



EVALUATE THE ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF EXTRACTS FROM THE SEAWEEDS

RAMANJANEYA VARMA N
RESEARCH SCHOLAR SUNRISE UNIVERSITY ALWAR

DR. VISHAL KUMAR CHHIMPA
ASSOCIATE PROFESSOR SUNRISE UNIVERSITY ALWAR

ABSTRACT

Much interest is focused on SEaweEDS, or bacteria closely related to seaweed, because of their ability to produce compounds with antimicrobial and anticancer properties. Microorganisms found in SEaweEDS have been studied for their potential as an antagonist, antibacterial, and anticancer agent. We identified 14 different epiphytic bacteria and 6 different entophytic bacteria. Anti-Staphylococcus aureus activity was found in three of the bacterial strains, while anti-Escherichia coli activity was found in one. Extracts of bacteria found in seaweed have showed potential in halting the growth of pathogens such Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. Suppressing the development of the SK-MEL-28 cell line was likewise a dose-dependent impact of the strains.

Keywords: - Antibacterial, Seaweeds, Bacteria, DMSO, Microorganisms

I. INTRODUCTION

Marine organisms may produce an abundance of metabolites that are biologically and structurally unique. From marine sources, scientists have isolated and investigated a wealth of chemical compounds with use in novel product and medicine development (Faulkner et al., 2000). Beneficial compounds have been isolated from marine microorganisms. More than 50,000 novel bioactive natural compounds have been discovered by scientists during the previous three decades, with 8,000 exhibiting antibacterial activity.

Marine bacteria that find a home in seaweeds may secrete novel physiologically active secondary metabolites that benefit the well-being of the linked host organism. In this way, chemicals with a broad variety of biological activity may be synthesized using microorganisms found in seaweed. Some forms of marine algae may obtain nutrients from bacteria that live in close proximity to them. They produce a plethora of active compounds with antimicrobial, antifungal, antibacterial, and cytotoxic properties to maintain symbiotic connections with epiphytic microenvironments.



II. MATERIAL AND METHODS

Sample collection

Hand-harvested at low tide along the shore of the Autonomous University of Baja California (UABC), the seaweeds *Endarachnebinghamiae* (*Petaloniabinghamiae*) (J. Agardh) *Vinogradova* (Eb), *Centrocerasclavulatum* (Agardh) *Montagne* (Cc), and *Laurencia Pacifica* *Kylin* (Lp) were obtained. A total of 100 g of the obtained samples were sealed in freezer bags. After washing the algal surface with clean water, a sterile swab was used to capture microorganisms from the surface. Following collection, the sample was given a final rinsing in sterile fresh water, the algal surface was patted dry with sterile paper, and the whole thing was stored in a sterile flask.

Antibacterial activity

At a dosage of 10 mg ml⁻¹ in DMSO, extracts from both algae and bacteria were examined for their efficacy against the pathogen bacteria *Staphylococcus aureus* (ATCC25923), *Klebsiellapneumoniae* (ATCC13883), *Proteus mirabilis* (ATCC 35659), and *Pseudomonas aeruginosa* (ATCC 27853). After incubation for 12 hours at 37 degrees Celsius, the dilution technique was used to determine the cell survival rate. Using DMSO as a negative control and vancomycin as a positive control, the absorbance was measured at 600 nm.

