4. The isolate's activity to produce chitinase was demonstrated by the formation of a clear zone when they were grown on chitin medium (Figure 3), and it was evaluated as a chitinolytic index. The chitinolytic indexes of SG1, SG2, SG3 and SG4 were 1.4, 7.5, 10.5 and 10.0, respectively (Table 1). The chitinolytic index indicates the capability of the bacteria to produce chitinase to break down chitin compounds in the medium. The activity varied from weak to strong, with SG2, SG3 and SG4 showing strong activity due to their index being above 2 (Wang et al 2018).

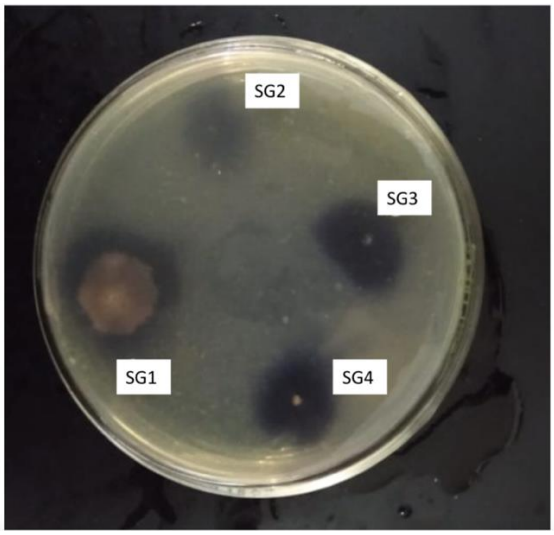

Figure 3. The appearance of a clear zone formed around bacterial colonies grown on chitin media.

Table 1

The chitinolytic index of bacterial isolates associated with seaweed *Gracilaria* sp.

Isolate	*Clear zone (cm)*	*Colony bacterium (cm)*	*Chitinolytic index*
SG1	2.8	2.0	1.4
SG2	1.5	0.2	7.5
SG3	2.1	0.2	10.5
SG4	2.0	0.2	10.0

The chitinolytic bacteria also had the ability to form chitosan. This ability is indicated by the formation of precipitation after the fermented broth is incubated for a certain amount of time. This showed the presence of chitosan. The precipitation of chitosan is represented by the colorization to a dark purple with the addition of 1% H_2SO_4, indicating a reaction (Wang et al 2018). The color binding of the chitin mechanism goes beyond simple absorption. The pH affects this mechanism (Miliao et al 2022). Under normal conditions, the amine group of chitins has both a neutral charge ($-NH_2$) and a cationic charge ($-NH_3^+$). However, in acidic conditions, the chitin polymer becomes positively charged, as the amine group (NH_2) gets protonated. The chitin polymer then undergoes an ion exchange with negative ions from anionic colorants to form an electrostatic

interaction, maintaining a neutral condition (Wang et al 2018). The chitin smallest unit, i.e., chitobiose, composed of two molecules of N-acetyl-D-glucosamine, possesses two hydroxyl groups, one amine group and one carbonyl group. To create a complex color, these groups create a reactive linkage with iodide (Agrawal & Kotasthane 2012).

The capability to form chitosan of the bacteria shows the potential for chitin deacetylase (CDA) production (Agrawal & Kotasthane 2012). The CDA enzyme catalyzes the chitin-chitosan conversion by deacetylating residues of N-acetyl-D-glucosamine. Therefore, the seaweed-associated bacteria in this research can be categorized as strains capable of producing CDA.

In chitosan synthesis, the deacetylation step is crucial, as it is essential for the chitosan quality (Harmsen et al 2019). The process of chitin deacetylation by bacteria capable of producing CDA can replace the conventional chemical process that uses NaOH for deacetylation to produce chitosan. The NaOH chemical process has the disadvantage of polluting the environment (Casadidio et al 2019).

