

# Screening of Antibacterial Activity from Bacteria Associated with the Seaweeds *Ulva* sp. and *Chaetomorpha* sp. from Gunungkidul Coast, Indonesia

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## Abstract

Seaweed is a strategic multi-industry commodity for food, pharmaceuticals, and cosmetics. The multifunctional properties of seaweed are influenced not only by its nutritional and bioactive content but also by microbes associated with it, both on the surface and as endophytes within its tissues, thus forming a functional holobiont unit. These microbes actively affect the metabolism, morphology, and bioactive compound content, which determine the seaweed quality for food, medicine, and cosmetics. This study explored bacteria associated with seaweed from Gunungkidul Coast, Indonesia, such as *Ulva* sp. and *Chaetomorpha* sp. The aim of this research was to obtain bacteria associated with *Ulva* sp. and *Chaetomorpha* sp. that possess antibacterial activity against pathogenic *Escherichia coli* and *Staphylococcus aureus*. Bacterial isolation was performed using the pour plate technique on de Man Rogosa Sharpe Agar (MRSA), Potato Dextrose Agar (PDA), Tryptic Soy Agar (TSA), Zobbel Marine Agar (ZMA), International *Streptomyces* Project-2 (ISP-2), and International *Streptomyces* Project-4 (ISP-4) media. Based on the number of colonies that grew, TSA medium was the most optimal, yielding 192 and 357 colonies from *Ulva* sp. and *Chaetomorpha* sp., respectively. Antibacterial testing showed that isolates U1 and U2 from *Ulva* sp. produced the largest inhibition zones, namely 12.71 mm and 5.27 mm against *E. coli* and 2 mm and 3.07 mm against *S. aureus*. Further testing of the cell-free supernatant (CFS) showed that these isolates were only active in inhibiting the growth of *E. coli*. Further genomic and metabolomic studies are needed to reveal more of the potential of bacteria from *Ulva* sp. as sources of antibacterial compounds for food and pharmaceutical applications.

**Keywords:** Seaweed, *Ulva* sp., *Chaetomorpha* sp., antibacterials

## Introduction

Seaweed has exceptionally high strategic importance as a multi-industry commodity because it contains hydrocolloid compounds (agar, carrageenan, and alginate) as well as unique bioactive substances not found in terrestrial plants (Lomartire & Gonçalves, 2023; Qasim *et al.*, 2024; Rhein-knudsen *et al.*, 2015). This versatility makes it a primary raw material of high economic value in the food, pharmaceutical/medicine, and beauty care sectors (Kezer *et al.*, 2026). In addition to its

nutritional content and hydrocolloid compounds, seaweed is also known to contain bioactive compounds, including phenolic substances and natural pigments, that act not only as antioxidants (Nufus *et al.*, 2017), and antibacterial agent (Afrin *et al.*, 2023) but also possess anticancer (Gengatharan *et al.*, 2025; Liu *et al.*, 2022) and anti-inflammatory properties (Pradhan *et al.*, 2023).

Identification of bioactive compounds from seaweed has been extensively studied and has yielded benefits in the pharmacological field, such as polyphenols, flavonoids, carotenoids,

alkaloids, terpenes, tocopherols, polysaccharides, and peptides (Afrin *et al.*, 2023). However, several studies generally use seaweed extracts as the test material (Liswandari *et al.*, 2018; Wulanjati *et al.*, 2020). Research investigating the antibacterial activity of bacteria specifically associated with seaweed, particularly *Ulva* sp. and *Chaetomorpha* sp., is still limited. These include the ethanol extract of *Ulva* sp. as an antibacterial agent against *Escherichia coli* (Wulanjati *et al.*, 2020), the extract of *Ulva lactuca* as an antibacterial agent against methicillin-resistant *Staphylococcus aureus* (Ardita *et al.*, 2021), and the extract of *Chaetomorpha aerea* against *Staphylococcus aureus* (Pierre *et al.*, 2011). However, that research focused solely on seaweed material as the active component and did not explore the involvement of endophytic microbes that might be responsible for its activity.

