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Isolation and identification of secondary metabolites producing organisms from marine sponge

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ABSTRACT

Sponges are among the simplest of multicellular animals and often described as the most primitive of all. This study is focused on the identification of the organisms which produce secondary metabolites and extraction of metabolites from marine sponges which exhibit antibacterial activities. This work also analyzes the confirmation of the secondary metabolites by thin layer chromatography. The cross streak method revealed that *Halobacterium Spp1, Halobacterium Spp2, Halobacterium Spp3, Marinomonas spp* has better antagonistic activity. TLC result shows that the secondary metabolites emit fluorescence while absorbing ultraviolet rays.

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Key words: Marine sponge; Antagonism; Secondary metabolites; Therapeutic agent.

Abbreviations: TLC - Thin Layer Chromatography; ZMA - ZoBell Marine Agar; SMA - Skim Milk Agar; NA - Nutrient Agar; I - Indole; MR - Methyl Red; VP - Voges Proskauer; CU - Citrate Utilization; O - Oxidase; C - Catalase

1. INTRODUCTION

Sponges are sessile marine animals which are commonly found in seas where there are rocks, coral or other suitable substrata. There are more than 5000 species and they are found in almost every sea from mid-tide levels to the deepest parts of the oceans. Sponges act as hosts to a variety of symbiotic/parasitic organisms including blue-green algae and bacteria. Marine invertebrates have been recognized as an important source of bioactive compounds having medicinal potential. Although no major therapeutic drugs have yet been developed from the sea, several compounds have so far entered clinical trials as anticancer drugs. Many of the compounds are present in small quantities and have complicated structures that preclude economical industrial syntheses. The development of biotechnological production of marine natural product such as aquaculture (bryostatin, esteinacidin), through symbiotic microorganism production (swinholide, manzamine) and also development of synthetical production (Ara-A) might become the solution of those plagues. Thus prospects of marine natural products as future medicine are still promising. Marine organisms have attracted special attention in the last years for their ability to produce interesting pharmacological lead compounds. Belarbi et al. (2003) reported that Sponges are most primitive of the multi-celled animals that have existed for 700-800 million years. Of the approximately 15,000 sponge species, most occur in marine environments. Only about 1% of the species inhabits freshwater. Pawlik et al. (2002) revealed that sponges produce secondary metabolites to repel and deter predators, compete for space with other sessile species and for communication and protection against infection. Hellio et al. (2005) reported that potentially therapeutic compounds identified in sponges include anticancer agents and immunomodulators. Some sponges seem to produce potentially useful antifouling agents. The objective of the work is to isolate and screen the microorganisms from the sponges which produce maximum secondary metabolites and showing antagonistic effect. This work also analyzes the extraction of secondary metabolites and confirm with thin layer chromatography.

2. MATERIALS AND METHODS

2.1. Collection of sponges

Marine sponge Dendrilla nigra was collected from the department of microbiology in Bharathidasan University, Trichy. The samples were stored at 4°C until use.

2.2. Isolation of sponge associated bacteria

1g of sponge sample was taken and ground well with distilled water in sterile mortar and pestle and the extract was taken. Nutrient agar was prepared with 2%Nacl and spread plate method was performed. Plates were kept for incubation at 37°c for 24 hours. Colonies were counted and sub cultured.

2.3. Screening of antibacterial activity by cross streak method

Secondary metabolites producing bacteria were screened by cross streak method. Nutrient agar with 2%Nacl was prepared and the marine bacteria isolated was streaked at the centre of the petri plate and incubated for 48hrs at room temperature. After incubation, the test organisms were streaked at both the sides of the marine bacteria which was streaked already and incubated for 24hrs at 37°c. After 24hrs of incubation the organisms showed maximum activity were selected and sub cultured.

2.4. Identification of antagonistic organisms

There are many practical applications for identifying antagonistic organisms. Characterizations of the selected isolates are confirmed by various physiological and biochemical test. Cultural characteristics of the organisms were studied by cultivating the organism in ZoBell marine agar, nutrient agar with 2%Nacl and skim milk agar with 2%Nacl.

2.5. Production of secondary metabolites from the bacteria

Tryptic digest broth was prepared with 2%Nacl in different conical flasks. The selected colonies were inoculated in the broth. Then the conical flasks were kept in the shaking incubator for 3 days at 180rpm. After 3days secondary metabolites was extracted.

2.6. Extraction of secondary metabolites by solvent extraction method

After the production of secondary metabolites, the broth was aseptically transferred to sterile centrifuge tubes and centrifuged at 10,000 rpm for 10mins. The supernatant was collected in sterile conical flask and the pellet was discarded. The supernatant was transferred to the separating funnel and mixed with ethyl acetate in the ratio 1:1 (supernatant: ethyl acetate). Then the mixture in the

separating funnel was shaked continuously for 15mins. After 15mins, keep the separating funnel undisturbed for 10-15mins. After 15mins, three layers were formed and the middle layer was removed in a sterile petri plate. The petri plate containing secondary metabolites in the suspension was allowed to dry in the air. This dried sample contains the secondary metabolites.