Some insects (Noh et al 2018) and fungi (Philibert et al 2017) have been reported to have the ability to produce chitin deacetylase (CDA). CDA-producing fungi can also be used for chitin N-deacetylation, and theoretically, they can also address the chitin-chitosan degradation problem through chemical processes. However, the majority fungal strains have limited capacity in CDA production, and their fermentation requirements can be challenging (Kaur et al 2012). Therefore, the function of fungal strains to produce CDA can be replaced by bacterial strains, as bacteria grow more easily and rapidly in large-scale fermentation systems at a lower cost compared to fungi. Modifications and optimizations of the process using bacterial CDA can be easily implemented without the need for enzyme purification in its application (Philibert et al 2017). Hence, seaweed-associated chitinolytic bacteria technique has good potential for degrading chitin and forming chitosan.

In this research, SG3 and SG4 had the biggest chitinolytic index; therefore, those bacteria were chosen to be identified. The successful completion of the SG3 and SG4 DNA gene sequences was achieved, along with the successful amplification of the 16S-rRNA gene. The 16S rRNA sequences results of SG3 and SG4 were visualized on a 1% agarose gel, exhibiting a size of approximately 1500 base pairs (Figure 4), which was enough for informatics analysis.

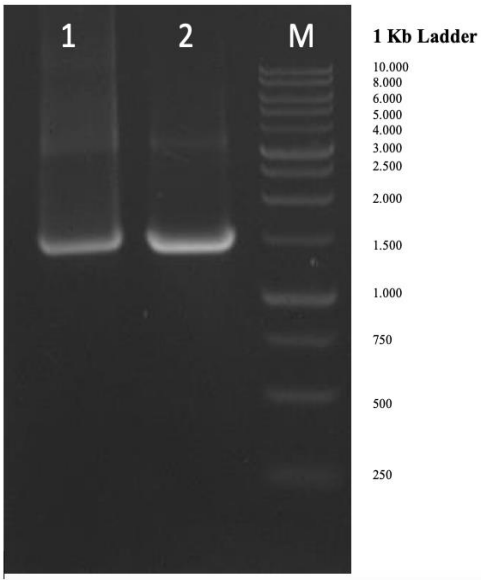

Figure 4. Agarose gel electrophoresis (1%) of the 16S-rRNA gene PCR product of the SG3 and SG4 bacterial isolates. One (1) µL PCR products were assessed by electrophoresis with 0.8% TBE agarose (line 1 = SG3, line 2 = SG4, M = DNA ladder).

The 16S rRNA partial gene sequence data were deposited with Accession Numbers of OR304223 (strain SG3) and OR304266 (strain SG4). Based on the 16S rRNA gene

sequences, which were compared to the BLASTN program sequence databases, SG3 indicated similarity (100% identity) to *Staphylococcus equorum*, and SG4 to *Bacillus tropicus*. A similar result was shown in the analysis of phylogeny, as shown in the phylogenetic tree (Figure 5). It showed that SG3 and *S. equorum* were in the same group, while SG4 and *B. tropicus* were in the same group. Hence, SG3 is named *S. equorum* strain SG3, and SG4 is named *B. tropicus* strain SG4.