Microbes associated with seaweed have a significant influence on its functional properties. Industries focused on sustainable seaweed holobionts have the potential to play a crucial role in the future bioeconomy, as they offer a more resource-efficient means of producing food, valuable chemicals, and medical materials (Ren *et al.*, 2022). The relationship between seaweed and its microbial community (both epiphytic on the surface and endophytic within the tissues) forms a functional unit known as the holobiont (Pradhan *et al.*, 2023). These microbes do not merely exist as commensals, but actively affect the metabolism, morphology, and the content of bioactive compounds that determine the quality of seaweed for food, pharmaceuticals, and cosmetics (Ravindra Pal Singh & Reddy, 2014). On the seaweed surface, bacterial communities from the phyla Proteobacteria and Firmicutes are typically the most prevalent (Ravindra Pal Singh & Reddy, 2014). Endophytic bacteria living within the tissues can produce bioactive compounds similar to those of their host. This is presumed to be the result of coevolution or genetic material transfer from the host (Rau *et al.*, 2018). Bacteria associated with *Chaetomorpha* sp. have not been widely studied, and research on the potential of *Chaetomorpha* sp. seaweed extract was last conducted by previous study (Triastinurmiatiningsih & Haryani, 2008).

Exploratory research on the potential of bacteria associated with the relatively abundant seaweed in Gunungkidul, especially *Ulva* sp. and *Chaetomorpha* sp., has not been widely

conducted. Therefore, the objective established in this study is to obtain bacteria associated with the seaweed *Ulva* sp. and *Chaetomorpha* sp. collected from beaches in Gunungkidul that possess antibacterial activity against pathogens, particularly *Escherichia coli* and *Staphylococcus aureus*. Through this exploratory research, it is hoped that new microbes can be identified—specifically those associated with seaweeds such as *Ulva* sp. and *Chaetomorpha* sp. that possess biological antibacterial properties, which could be further utilized in the food and pharmaceutical industries.

## Materials and Methods

**Sample Collection.** Seaweed samples were collected on June 14, 2023 at Sepanjang Beach, Gunungkidul, Yogyakarta, Indonesia. The base of the seaweed was cut with scissors to avoid damaging the thallus, after which the samples were placed in jars filled with seawater to prevent the seaweed from lysing due to insufficient salt ions (Sirri *et al.*, 2022). The samples were placed in a coolbox for transport to the laboratory for further processing.

**Seaweed Identification.** The seaweed samples were placed in sterile petri dishes for observation, documentation, and genus identification. Seaweed identification can be performed visually, by observing the features or characteristics of each sample, as the morphology of the thallus differs among genera (Alrasyid *et al.*, 2025). The observational results must still be validated by providing supporting references.

**Bacterial Isolation and Purification.** The collected seaweed was weighed. Stepwise rinsing with sterile seawater was carried out three times (Safitri *et al.*, 2023). The dilution process was performed by preparing a sterile stomacher plastic bag containing 0.9% NaCl solution (Merck, Germany) at a volume:weight ratio of 10:1. After dissolving, 1 mL first solution was transferred to sterile tube containing 9 mL sterile 0.9% NaCl solution (secondary dilution) until a  $10^{-3}$  dilution was reached. Bacterial isolation was performed using the pour plate method on ZoBell Marine Agar (ZMA, BD Difco, USA), Potato Dextrose Agar (PDA) (Merck, Germany), Tryptic Soy Agar (TSA) (BD Difco, USA), International Streptomyces

Project-2 (ISP-2 (BD Difco, USA), International Streptomyces Project-4 (ISP-4) (BD Difco, USA), and The Man Rogose Sharpe Agar (MRSA) (Oxoid, UK) media and incubated at 30°C for 24 h. After incubation, colonies growing on the media were observed and counted. Single colonies were then selected and purified by the streak plate method on the same media and incubation conditions. The resulting single isolates were stored in 50% glycerol medium in a -80°C freezer as stock cultures.