2.7. Antibiotic sensitivity assay by well diffusion method

The Muller Hinton agar medium was prepared and the test organisms (*Vibrio cholerae, klebsiella pneumoniae, staphylococcus aureus*) were spread on individual plates. Then wells were made with help of gel puncher. 100microlitre of crude extract from each sample was added in a separate well. It was incubated at 37ºC for 24hrs. Zone of inhibition was noted and the diameter was measured. Ethyl acetate served as control.

2.8. Extraction of alkaloids from the sponge by solvent extraction method

The marine sponge was taken and cut into small pieces. Then the sponges were ground with ethyl acetate. The extract was collected in the sterile centrifuge tube and was transferred in to the separating funnel. Add an equal volume of ethyl acetate and the mixture in the separation funnel was shaked continuously for 15mins. After that the separation funnel was kept in a burette stand for 5-10mins. After 10mins three layers were formed and the middle layer was removed in a sterile centrifuge tube, which was used for further analysis.

2.9. Solubility

The solubility of the secondary metabolites was tested using distilled water, petroleum ether, ethyl acetate and chloroform.

2.10. UV absorbing compound in thin layer chromatography

Silica gel was prepared and poured in the glass slide and spreaded evenly using spreader and kept in hot air oven for 45 minutes. The crude sample of sponge was mixed with ethyl acetate and was added to the TLC slide. The slide was placed in the beaker at one end of the slide immersed in the solvent prepared. The prepared solvent contains ethyl acetate, methanol, and distilled water in the ratio of 10:0.25:0.1 by volume. Run the TLC plate for more than 30 minutes and remove the slides from the solvent. The fluorescence was observed and viewed under UV trans illuminator.

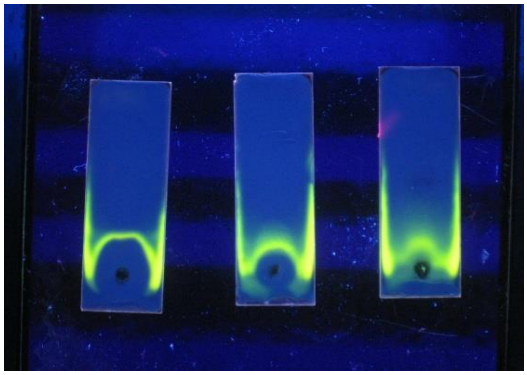

Figure 1
Fluorescence emitting secondary metabolites from sponges in TLC plate

3. RESULTS AND DISCUSSION

The secondary metabolites producing organism was isolated from marine sponge and was screened by spread plate method followed by cross streak method. Efficient antagonistic bacteria were identified by staining method, cultural characteristic and biochemical test (Table 1). Antibacterial activity among marine bacteria is a well-known phenomenon and has been demonstrated in a number of studies (Isnansetyo et al., 2003: Uzair et al., 2006). The cross streak method revealed that *Halobacterium Spp1, Halobacterium Spp2, Halobacterium Spp3, Marinomonas spp* has better antagonistic activity. Solubility test shows that the extracted secondary metabolites are insoluble in chloroform and ethyl acetate, petroleum ether and water shows moderate solubility (Table 2). TLC result shows that the extracted secondary metabolites from the sponges have the capability to absorb the ultraviolet rays and emit fluorescence (Fig.1).

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Table 1 Identification of secondary metabolites producing organisms

Cultural characteristic			Biochemical test						Organism
ZMA	SMA	NA	I	MR	VP	CU	O	C	
Milky white	orange	Milky white	-	-	-	-	-	+	Halobacterium spp 1
Milky white	orange	Milky white	-	+	-	-	-	+	Marinomonas
Milky white	orange	Milky white	-	-	-	-	-	+	Halobacterium Spp 2
Milky white	colorless	Milky white	-	-	-	-	-	-	Halobacterium Spp 3

Table 2 Solubility test for the extracted secondary metabolites

SOLVENT	SOLUBILITY
Petroleum ether	Moderately soluble
Chloroform	Insoluble
Ethyl acetate	Insoluble
Water	Moderately soluble

4. CONCLUSION

Marine bacteria have been recognized as an important and untapped resource for novel bioactive compound .The chemical compound of marine organism are less well known than those of their terrestrial counterparts however, in the last decade several bioactive compound have been isolated from marine bacteria and are new resources for the development of medically useful compound. The cross streak method revealed that *Halobacterium Spp1, Halobacterium Spp2, Halobacterium Spp3, Marinomonas spp* has better antagonistic activity. Sponge associated bacteria were identified as *Halomonas spp* and *Marinomonas spp*. Secondary metabolites were produced from these bacteria using tryptic digest broth. They showed broad spectrum activity against both Gram positive and Gram negative organisms, Compound nature was screened using TLC method which showed UV absorbing compounds.

SUMMARY RESEARCH

1.This work has done to analyze the confirmation of the secondary metabolites in marine sponges by thin layer chromatography.
2.The cross streak method revealed that *Halobacterium Spp1, Halobacterium Spp2, Halobacterium Spp3, Marinomonas spp* has better antagonistic activity.
3.TLC result shows that the secondary metabolites emit fluorescence while absorbing ultraviolet rays.

FUTURE ISSUES

1. In the future, metabolite production from cultured sponge cells and primmorphs may become feasible.
2. Is the use of microorganisms for biological purpose has become an effective alternative to control pathogens for all diseases?

DISCLOSURE STATEMENT

There is no financial support for the proposed research work.

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