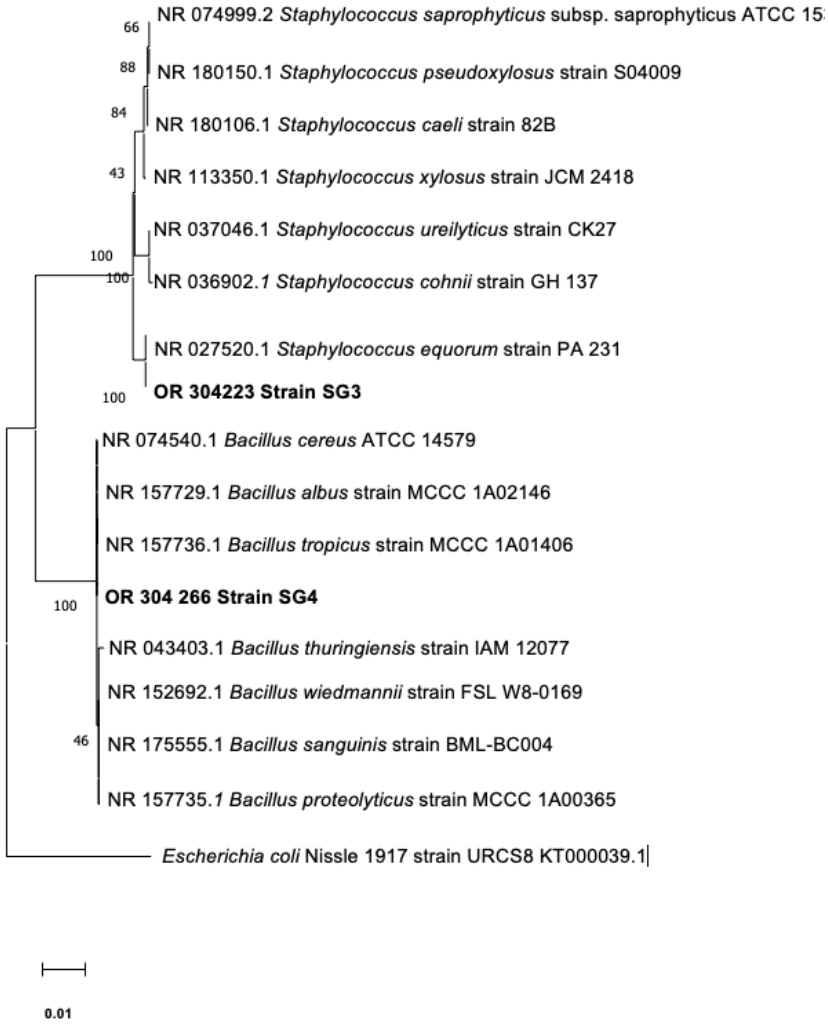

Figure 5. Neighbor-joining phylogenetic tree from analysis of 16S rRNA gene sequence of bacterial strains SG3 and SG4. To assess the reliability of phylogenetic tree reconstruction, a resampling method with bootstrapping values from 1000 replicates was applied to the branch. *Escherichia coli* was utilized as the 'outgroup'.

The endophytic bacteria genera, such as *Bacillus*, *Enterobacter*, *Burkholderia*, *Stenotrophomonas*, *Streptomyces* and *Micromonospora* have been reported to exhibit chitinase activities and effectiveness when opposed to fungal phytopathogens (Tabli et al 2018). *Staphylococcus* sp. has been found to be capable of producing chitinase. *Staphylococcus* is a Gram-positive cocci bacterium, positive for the tube coagulase test, catalase, DNAse, and nitrate reduction test (Oluduro et al 2023). This bacterium has been found in marine water and sand of intertidal beaches (Thapaliya et al 2017; Das et al 2019).

Staphylococcus sp. were found associated with marine sponges and seaweed. *S. aureus* ATCC 25923 and *S. mutans* FNCC 0405 were associated with *Eucheuma cottonii* seaweed and showed antibacterial activity (Purnami et al 2022). On the other hand, *S. equorum* was associated with a marine sponge that exhibited antibacterial activity against *Bacillus subtilis*. Additionally, seaweed-associated *S. haemolyticus* MSM was a

bacteriocin producer (Suresh et al 2014). Bacteriocins are ribosomally synthesized antimicrobial peptides which are not lethal to producer cells. These peptides are usually active when opposed to species closely related to microorganism production (Maughan & Van der Auwera 2011). However, based on our knowledge, there is no information about *S. equarium* being a chitinolytic bacteria and having the ability to form chitosan through CDA production.

Bacillus are rod-shaped bacteria, comprising a heterogeneous collection of Gram-variable or Gram-positive, phenotypically large, capable of spore formation, aerobic or anaerobic facultative bacteria (Maughan & Van der Auwera 2011). Some species of *Bacillus* are found in marine and coastal environments (Liu et al 2017). *Bacillus* species have been identified in various habitats, including in marine and shrimp waste. *B. tropicus* was isolated from a marine environment. The marine bacterium *B. cereus* TK19 was found to have activity in producing chitinase and chitin deacetylase (Ravikumar & Perinbam 2016). Additionally, *Bacillus* is known to have the highest chitinolytic activity (Setia & Suharjono 2015), but there is no information about the ability of *B. tropicus* to form chitosan through CDA production. However, *B. tropicus* was reported to have keratinase activity (Liya et al 2023).

