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ANTIBACTERIAL PROPERTIES OF SECONDARY METABOLITES FROM SEAGRASS *Cymodoceaserrulata* AGAINS HUMAN URINARY TRACT INFECTION

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1.1 background

Microbes are tiny organisms that are too small to be seen with the unaided eye. Also called *microorganisms,* microbes encompass a wide diversity of organisms, such as bacteria, fungi, archaea, protists, microscopic plants, and animals that are generally unrelated except for their small size. Although they are tiny, they do big things for us and our environment. Microbes helped create the ozone layer that protects us from the Sun's ultraviolet rays; they also form the base of our food web. Virtually every other organism depends on microbes (either directly or indirectly) for food Microbes are found absolutely everywhere, in every land and ocean environment imaginable. Although some microbes can be harmful, the overwhelming majority are helpful. Life on Earth could not exist without them. This Article focuses on marine microbes to increase awareness of their incredible diversity and their important roles in ocean ecosystems. Marine microbes are Earth's biggest photosynthesizers: Like plants, they consume carbon dioxide and produce oxygen. In fact, marine microbes produce as much oxygen as all land plants combined and by consuming carbon dioxide (a greenhouse gas), they help regulate our global climate. Interestingly, only a small percentage of marine microbes have been discovered which means that a wealth of discoveries awaits scientists and endless opportunities exist to explore, inquire about, and study these fascinating and fundamental organisms.

Microbes significantly impact our global climate. Marine microbes are very small and have been around for a long time. Life on Earth could not exist without microbes. Most marine microbes are beneficial. Microbes are everywhere: They are extremely abundant and diverse. There are

new discoveries every day in the field of microbial oceanography.

In the ocean, plant-like organisms called *phytoplankton* form the base of the marine food web. These microbes serve as food for zooplankton and small fish, which in turn are eaten by larger fish, birds, and mammals. Thus, without phytoplankton, the entire marine food web would collapse. (Kimberley Weersing *et al*., 2008).

2.0 AIM AND OBJECTIVES

Aim of this study is to identify the marine bioactive compounds from sea grass against urinary tract infection. The present investigation has been under taken with the following objectives.

- Isolation and identification of seagrass *(Cymodocea serrulata)* associated microorganisms from Palk Strait coast of South India.
- To find out the potential THB strain showing sensitivity against urinary tract pathogens.
- Assessment of concentration dependent antibacterial sensitivity by following Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

3.0 REVIEW OF LITERATURE

Pathogen frequency and resistance patterns may vary significantly from country and also in different hospitals within a country. Thus, regional surveillance programs are essential programs are essential to guide empirical therapy and infection control measures. Rank order occurrence and antimicrobial susceptibility of pathogenic species causing bloodstream infections (BSI, lower respiratory tract infections (LRTI), wound or skin and soft tissue infections (wssti), and urinary tract infections (UTI), in hospitalized patients were determined by collecting consecutive isolates over a specified period of time, as part of the SENTRY Antimicrobial Resistance Surveillance 9SENTRY). All isolates were tested by reference broth micro dilution.**(Helio S. Sader *et al*., 2001).**

Surveillance program are essential to detect the increase of antimicrobial resistance, and several different programs are being conducted in many countries. The RESISTANT is a surveillance program for bacterial resistance against several antimicrobial agents initiated in 1998 among Latin American countries. All results were analyzed using the WHO NET program. A total of 894 *Escherichia coli*, 386 *Klebsiella pneumoniae.* The results of this survey show significant

resistance rates among these bacteria which are responsible for several types of human infections. **(Carmen Paz Oplustil *et al.*, 2001).**

4.0 METHODOLOGY

4.1 COLLECTION AND PRESERVATION OF SEA GRASS

Flowering in sea grasses is rare and ephemeral and hence sufficient care should be taken to collect sea grasses with all the developmental stages of male and female flowers and fruits. During collection, sea grasses should be uprooted with care to keep the underground parts intact and washed in the field itself to remove sediments and epiphytes without making any damage to the plants. Then the specimens should be poisoned with 1% mercury chloride solution and pressed and dried for preservation. The preserved materials could be pasted on mounting boards. If the specimens are slender and fragile, then they should be spread neatly on a mounting board submerged in a tray containing water and the board should be gradually lifted allowing the excess of water to drain. The board with the specimen should then be kept in blotters for drying. After drying, rectified sprit saturated with mercuric chloride can be brushed on the plants and allowed to dry. The poisoned, pressed and dried specimens can neatly be pasted on mounting boards. Fresh materials of various developmental stages with fruits can also be fixed in 25% rectified sprit mixed with seawater.

5.0 ISOLATION OF ENDOPHYTIC ORGANISMS

Sea grass species of *Cymodocea serrulata* were brought to the laboratory in sterilized containers and washed thoroughly with sterile distilled water for the isolation of endophytic heterotrophic bacteria. Leaves and stems were carefully separated and surface sterilized with disinfectant (2% sodium hypochlorite containing 0.1% Tweent 20). To remove the disinfectant, samples were rinsed five times with de-ionised distilled water followed by sterile water. Approximately 1g of samples were surface washed with sterile distilled water and 1ml of samples were plated on to Zobell Marine Agar 2216^{e} (Hi media) to recover endophytic bacteria. 1ml of the serially diluted samples were plated on to the same agar media and incubated at 37±2˚C for 24 hours.