**Bacterial Characterization.** The characteristics of single bacterial colonies were observed macroscopically on respective agar medium to determine the form, edge, elevation, color, and size of each colony (Rau *et al.*, 2018). Bacterial characterization was also carried out using Gram staining as detailed previously (Froböse *et al.*, 2020)

**Antibacterial Activity Testing.** Antibacterial tests were performed using the disk diffusion method (Nurhayati *et al.*, 2020), using both whole cell culture and cell-free supernatant (CFS). Cell-free supernatant was obtained by separating the supernatant from the cells using centrifugation at 5,000 rpm for 10 mins, and then filtered with a sterile 0.45-micron filter (Labselect, China) before use. The pathogenic bacteria used in this study were *E. coli* FNCC 0091 and *S. aureus* FNCC 0047. A 100 µL aliquot of the test bacteria from a glycerol stock culture was inoculated into 1000 µL of Mueller-Hinton Broth (MHB) (Himedia, India) and incubated overnight; subsequently, a 5% subculture was prepared in fresh MHB medium and incubated for 24 hours. To prepare for assay, test bacteria (100 µL) at a density of  $10^7$  CFU/mL were seeded onto Mueller-Hinton Agar (MHA) (Himedia, India) medium. Sterile blank paper disks (Oxoid) were placed on the surface of the medium containing the test bacteria, and immediately 10 µL bacterial culture (for whole cell) or cell free supernatant (CFS) was pipetted onto the disks (Nurhayati *et al.*, 2020). Two replicates were performed with each replicate on different plates. Plates were then incubated for 24 h at 37°C. Following incubation, the inhibition zones formed around the disks were measured using calipers and recorded. The

diameter of the inhibition zone was interpreted as follows: 0 mm means no antibiotic activity, 1.4–6.2 mm means weak, 6.3–10.3 mm means moderate, and 10.4–26.8 mm means strong, and >26.8 mm means very strong (Tchaou *et al.*, 1996).

## Results

### Seaweeds Characterization

The seaweeds collected and identified in this study were *Ulva* sp. and *Chaetomorpha* sp. (Figure 1). *Ulva* sp. has light green color characteristics, a membranous type thallus or shaped like a thin, smooth leaf lamina, with undulating leaf edges, an elongated rectangular shape, and a holdfast (rootlike part) with a disk shape for attachment to rocks (Febrianti *et al.*, 2026; Ibrahim *et al.*, 2024; Nabilla *et al.*, 2023). *Chaetomorpha* sp. is characterized by a green color and a filamentous thallus or fibrous, threadlike appearance with irregular shape (Bingxin *et al.*, 2016).

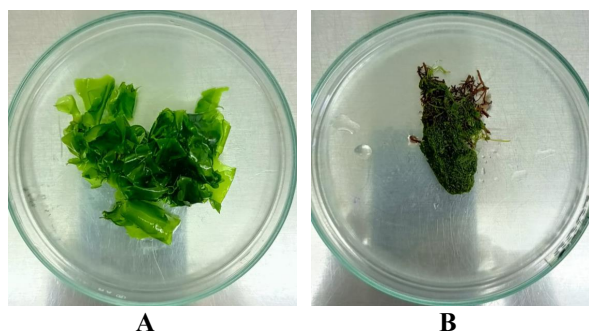


**Figure 1.** Seaweed Identification. (A) The genus *Ulva* sp. can be identified macroscopically by its sheet-like thallus and green color (B) The genus *Chaetomorpha* sp. can be identified macroscopically by its grass-like thallus with a coarse texture and green color

### Total Bacterial Colonies Associated with Seaweed

The morphology of bacterial colonies successfully isolated from *Ulva* sp. and *Chaetomorpha* sp. is shown in Figure 2. The number of colonies grown on various media appears to vary, as indicated by differing colony counts; in some media, there is no growth at all.