B. tropicus can live while being associated with other organisms. *B. tropicus* was successfully isolated from *Acaudina molpadioides* sea cucumber's intestine (Lambuk et al 2023). However, *B. tropicus* has not been isolated from seaweed, especially *Gracilaria* sp., and to our knowledge, there is no information about *B. tropicus* as a chitinolytic bacterium and its ability to form chitosan through CDA production. On the other hand, *B. subtilis* has been reported to be associated with sponges and has chitinase enzyme that can convert chitin to chitosan through CDA. *B. subtilis* produces chitinase that is superior to commercial chitinase derived from *Streptomyces griseus* and *Serratia marcescens* (Wang et al 2018).

The results of this research demonstrate the capability of bacterial strains isolated from *Gracilaria* sp., from Tongkaina water, North Sulawesi, which is the *S. equorum* strain SG3 and *B. tropicus* strain SG4, in producing chitinase and forming chitosan. This finding can provide significant value in which these bacteria can be utilized for chitosan production through an economical and environmentally friendly process without damaging the seaweed ecosystem.

Conclusions. The present research confirmed that *S. equoum* strain SG3 and *B. tropicus* strain SG4 were isolated from *Gracilaria* sp. from Tongkaina waters, North Sulawesi. The bacteria had the ability to produce chitinase and were able to degrade chitin that will then form chitosan. The bacteria could act as a CDA producer in forming chitosan, meaning that it could be a promising candidate for an economical and eco-friendly process of chitosan production. This process can replace the usage of NaOH chemical method which is not environmentally and economically friendly for chitosan production from chitin.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Agrawal T., Kotasthane A. S., 2012 Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. SpringerPlus 1(1):73.

Badawi M. A., Negm N. A., Kana M. T. H. A., Hefni H. H., Moneem M. M. A., 2017 Adsorption of aluminum and lead from wastewater by chitosan-tannic acid modified biopolymers: isotherms, kinetics, thermodynamics, and process mechanism. International Journal of Biological Macromolecules 99:465-476.