III. RESULTS

Identification of bacterial strains

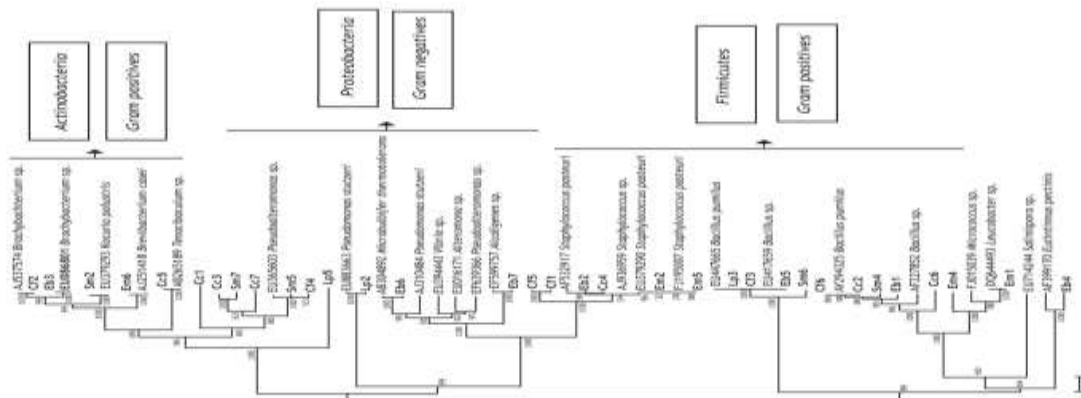
In all, 35 bacterial strains were isolated from the 6 types of sea algae tested. There are 16 Gram-positive bacteria and 19 Gram-negative bacteria, as determined by the Gram stain.

Analysis of 16S rDNA sequences showed that only 33 bacteria could be classified within three main phyla (Fig. 1): Actinobacteria (Cf2, Eb3, Sm2, Em6, Cc5, and Cc1; Gram positive), Proteobacteria (Cc3, Sm7, Cc7, Sm5, Cf4, Lp5, Lp2, Eb6, Eb7, and Cf5; Gram negative), and Firmicutes (Cf1, Eb2, Cc4, Em2, Em5, Lp3, Cf3, Eb5, Sm6, Cf6, Cc2, Sm4, Eb1, Cc6, Em4, Em1, and Eb4; Gram positive).

Based on genetic data, the phylogenic tree was split into two primary branches, one for Gram-positive bacteria and the other for Gram-negative bacteria. None of the algae species were associated with a specific bacteria. The distribution of bacterial species and strains shows no clear trends, as seen in Fig. 1.



Figure 1. Phylogram of the evolutionary relationships of 58 taxa of marine bacteria with sequenced clones using universal primers 27F and 1387R for marine samples. The evolutionary history was assessed using the method of construction described by neighborjoining (Saitou & Nei 1987), based on a total of 625 base pairs in MEGA4 (Tamura et al. 2007) / Filograma de las relaciones evolutivas de 58 taxas de bacterias marinas con las clones secuenciadas usando los cebadores universales de bacterias 27F y 1387R para muestras marinas. La historia evolutiva fue determinada usando el método del vecino más cercano (Saitou & Nei 1987) con un total de 625 pares de bases en MEGA4.



Biological activity

The IC50 values for strains Em6, Cc3, and Cc5 (isolated from *E. menziesii* and *C. clavulatum*) against colon cancer cells (HCT-116), respectively, were 58.0, 101.6, and 56.7 g ml⁻¹, indicating that these chemical extracts were ineffective. By far the most potent anticancer activity was shown by Eb6 (isolated from *E. binghamiae*), with an IC50 value of 2.8 g ml⁻¹; the most cytotoxic strains were Cc1 (isolated from *C. clavulatum*) and Sm6 (isolated from *S. muticum*), with IC50 values of 6.4 and 5.5 g ml⁻¹, respectively (Table 1).

Against *Proteus mirabilis*, 14 bacterial strains and 5 algae (with the exception of *E. menziesii*) shown antibacterial activity. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were resistant to marine microbe and algae extracts. Extracts from strains Em2, Cf1, Cf6, Sm2, Sm6, Eb4, Eb5, Eb6, CC1, CC2, CC3, CC7, Lp2, and Lp3 showed more than 50% bacterial growth when tested against *P. mirabilis* (Table 1). Also, the LD50 values for seaweed extracts in Cf, Sm, Eb, Cc, and Lp were 140.1, 140.1, 178.5, 181.9, and 107.1 g ml⁻¹. Antibacterial activity was found in just two *E. menziesii* strains, Em1 and Em4, with LD50 values of 211.5 and 203.0 g ml⁻¹, respectively (Table 1). *K. pneumoniae* was not very sensitive to any of the extracts used in this study.