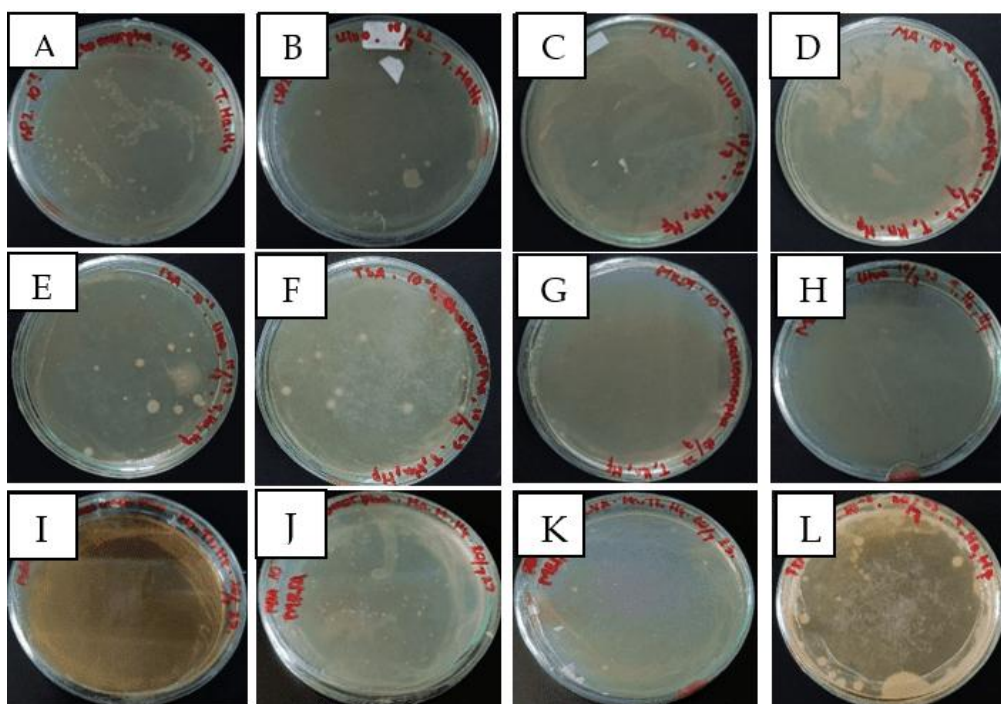


**Figure 2.** Colony growth from seaweed samples on different dilutions and media. Dilution  $10^{-2}$  (ABCDEFGH),  $10^{-1}$  (IJK),  $10^{-3}$  (L); (A) International Streptomyces Project-2 (ISP-2)\_*Chaetomorpha* sp. (B) ISP-2\_*Ulva* sp. (C) ZoBell Marine Agar (ZMA)\_*Ulva* sp. (D) ZMA\_*Chaetomorpha* sp. (E) Tryptic Soy Agar (TSA)\_*Ulva* sp. (F) TSA\_*Chaetomorpha* sp. (G) De Man Rogosa Sharpe Agar (MRSA)\_*Chaetomorpha* sp. (H) MRSA\_*Ulva* sp. (I) International Streptomyces Project-4 (ISP-4)\_*Chaetomorpha* sp. (J) MRSA\_*Chaetomorpha* sp. (K) MRSA\_*Ulva* sp. (L) TSA\_*Ulva* sp.

After incubation at 30°C for 24 hours in various media and dilutions, it appears that bacteria from *Ulva* sp. and *Chaetomorpha* sp. can survive on various types of media (Table 1). TSA medium was found to be the most optimal because it produced the highest number of colonies, namely 192 and 357 colonies from *Ulva* sp. and *Chaetomorpha* sp., respectively. However, MRSA medium yielded only a small number of colonies, namely 7 and 6 colonies for *Ulva* sp. and *Chaetomorpha* sp. In addition, fungal growth was observed on PDA medium without cycloheximide (anti-fungal).

### Characterization of Purified Bacteria

Endophytic bacteria that were successfully isolated were purified to obtain single colonies for further identification. The macroscopic appearance of the colonies of each isolate

(Figure 3) can be seen to differ. The macroscopic colony characteristics of isolated bacteria are shown in Table 2.

**Table 1.** Total of bacteria colonies in different types of media

| On media                        |                         | Total Colony in Media |      |     |      |     |     |
|---------------------------------|-------------------------|-----------------------|------|-----|------|-----|-----|
| No                              | Sample                  | ISP4                  | ISP2 | ZMA | MRSA | TSA | PDA |
| Dilution level 10 <sup>-1</sup> |                         |                       |      |     |      |     |     |
| 1.                              | <i>Ulva</i> sp.         | -                     | -    | -   | 7    | -   | -   |
| 2.                              | <i>Chaetomorpha</i> sp. | 90                    | -    | -   | 6    | -   | -   |
| Dilution level 10 <sup>-2</sup> |                         |                       |      |     |      |     |     |
| 1.                              | <i>Ulva</i> sp.         | -                     | 57   | 82  | -    | 192 | 39  |
| 2.                              | <i>Chaetomorpha</i> sp. | -                     | 215  | 43  | -    | 357 | 31  |
| Dilution level 10 <sup>-3</sup> |                         |                       |      |     |      |     |     |
| 1.                              | <i>Ulva</i> sp.         | -                     | -    | -   | -    | 26  | -   |
| 2.                              | <i>Chaetomorpha</i> sp. | -                     | -    | -   | -    | -   | -   |