Baharlouei P., Rahman A., 2022 Chitin and chitosan: prospective biomedical applications in drug delivery, cancer treatment, and wound healing. Marine Drugs 20(7):460.
Burke C., Thomas T., Lewis M., Steinberg P., Kjelleberg S., 2011 Composition, uniqueness, and variability of the epiphytic bacterial community of the green alga *Ulva australis*. The ISME Journal 5:590-600.
Casadidio C., Peregrina D. V., Gigliobianco M. R., Deng S., Censi R., Di Martino P., 2019 Chitin and chitosans: characteristics, eco-friendly processes, and applications in cosmetic science. Marine Drugs 17(6):369.
Das P., Chatterjee S., Behera B. K., Dangar T. K., Das B. K., Mohapatra T., 2019 Isolation and characterization of marine bacteria from East Coast of India: functional screening for salt stress tolerance. Heliyon 5(6):e01869.
Harmsen R. A. G., Tuveng T. R., Antonsen S. G., Eijsink V. G. H., Sorlie M., 2019 Can we make chitosan by enzymatic deacetylation of chitin? Molecules 24(21):3862.
Hartl L., Zach S., Seidl-Seiboth V., 2012 Fungal chitinases: diversity, mechanistic properties, and biotechnological potential. Applied Microbiololy and Biotechnology 93(2):533-543.
Kaur K., Dattajirao V., Shrivastava V., Bhardwaj U., 2012 Isolation and characterization of chitosan-producing bacteria from beaches of Chennai India. Enzyme Research 2012:421683.
Kepel R. C., Mantiri D. M. H., Nasprianto, 2018 [Biodiversity of macroalgae in coastal waters of Tongkaina, Manado City]. Jurnal Ilmiah Platax 6(1):160-173. [in Indonesian]
Khan A. L., Shahzad R., Al-Harrasi A., Lee I. J., 2017 Endophytic microbes: a resource for producing extracellular enzymes. In: Endophytes: crop productivity and protection. Maheshwari D. K., Annapurba K. (eds), Springer International Publishing, pp. 95-110.
Korany S. M., Mansour A. N., El-Hendawy H. H., Kobisi A. N. A., Aly H. H., 2019 Entomopathogenic efficacy of the chitinolytic bacteria: *Aeromonas hydrophila* isolated from Siwa Oasis, Egypt. Egyptian Journal of Biological Pest Control 29:16.
Kumar M., Ralph P., 2017 Systems biology of marine ecosystems. Springer, Berlin, 372 pp.
Kumar M., Brar A., Yadav M., Chawade A., Vivekanand V., Pareek N., 2018 Chitinases - potential candidates for enhanced plant resistance towards fungal pathogens. Agriculture 8(7):88.
Lachnit T., Meske D., Wahl M., Harder T., Schmitz R., 2011 Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. Environmental Microbiology 13(3):655-665.
Lambuk F., Mazlan N., Young T. T., Anua S. M., Ishak R., 2023 Isolation and characterisation of bacteria and fungus from the intestine of sea cucumber *Acaudina molpadioides*. Malaysian Journal of Fundamental and Applied Sciences 19: 103-115.
Liu Y., Lai Q., Du J., Shao Z., 2017 Genetic diversity and population structure of the *Bacillus cereus* group bacteria from diverse marine environments. Science Reports 7:689.
Liya S. M. M., Umesh M., Nag A., Chinnathambi A., Alharbi S. A., Jhanani G. K., Shanmugam S., Brindhadevi K., 2023 Optimized production of keratinolytic proteases from *Bacillus tropicus* LS27 and its application as a sustainable alternative for dehairing, destaining and metal recovery. Enviromental Research 221:115283.
Malerba M., Cerana R., 2018 Recent advances of chitosan applications in plants. Polymers 10(2):118.
Maughan H., Van der Auwera G., 2011 *Bacillus* taxonomy in the genomic era finds phenotypes to be essential though often misleading. Infection, Genetics and Evolution 11(5):789-797.