4.6 ISOLATION OF URINARY TRACT PATHOGENS

The collected urine samples were taken to laboratory for processing and the isolation of bacteria was done by different selective media. The different selective media were used to identify the growth and colony morphology of all organisms.

6.0 RESULT

In the present reports on antibacterial activity against urinary tract pathogens. The endo symbiotic heterotropic bacteria have been isolated from seagrass *Cymodocea serrulata*. Based on the morophologial characters, 15 THB strains were isolated and all of them have been tested for the antimicrobial sensitivity against urinary tract pathogens viz., *Pseudomonas* sp, *Escherichia coli, Streptococcus* sp and *Staphylococcus* sp, by Cross Streak Assay.

All the isolated endo symbiotic THB strains which shown sensitivity against one or other pathogenic bacteria were subjected for the Minimum Inhibitory Concentration (MIC) assay by following standard methodology. It shows that the strain number ENC 8 has MIC value of 500µg against *Pseudomonas* sp, the strain ENC 8 has MIC value of 1000µg against *Escherichia coli*, the strain ENC 1 has MIC value of 250µg against *Streptococcus* sp, the strain ENC 1, ENC 5 has MIC value of 1000µg against *Staphylococcus* sp.

The strain number ENC 8 has MBC value of 500µg against *Pseudomonas* sp, the strain ENC 8 has MBC value of 1000µg against *Escherichia coli*, the strain ENC 1 has MBC value of 250µg against *Streptococcus* sp, the strain ENC 1, ENC 5 has MBC value of 1000µg against *Staphylococcus* sp.

7.0 DISCUSSION

The earliest bioactive marine metabolite, isolated by Burkholder, was the highly brominated antibiotic, 2, 3, 4-tribromo-5-(1'hydroxyl-2', 4'-dibromophenyl) pyrrole. The compound showed in vitro properties against Gram positive bacteria, with minimum inhibitory concentration (MIC) ranging from 0.0063-0.2 mg ml^{-1} (Burkholder, 1966). Based on the morphological characters, 32 strains of endo and exo symbiotic heterotrophic bacteria were isolated by the present study. All the isolated strains were tested for their antagonistic activity against five antibiotic resistance human pathogens. Among 32 strains, 10 strains were shown antagonistic activity against one or more antibiotic resistance human pathogens. Balagurunathan and Subramanian (2001) reported that, out of 57 isolates from the littoral sediments of Parangipettai coastal water, 8 strains showed very promising antibiotic activity against pathogenic bacteria and fungi.

Liasu and Ayandele (2008) reported that, the minimum inhibitory concentration of the ethanolic plant extract ranged from 0.01 mg ml^{-1} to 100 mg ml^{-1} against pathogenic bacteria and fungi. Gandhimathi *et al.,* (2008) reported that the endosymbiotic marine actinomycetes from sponges exhibited potent antimicrobial activity against the growth of human pathogens. Generally, the endophytic bacteria isolated from seagrasses showed maximum sensitivity against several human bacterial pathogens compared with the epiphytic bacteria. Moreover, the bioactive compounds from endophytic bacteria showed maximum sensitivity with minimum concentration than the bioactive compounds from epiphytic bacteria and other biological origin. Hence, steps have been undertaken to find out the reason for the maximum activity of endophytic bacteria from seagrasses.

Natural products are considered as an important source of new antibacterial agents. Drugs derived from natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs [Cragg *et al.,* 1997]. Marine forms comprising approximately a half of the total global biodiversity, large-scale screening will continue to play an important role in development of

new drugs [Xu *et al.,* 2004]. Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemically unique compounds of marine origin with different biologically activity have been isolated and a number of them are under investigation and are being developed as new pharmaceuticals [Faulkner, 2000a, b; Rocha *et al.,* 2001; Schwartsmann *et al.,* 2001]. Several species of seagrass produce antimicrobial compounds that may act to reduce or control microbial growth. Marine organisms collected from the South East coast of India have been shown to possess a number of biological activities [Ely *et al.,* 2004].

The above results showed that the antibacterial activity and bioactive chemical characterization of seagrass C*ymodocea serrulata* have their own characteristic spectra and spectral parameters. This report also demonstrating that the antimicrobial activity of the seagrass is an encouraging trend unravelling the potential of the Indian coastline as a source of marine organisms worthy of further investigation. These organisms are currently being investigated in detail with the objective of isolating biologically active molecules which would prove to be lead chemicals for drug discovery.

The collected samples and information's were carefully screened and recorded. To find out the extracellular proteins responsible for the antibacterial sensitivity against the urinary tract pathogens have been isolated by standard procedures.

8.0 Conclusion

The results of the present study are summarized as follows:

- In the present reports on antibacterial activity against urinary tract pathogens viz., *Pseudomonas* sp, *Escherichia coli*, *Streptococcu*s sp and *Staphylococcus* sp, by Cross Streak Assay and also biochemical tests were reported.
- The endo heterotrophic bacteria have been isolated from sea grass species. 25strains were of which 15 strains were shown sensitivity against one or more chosen antibiotic resistant pathogenic bacteria.
- The strain no ENC 8, have shown the MI
- C value of 500µg against *Pseudomonas* sp. The ENC 8, showed MIC value of 1000µg against *Escherichia coli* sp. The ENC 1 showed MIC value of 250µg against *Streptococcus* sp. The ENC1 and ENC 5 showed the MIC value of 1000µg against *Staphylococcus* sp.
- The ENC 1 showed MIC value was found lower by 250µg against *Streptococcus* sp.

9.0 REFERENCE

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