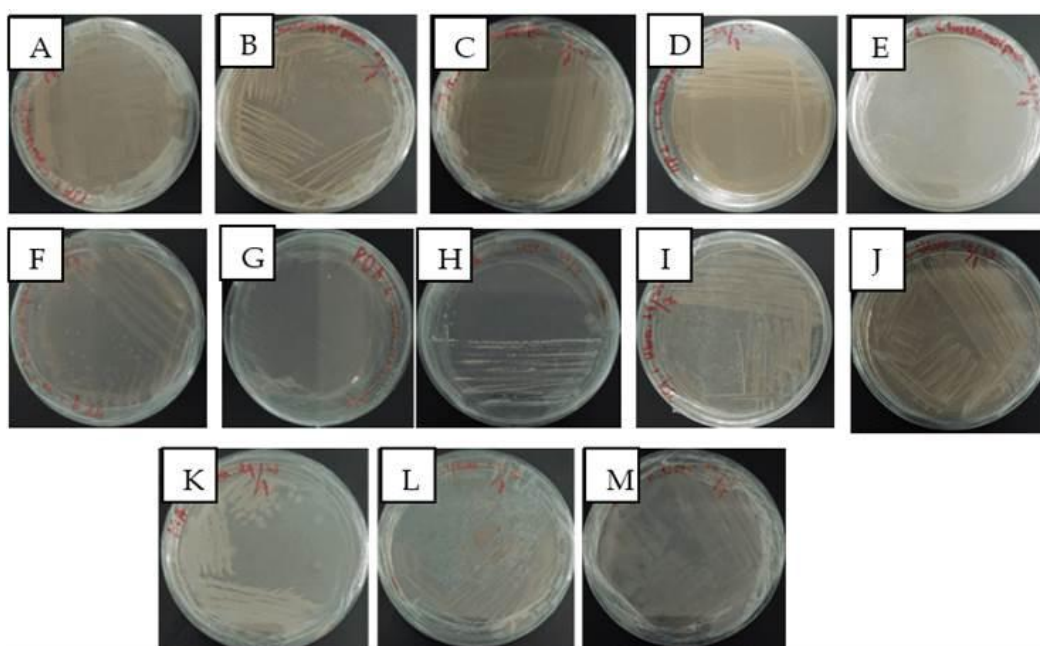


**Figure 3.** Purified colonies isolated from *Chaetomorpha* sp. (ABCDEF), *Ulva* sp. (GHIJKLM) from various media and different dilution series; (A) 2\_Tryptic Soy Agar (TSA)  $10^{-2}$  (B) 1\_TSA  $10^{-2}$  (C) 3\_TSA  $10^{-2}$  (D) 1\_International Streptomyces Project-2 (ISP-2)  $10^{-2}$  (E) 2\_ISP2  $10^{-2}$  (F) 1\_International Streptomyces Project-4 (ISP-4)  $10^{-1}$  (G) 1\_Potato Dextrose Agar (PDA)  $10^{-1}$  (H) 2\_PDA  $10^{-1}$  (I) 1\_TSA  $10^{-2}$  (J) 2\_TSA  $10^{-2}$  (K) 2\_ZoBell Marine Agar (ZMA)  $10^{-2}$  (L) 1\_ZMA  $10^{-2}$  (M) 3\_ZMA  $10^{-2}$