Miliao G. L., Soares L. S., Balbino D. F., Barbosa E. A. A., Bressan G. C., Teixeira A. V. N. C., Coimbra J. S. R., de Oliveira E. B., 2022 pH influence on the mechanisms of interaction between chitosan and ovalbumin: a multi-spectroscopic approach. Food Hydrocolloids 123:107137.
Noh M. Y., Muthukrishnan S., Kramer K. J., Arakane Y., 2018 Group I chitin deacetylases are essential for higher order organization of chitin fibers in beetle cuticle. Journal of Biological Chemistry 293(18):6985-6995.
Oluduro A. O., Adesiyan Y. M., Omoboye O. O., Odeyemi A. T., 2023 Phenotypic and molecular characterization of *Staphylococcus aureus* from mobile phones in Nigeria. AIMS Microbiology 9(3):402-418.
Pereira L., 2017 Therapeutic and nutritional uses of algae. CRC Press, Boca Raton, Florida, USA, 672 pp.
Philibert T., Lee B. H., Fabien N., 2017 Current status and new perspectives on chitin and chitosan as functional biopolymers. Applied Biochemistry and Biotechnology 181(4): 1314-1337.
Purnami P. P. C. P., Indraningrat A. A. G., Darmayasa I. B. G., 2022 Antibacterial activity screening of bacterial isolates associated with seaweed *Eucheuma cottonii* from coastal area in Buleleng, Bali. Biotropika: Journal of Tropical Biology 10(2):132-140.
Ravikumar M., Perinbam K., 2016 Production, optimization, and characterization of chitin deacetylase from marine bacteria *Bacillus cereus* TK19. Journal of Academia and Industrial Research 5:72-76.
Satheesh S., Ba-akdah M. A., Al-Sofyani A. A., 2016 Natural antifouling compound production by microbes associated with marine macroorganisms - a review. Electron Journal of Biotechnology 21:26-35.
Schmitz C., Auza L. G., Koberidze D., Rasche S., Fischer R., Bortesi L., 2019 Conversion of chitin to defined chitosan oligomers: current status and future prospects. Marine Drugs 17(8):452.
Setia I. N., Suharjono, 2015 Chitinolytic assay and identification of bacteria isolated from shrimp waste based on 16S rDNA sequences. Advances in Microbiology 5(7):541-548.
Singh B., Bauddh K., Bux F. (eds), 2015 Algae and environmental sustainability. Springer, India, 181 pp.
Singh R. P., Reddy C. R. K., 2014 Seaweed-microbial interactions: key functions of seaweed-associated bacteria. FEMS Microbiology Ecology 88(2):213-230.
Subramanian K., Sadaiappan B., Aruni W., Kumarappan A., Thirunavukarasu R., Srinivasan G. P., et al., 2020 Bioconversion of chitin and concomitant production of chitinase and N-acetylglucosamine by novel *Achromobacter xylosoxidans* isolated from shrimp waste disposal area. Scientific Reports 10:11898.
Suresh M., Iyapparaj P., Anantharaman P., 2014 Optimization, characterization, and partial purification of bacteriocin produced by *Staphylococcus haemolyticus* MSM an isolate from seaweed. Biocatalysis and Agricultural Biotechnology 3(4):161-166.
Suvega T., Arunkumar K., 2019 Probiotic bacteria promote the growth of associating host (red seaweed, *Gracilaria edulis*) also synthesize antibacterial protein. Biocatalysis and Agricultural Biotechnology 19:101136.
Tabli N., Rai A., Bensidhoum L., Palmieri G., Gogliettino M., Cocca E., Consiglio C., Cillo F., Bubici G., Nabti E., 2018 Plant growth promoting and inducible antifungal activities of irrigation well water-bacteria. Biological Control 117:78-86.
Thapaliya D., Hellwig E. J., Kadariya J., Grenier D., Jefferson A. J., Dalman M., Kennedy K., DiPerna M., Orihill A., Taha M., Smith T. C., 2017 Prevalence and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* on public recreational beaches in Northeast Ohio. Geohealth 1(10):320-332.
Wang D., Li A., Han H., Liu T., Yang Q., 2018 A potent chitinase from *Bacillus subtilis* for the efficient bioconversion of chitin-containing wastes. International Journal of Biological Macromolecules 116:863-868.
Yang T. L., 2011 Chitin-based materials in tissue engineering: applications in soft tissue and epithelial organ. International Journal of Molecular Science 12(3):1936-1963.

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Authors:
Elvy Like Ginting, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Kampus Unsrat-Bahu street, Manado-95115, North Sulawesi, Indonesia, e-mail: like.ginting@unsrat.ac.id
Deiske Adeliene Sumilat, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Kampus Unsrat-Bahu street, Manado-95115, North Sulawesi, Indonesia, e-mail: deiske.sumilat@unsrat.ac.id
Natalie Detty Carolina Rumampuk, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Kampus Unsrat-Bahu street, Manado-95115, North Sulawesi, Indonesia, e-mail: dety.natalie@unsrat.ac.id
Riorifki Kabense, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Kampus Unsrat-Bahu street, Manado-95115, North Sulawesi, Indonesia, e-mail: riorifkikabense@gmail.com
Emma Mauren Moko, Faculty of Mathematics and Natural Science, Biology Department, Manado State University, Raya Tondano street, North Sulawesi, Indonesia, e-mail: emmamoko@unima.ac.id
Mayse Sofien Siby, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Kampus Unsrat-Bahu street, Manado-95115, North Sulawesi, Indonesia, e-mail: Sibymasye006@gmail.com

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