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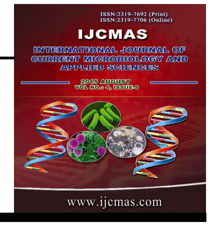

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Original Research Article

Antimicrobial Activity of Marine Algae Associated Endophytes and Epiphytes from the Kenya Coast

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Keywords

Marine microbes, Antimicrobial activity, pathogens, Marine algae, Endophytes, Epiphytes

A B S T R A C T

The current study entails isolation of epiphytic and endophytic microbes associated with marine algae collected from the Kenyan coastal region and screening for their antimicrobial activities. The marine microbes were isolated from the red, green and brown marine algae. A total of 543 isolates were obtained and screened for their antimicrobial activity through the Kirby-Bauer disc diffusion method, of which 120 isolates (22%) exhibited inhibitory effect against the used test organisms (*Staphylococcus aureus* ATCC 25922, *Escherichia Coli* ATCC 25923, *Candida albicans* ATCC 90028). An isolates with an inhibition zone of more than 7mm was considered positive. Most of the isolates showed inhibition against *Escherichia coli* (4%) followed by *Staphylococcus aureus* (4%) and *Candida albicans* (2%).

Introduction

Natural products play a dominant role in the discovery of leads for the development of drugs in the treatment of human diseases. Many microbes are known to collaborate with many plants including marine algae to form mutually beneficial associations. Many of such microbes are endophytes that reside in the internal tissues of living plants without causing any immediate negative effects. They have been found in every plant species examined to date and recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry with more and more bioactive natural products isolated from the microorganisms (Bacon and White, 2000). They are also chemical synthesizers inside plants (Owen and Hundley, 2004). Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proven useful for novel drug discovery. Surfaces of most plants are also characterized by an associated epiphytic micro flora (Melanie *et al.,* 2008). In aquatic ecosystems, bacteria occur often associated with surfaces, for example in biofilms (Costerton *et al.,* 1995). Epiphytic microorganisms live on the surface tissues of their hosts. Current studies indicate that complex interactions exist between the host and their epiphytic microorganisms, where the host provides organic nutrition and epiphytes act as chemical guards (Simon *et al.,* 2002).

The epiphytic marine microorganisms have drawn more interest from natural product chemists in the search for novel antimicrobial or other active compounds. In the past, it was presumed that the marine environment was a "desert" with scarcity of life forms (Zobell, 1946). However, it is now clear that the oceans are thriving with tremendous diversity of living microorganisms, with cell counts of 10^6–10^9 cells per milliliter (Rheinheimer, 1992, Fenical and Jensen, 2006). This microbial diversity is presumed to translate into metabolic diversity resulting in the potential for new bioactives to be discovered. These microbes associated with marine algae have been known to produce biologically active compounds that have a broad range of biological effects: antimicrobial antiprotozoan, antiparasitic, antiviral & antitumor activities (Fremlin *et al.,* 2009). Bacterial resistance is being encountered for most classes of antibiotics, raising the need for search for new drugs. Metabolites produced by marine microbes are being recognized as a rich source of antimicrobial agents and since no studies have been reported on the antimicrobial activity of microbes from Kenyan seaweeds has also prompted this research to explore the marine environment.

Materials and Methods

Sample collection

The marine algae samples were collected from the intertidal zone at Kibuyuni, 100 km south of Mombasa Kenya. The samples were collected in sterile plastic bags, stored in a cooler box with ice packs and transported aseptically to the laboratory for further processing.

Specimen of marine algae collected

Different classes and species of marine algae were collected. Red algae (*Solieria robusta, Gracilaria salicornia, Gracilaria corticata, Acanthophora spicifera, Halymenia durvillaei, Chrondrophycus papillosus, Botrycladia leptopoda, Hypnea pannosa Eucheuma denticulatum)*; green algae (*Halimeda macrolaba, Caulerpa racemosa, Ulva sp)* and brown algae (*Cystoseira myrica, Hydroclathrus clathratus, Sargassum oligocystum, Kappaphycus alvarezii, Padina tetrastromatica).*

Isolation of marine endophytes and epiphytes

Marine endophytes: the marine algae were washed with sterile ocean water, followed by two minutes wash in 70% ethanol and washing in 2% sodium hypochlorite for one minute. The samples were then rinsed with sterile ocean water for five minutes with shaking and dried with sterile paper towels (Denise *et al.,* 2002). The samples were cut into sections 2–3 cm long using a sterilized knife/scalpel. The cut sections were then placed on the surface of Nutrient Agar, Tryptic Soy Agar and Potato Dextrose Agar media and were all prepared using Ocean water.