**Table 2.** Morphological characteristic of bacteria isolated from *Ulva* sp. and *Chaetomorpha* sp.

| No. | Isolates                   | Morphological characteristics |        |           |                 |        |      |
|-----|----------------------------|-------------------------------|--------|-----------|-----------------|--------|------|
|     |                            | Shape                         | Edge   | Elevation | Color           | Size   | Gram |
| 1.  | ISP2_10 <sup>-2</sup> _1 C | Irregular                     | wavy   | Flat      | cloudy white    | Medium | -    |
| 2.  | TSA_10 <sup>-2</sup> _1 C  | Irregular                     | wavy   | Convex    | cloudy white    | Medium | +    |
| 3.  | TSA_10 <sup>-2</sup> _2 C  | Filamentous                   | wavy   | Flat      | off-white       | Medium | +    |
| 4.  | TSA_10 <sup>-2</sup> _3 C  | Irregular                     | wavy   | Convex    | cloudy white    | Medium | +    |
| 5.  | ISP4_10 <sup>-1</sup> _1 C | Irregular                     | wavy   | Flat      | off-white       | Medium | -    |
| 6.  | MA_10 <sup>-2</sup> _1 U   | Irregular                     | wavy   | Flat      | cloudy white    | Medium | +    |
| 7.  | MA_10 <sup>-2</sup> _2 U   | Irregular                     | jagged | Flat      | grey            | Large  | +    |
| 8.  | MA_10 <sup>-2</sup> _3 U   | Irregular                     | wavy   | Flat      | off-white       | Large  | +    |
| 9.  | TSA_10 <sup>-2</sup> _1 U  | Circular                      | wavy   | Convex    | yellowish-white | Small  | -    |
| 10. | TSA_10 <sup>-2</sup> _2 U  | Irregular                     | wavy   | Flat      | yellowish-white | Large  | -    |
| 11. | PDA_10 <sup>-1</sup> _1 U  | Circular                      | wavy   | Flat      | off-white       | Small  | +    |
| 12. | PDA_10 <sup>-1</sup> _2 U  | Circular                      | wavy   | Convex    | cloudy white    | Small  | +    |

**Table 3.** Antibacterial assay results against *S. aureus* and *E. coli*

| No. | Isolate                                      | Code | Clear zone diameter (mm) |                |                       |                |
|-----|----------------------------------------------|------|--------------------------|----------------|-----------------------|----------------|
|     |                                              |      | Whole culture            |                | Cell-free supernatant |                |
|     |                                              |      | <i>S. aureus</i>         | <i>E. coli</i> | <i>S. aureus</i>      | <i>E. coli</i> |
| 1.  | PDB_10 <sup>-1</sup> _2 <i>Ulva</i>          | U1   | 4.09 ± 0.54              | 1.69 ± 0.21    | -                     | 4.70 ± 0.31    |
| 2.  | PDB_10 <sup>-1</sup> _1 <i>Ulva</i>          | U2   | 8.99 ± 5.26              | 2.54 ± 0.76    | -                     | 7.99 ± 2.31    |
| 3.  | MB_10 <sup>-2</sup> _1 <i>Ulva</i>           | U3   | -                        | -              | -                     | -              |
| 4.  | MB_10 <sup>-2</sup> _2 <i>Ulva</i>           | U4   | -                        | -              | -                     | -              |
| 5.  | MB_10 <sup>-2</sup> _3 <i>Ulva</i>           | U5   | -                        | -              | -                     | -              |
| 6.  | TSB_10 <sup>-2</sup> _1 <i>Ulva</i>          | U6   | -                        | 1.65 ± 0.24    | -                     | -              |
| 7.  | TSB_10 <sup>-2</sup> _2 <i>Ulva</i>          | U7   | -                        | -              | -                     | -              |
| 8.  | ISP2_10 <sup>-2</sup> _1 <i>Chaetomorpha</i> | C1   | -                        | -              | -                     | -              |
| 9.  | TSB_10 <sup>-2</sup> _1 <i>Chaetomorpha</i>  | C2   | 1.77 ± 0.52              | -              | -                     | -              |
| 10. | TSB_10 <sup>-2</sup> _2 <i>Chaetomorpha</i>  | C3   | 1.48 ± 0.07              | -              | -                     | -              |
| 11. | TSB_10 <sup>-2</sup> _3 <i>Chaetomorpha</i>  | C4   | -                        | -              | -                     | -              |
| 12. | ISP4_10 <sup>-1</sup> _1 <i>Chaetomorpha</i> | C5   | -                        | -              | -                     | -              |

Based on macroscopic characterization, isolated bacteria were observed to have similar characteristics. Mostly, the characteristics of irregular shape, undulate margins, flat elevation, cloudy white color, and medium size were commonly found. Isolated bacteria from *Ulva* sp. were found to be Gram-positive, while samples from *Chaetomorpha* sp. were a mixture of both Gram-positive and Gram-negative bacteria.