IV. DISCUSSION

After being stained with Gram-stain, 16 of the 35 bacteria examined were determined to be Gram-positive, whereas 19 were determined to be Gram-negative. However, contrary to the findings of earlier bacterioplankton studies, recent research on the microbial communities of marine sediments suggests that the majority of the bacteria in this environment are Gram positive (Gontang et al., 2007). Unlike the other seaweeds, surface strains of *Laurencia Pacifica* tended to be Gram-negative. The Gram classification has the potential to be useful if it is used to distinguish between the two main categories of bacteria based on their structural and chemical differences. These chemical characteristics may be species- or genus-specific and facilitate communication between bacteria and algae.

The observed rise and fall of some bacterial families in marine algae may be explained by these chemical interactions. Gallardo et al. (2004) found predominantly Gram-negative bacteria, specifically those belonging to the genera *Vibrio* (20%), *Escherichia coli* inactive (18%), *Flavobacterium* (11%), *Flexibacter* (9%), *Moraxella* (9%), *Pseudomonas* (9%), *Aeromonas* (2%), *Acinetobacter* (2%), *Cotophaga* (2%), *Photobacterium* (2%) and *Alteromonas* (2%). The Gram-positive bacteria *Staphylococcus aureus* was also found in the seaweed *Monostroma undulatum*.

According to phylogenetic research, 33 of the 35 bacterial strains recovered from the 6 seaweeds (the latter are considered major manufacturers of bioactive secondary metabolites) belong to the phyla Firmicutes, Proteobacteria, and Actinobacteria. The 15 different bacterial species found showed no discernible trend in their colonization of different algae. However, it was discovered that certain bacteria can only thrive in certain algae; for example, *Leucobacter* sp. could only be found in *Egregiamenziesii*. This kind of Actinobacteria was linked to the nematode *Caenorhabditiselegans*.



Seaweed and bacteria strains	HCT-116 Colorectal cancer cells IC ₅₀ (µg ml ⁻¹)	<i>Klebsiella pneumoniae</i> MIC (µg ml ⁻¹)	<i>Proteus mirabilis</i> MIC (µg ml ⁻¹)
<i>Egregia menziesii</i>	*	*	*
Em1	*	211.5	*
Em2	*	*	212.3
Em4	*	203.0	*
Em5	*	*	*
Em6	58.0	*	*
<i>Codium fragile</i>	*	*	140.1
Cf1	*	*	196.0
Cf2	*	*	*
Cf3	*	*	*
Cf4	*	*	*
Cf5	*	*	*
Cf6	*	*	213.9
<i>Sargassum muticum</i>	*	*	140.1
Sm2	*	*	165.7
Sm4	*	*	*
Sm5	*	*	*
Sm6	5.5	*	174.0
Sm7	*	*	*
<i>Endarachne binghamiae</i>	*	*	178.5
Eb1	*	*	*
Eb2	*	*	*
Eb3	*	*	*
Eb4	*	*	188.1
Eb5	*	*	209.7
Eb6	2.8	*	178.9
Eb7	*	*	*
<i>Centroceras clavulatum</i>	*	*	181.9
Cc1	6.4	*	180.5
Cc2	*	*	217.1
Cc3	101.6	*	153.1
Cc4	*	*	*
Cc5	56.7	*	*
Cc6	*	*	*
Cc7	*	*	159.1
<i>Laurencia pacifica</i>	*	*	107.1
Lp2	*	*	185.4
Lp3	*	*	288.1
Lp4	*	*	*
Lp5	*	*	*
Lp6	*	*	*

IC₅₀ : Medium inhibitory concentration
 MIC : Minimum inhibitory concentration
 * : No significant activity

V. CONCLUSION

This study's findings suggest that bacteria associated with marine macroalgae isolated from the Kadiapattinam coast in the Kanyakumari district of Tamil Nadu, India, are useful resources for the production of various bioactive compounds that can inhibit the growth of pathogenic microbes and induce the apoptosis of cancer cells. This paves the way for further study of these species, with the ultimate goal of discovering novel therapeutic compounds.

Our research led us to the conclusion that species found in the same location have similar antibacterial activity against *Proteus mirabilis*. This bacterium has the potential to cause a wide



range of medical issues in people, including but not limited to kidney stones, septicemia, pneumonia, and even wound infections.

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