Marine epiphytes: The marine algae were first rinsed with sterile sea water for a few seconds. This was followed by swabbing/rubbing the surface of the algae using a wet sterile cotton swab and spread plated on Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar media and were all prepared using Ocean water.

All the plates were incubated for 24–48 hours at 30°C. After incubation the colonies were sub- cultured to obtain pure isolates and stored at 4°C for further study.

Screening of isolates for their antimicrobial substance production

All the isolates obtained were screened for their antimicrobial activity.

In preliminary screening, determination of the antimicrobial activity of pure isolates was done by disc diffusion method on Mueller Hinton agar (MHA) using the pathogens *Staphylococcus aureus* (ATCC 25922), *Escherichia coli* (ATCC 25923) and fungi *Candida albicans* (ATCC 90028).

The isolates were cultured overnight in Mueller Hinton broth prepared using ocean water, for their antimicrobial substance production. The pathogens were also cultured overnight in Tubes containing Mueller Hinton broth.

For overnight cultures of the pathogens, 0.5 MacFarland standards were prepared which were uniformly spread on sterile Mueller Hinton agar plates using sterile cotton swabs. The plates were seeded with 6mm sterile discs loaded with 20 µl of the 24 hour marine isolate culture.

The plates were incubated by inverting for 24 hours at 37°C and the zones of inhibition were noted and recorded as positive if it had a diameter of more than 7mm.

Result and Discussion

The surfaces of marine plants and animals are known to be covered with microbes. These microbes play a significant role in the plant development since they evolve allelochemicals capable of protecting the producer from the fierce competition that exists between microorganisms on the surfaces of marine eukaryotes (Anahit *et al.*, 2010). Such chemically driven interactions are important for the establishment of cross-relationships between microbes and their eukaryotic hosts, in which organisms producing antimicrobial compounds protect the host surface against over colonization in return for a nutrient rich environment (Guanatilaka, 2006). Bioactive compound production in these marine bacteria could be attributed to the competition among them for space nutrition (Burgess *et al.*, 1999). The production of secondary metabolites inhibits the settlement of other bacteria (Holmstrom and Kjelleberg, 1994).

In the present study, a total of 543 endophytic and epiphytic microbes associated with the red, green and brown classes of marine algae were isolated (Table 1). Among them were 311 endophytic microbes and 232 epiphytic microbes. The microbes were screened for their antibiotic production against disease causing pathogens (*Staphylococcus aureus* (ATCC 25922), *Escherichia coli* (ATCC 25923) and fungi *Candida albicans* (ATCC 90028) and the results (Figure 1) indicate that120 microbes (22%) exhibited inhibition against the test pathogens. Inhibition activity was observed for both gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria and also the yeast *C. albicans*. Most of the isolates had considerable activity against *E. coli* (Figure 1).

Table.1 Summary of microbe isolation

Number of algae species collected	18
Classes of algae collected	Red, Green and Brown
Microbial isolates obtained	543
Endophytes isolated	311
Epiphytes isolated	232

Table.2 Summary of bioactive microbes based on place of isolation

Classes of algae	Endophytes isolated	Bioactive endophytes	Epiphytes isolated	Bioactive epiphytes
Red	147	32	145	35
Green	44	11	28	6
Brown	100	26	59	10
TOTAL	311	69	232	51

Figure.1 Inhibition activity against the test organisms

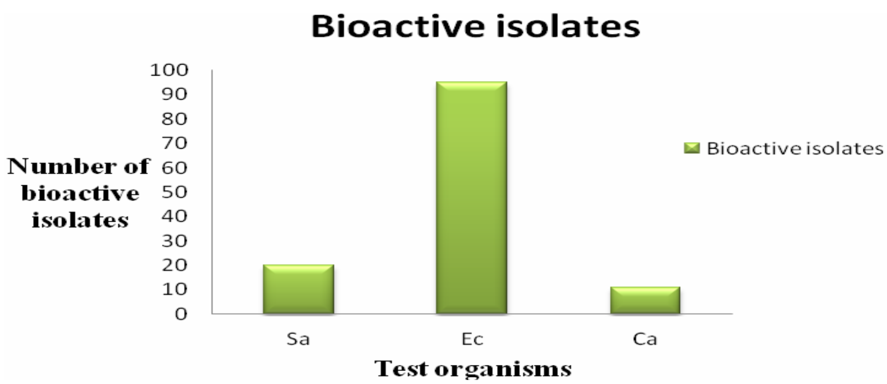


Figure.2 Bioactivity of marine isolates against the test organisms based on class of algae