### Antibacterial Activity Testing

Table 3 shows the antibacterial activity data of isolates obtained from *Ulva* sp. and *Chaetomorpha* sp. Whole cultures of isolates U1 and U2 from *Ulva* sp. and C2 and C3 from *Chaetomorpha* sp. were found to have antibacterial activity against *S. aureus*., however, antibacterial activity against *E. coli* was found only by isolates obtained from *Ulva* sp., namely U1, U2, and U6. Further testing regarding the antibacterial effects of CFS yielded negative results against *S. aureus*, and positive results against *E. coli* for the CFS from isolates U1 and U2. The highest overall antibacterial activity was produced by isolate U1 and U2, both from whole culture and CFS against *E. coli*, as shown in Figure 4.

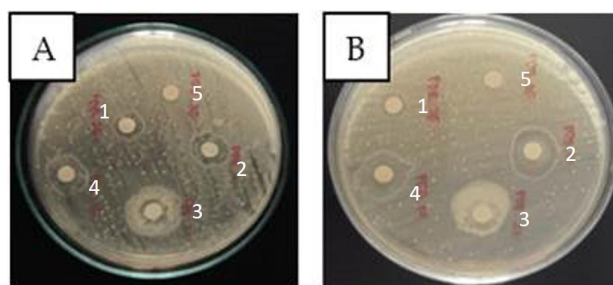


**Figure 4.** Results of antibacterial testing of whole culture (WC) and cell free supernatant (CFS) of U1 and U2 isolates against *E. coli* bacteria. (1) isolate U1\_WC, (2) isolate U1\_CFS, (3) isolat U2\_WC(4) isolat U2\_CFS. (A) Replication 1 (B) Replication 2

### Discussion

The primary objective of this study was to isolate and characterize bacteria associated with the seaweeds *Ulva* sp. and *Chaetomorpha* sp. from the Gunungkidul Coast and evaluate their antibacterial potential against *Escherichia coli* and *Staphylococcus aureus*. Regarding sample validation, visual observation confirmed that the collected macroalgae belonged to the genera *Ulva* and *Chaetomorpha*, aligning with their documented presence in the southern coastal

area of Gunungkidul. Previous studies have reported that at least 11 species of macroalgae are found at Ngrumput Beach, one of the beaches in the southern coastal area of Gunungkidul, namely the species *Ulva lactuca*, *Boergesenia forbesii*, *Chaetomorpha atennina*, *Enteromorpha compressa*, *Padina australis*, *Padina minor*, *Turbinaria ornata*, *Sargassum muticum*, *Callophyllis crispata*, *Acanthophora spicifera*, and *Halymenia maculata* (Muhromi, 2021). The characteristics of the seaweed samples used in this study are consistent with the parameters outlined by Sarita and colleagues (Sarita *et al.*, 2021) used to enable identification of *Ulva* sp. and *Chaetomorpha* sp., confirming that the samples used in this study were indeed those two genera.

Bacteria associated with seaweed—whether epiphytes or pathogens, such as those causing "ice-ice" disease—exhibit diverse nutritional requirements (Ihsan *et al.*, 2022). To optimize the recovery of these seaweed-associated microbes, the study aimed to evaluate different growth media, discovering that Tryptic Soy Agar (TSA) was the most effective medium, yielding the highest colony counts of 192 from *Ulva* sp. and 357 from *Chaetomorpha* sp.. Although they can grow on several types of media, Tryptic Soy Agar (TSA) was found to be the most optimal medium for *Ulva* sp. and *Chaetomorpha* sp.. TSA is considered one of the most optimal media for isolating and cultivating seaweed-associated bacteria due to its status as a non-selective, universal medium that is rich in complex nutrients and allows for easy salinity adjustment. In studies of microbiome dynamics in seaweed of the genus *Ulva* sp., TSA is consistently chosen as the baseline culture medium for enumerating total viable culturable aerobic bacteria. Its capacity for recovering a diverse range of colonies from the surface of macroalgal tissues has proven superior, as it does not impose selective pressure (such as the acidity found in MRSA or the carbon limitations present in ISP media) (Jager *et al.*, 2024).

In terms of physical characterization, the research aimed to classify the isolated strains macroscopically and microscopically, finding a predominance of irregular, cloudy white colonies and revealing that all *Ulva*-derived isolates were Gram-positive, whereas *Chaetomorpha* yielded a mixture of Gram-positive and Gram-negative bacteria. The characteristics of irregular shape, undulate edges, flat elevation, and cloudy white color, as well as

medium size, predominate among the bacterial samples isolated from the seaweed. However, even though they share similar characteristics, these bacteria are not necessarily of the same species (Ariyanti & Arsita, 2023). Based on the results of Gram staining, all samples from *Ulva* sp. fall into the Gram-positive bacteria group, while in the bacterial samples from *Chaetomorpha* sp., there are both Gram-positive and Gram-negative bacteria. Microscopic observation is required for further identification of the bacterial colony characteristics. Microscopic observation can be carried out using the Gram staining method. Staining with crystal violet, iodine, alcohol, and safranin will induce color changes in the bacterial cell walls due to structural differences (Pari *et al.*, 2023). This study has limitations as it focuses solely on morphological identification; therefore, further research is required to determine the species based on molecular data.

For the core bioactivity screening, the study aimed to determine the efficacy of the isolates against pathogens, demonstrating that *Ulva*-associated isolates U1 and U2 possessed the strongest antibacterial action, though their cell-free supernatants (CFS) selectively maintained activity only against *E. coli*. The indicated antibacterial activity shows a difference in result between the whole culture and CFS, as CFS seemed to exhibit activity against *E. coli* but not *S. aureus* tested. The antibacterial test was carried out on both the cells and the supernatant, with the aim of determining the different effects of these two sources, namely, the cells containing active bacteria and the supernatant, which contains residual secondary metabolite products (Purwaningsih & Wulandari, 2021). Further investigation into the metabolites produced by this bacterial supernatant is required. This will reveal the metabolite profile, which may potentially possess antibacterial properties.

Finally, to address the exploratory nature of this research, the study successfully mapped out the baseline potential of specific seaweed-associated bacteria from the Gunungkidul Coast, highlighting distinct candidate isolates for future development. The screening results clearly demonstrated that isolates U1 and U2 derived from *Ulva* sp. are the most promising candidates, as they exhibited tangible antibacterial activities against both *E. coli* and *S. aureus* in whole-culture tests, with isolate U2 showing the highest zone of inhibition. Interestingly, the

retention of anti-*E. coli* activity in their cell-free supernatants (CFS) strongly indicates that these specific isolates secrete stable, diffusible extracellular metabolites capable of disrupting Gram-negative cell walls, whereas their anti-*S. aureus* mechanism might be cell-bound or requires live bacterial interaction. Consequently, rather than broad exploratory screening, future development pathways must now pivot toward intensive characterization of these specific high-performing strains. Subsequent research should prioritize molecular identification (e.g., 16S rRNA gene sequencing) of isolates U1 and U2 to pinpoint their genus and species. Furthermore, a detailed metabolomic tracking is highly recommended to isolate, profile, and identify the exact bioactive compounds responsible for the clear zone inhibition observed in the CFS. Unveiling these chemical profiles will provide the structural and functional validation necessary to establish these *Ulva*-associated bacteria as standardized, scalable sources of natural antibacterial agents, paving the way for their targeted application in food preservation and pharmaceutical formulations.

## Conclusions

This study successfully isolated, characterized, and screened bacteria associated with the seaweeds *Ulva* sp. and *Chaetomorpha* sp. from Sepanjang Beach, Gunungkidul, fulfilling the research objective to find marine-derived microbes with antibacterial properties. Tryptic Soy Agar (TSA) was determined to be the most optimal medium, recovering the highest microbial diversity with 192 colonies from *Ulva* sp. and 357 colonies from *Chaetomorpha* sp.. Bacteria associated with *Ulva* sp. demonstrated much stronger antibacterial potential compared to those from *Chaetomorpha* sp.. Whole-culture assays revealed that isolates U1 and U2 from *Ulva* sp. successfully inhibited both pathogens, with isolate U2 achieving the highest inhibition zones of 8.99 mm against *S. aureus* and 2.54 mm against *E. coli*. Testing of cell-free supernatants (CFS) confirmed that the antibacterial compounds secreted by isolates U1 and U2 selectively maintain efficacy against the Gram-negative pathogen *E. coli*, while losing activity against *S. aureus*. In conclusion, this exploratory research successfully identified high-performing bacterial isolates (U1 and U2) from *Ulva* sp. as viable candidates for natural

antibacterial agents. To fully realize their application in the food and pharmaceutical industries, future studies must advance beyond morphological screening to perform formal molecular identification and metabolomic profiles to identify the specific active compounds.

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