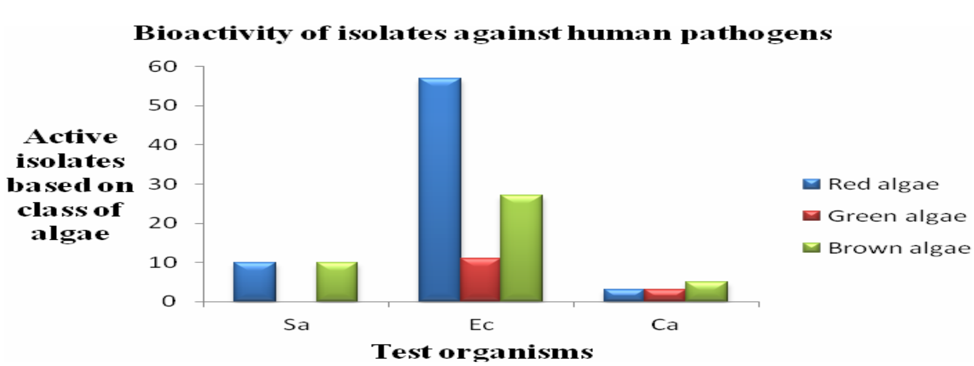
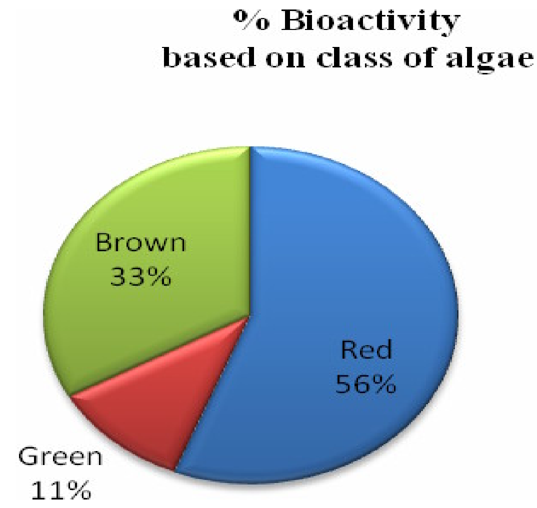


Figure.3 Bioactivity of isolates based on algae class



The result showed that the microbes indeed produce compounds that had antimicrobial activity. This is supported by numerous studies indicating that marine microbes produce compounds with some degree of bioactivity against other microorganisms or acting against certain physiological states of a diseased body. The activity also varied from the place of isolation. From table 2, it's clear that more endophytes showed inhibition activity compared to the epiphytes.

Different classes of algae also exhibit different levels of activity (Figure 2). Isolates from the red algae and brown algae showed activity against all the test organisms. Based on the algae class, the red algae had more active isolates (56%) compared to the brown (33%) and green algae (11%) (Figure 3). A wide variety of biological activities is associated with the marine red algae metabolites since they contain more chemical compounds. In addition, the three taxonomic groups also show seasonal changes in the production of antimicrobial substances (Salvador *et al.*, 2007) but Phaeophytas (Brown algae) and Rhodophytas (Red algae) present significantly more quantity of bioactive compounds than Chlorophytas (Green algae).

The fact that many marine microorganisms–host associations are based on metabolic or chemical interactions may explain the abundance of bioactive producing bacteria associated on living surfaces. Therefore, an understanding of the mechanisms of interactions involved should accelerate the search for novel bioactives.

From the study, it's clear that marine algae harbor epiphytic and endophytic microbes that produce antimicrobial substances that can inhibit human pathogens. Hence, this creates an avenue for the exploration of the marine algae as a source of antimicrobial compounds that can be used against multi-drug resistant microorganisms and come up with novel compounds that can replace the existing drugs once resistance builds up.

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