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Sponges of Tioman and their actinomycete inhabitants

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Abstract: Actinomycetes were isolated from marine sponges of Tioman Island and taxonomically characterized up to genus level. Four hundred and seven putative actinomycetes were recovered from 39 marine sponge samples collected from nine sites in Tioman Island using standard isolation protocols developed in the study. The actinomycetes population ranged from 2.72×10^2 to 0.6×10^2 colony forming unit (cfu)/g of dry sample. Isolates were then assigned into 11 color groups. Fifty three morphologically different representatives observed under the binocular light microscope were selected for chemotaxonomic, cultural, morphological and physiological characteristization. Chemotaxonomically, 21 (41%) isolates with L,L- diaminopimelic acid (DAP) and no diagnostic sugars pattern in the whole-cell wall hydrolysate were tentative streptomycetes (chemotype I), while 32 (59%) isolates with meso-DAP with observed three different sugars pattern were tentative non-streptomycetes (chemotype II/D, IV/A and III/B). Based on salt tolerance and carbon utilization test, forty five halo-groups and forty one carbo-groups were observed, indicating probable forty one to forty five different species or strains. The tentative genus diversity of rare actinomycetes in the marine sponges was *Actinoplanes*, *Micromonospora*, *Sprilliplanes*, *Pseudonocardia*, *Nocardia*, *Rhodococcus*, *Saccharomonospora* and *Salinispora*. Thirty eight crude extracts were tested against 13 bacterial strains and three fungal strains. Thirty four (64.2%) isolates exhibited antibacterial and antifungal activities, out of which eight (18.9%) isolates exhibited broad spectrum bioactivity.

Introduction

Marine macroorganisms, including invertebrates such as corals, sponges, tunicates and acsidans have been studied in the hope of unraveling novel metabolites and lead structures. To date many novel and useful bioactive compounds produced by the above macroorganisms have been identified. Further investigation showed that the invisible symbionts and commensals living in the macroorganisms to be the true chemical factories producing various secondary metabolites of pharmacological and non-pharmacological applications (Fenical, 1997). The salinosporamide, a potent anticancer agent was produced by *Salinospora* spp., a novel actinomycetes isolated from marine sediments and marine sponges in the tropical and subtropical regions. This discovery has given new revenue to the pharmaceutical industry in the search of novel compounds and drugs (Fehling *et al.* 2003). This is, however, just the tip of the iceberg and extensive studies using both traditional and molecular methods can aid in the identification of novel microorganisms and in particular the actinomycetes living on or within the macroorganisms (Sponga *et al.* 1999).

Among the invertebrates, the marine sponges were the most prolific producers of many compounds with diverse antagonistic properties. The ability to produce a

diverse assemblage of compounds may be due to the fact that 40% of the sponge biomass harbors a diverse assemblage of microorganisms, such as the proteo-bacteria, cyanobacteria, fungi, microalgae and actinomycetes (Sponga *et al.* 1999).

At University Malaya, we have turned to the seas to tap their vast and unexploited microbial diversity and in particular the actinomycetes which are prolific producers of bioactive agents. Recent reports have shown that true marine actinomycetes do exist. Seaweeds, corals, nudibranchs and sponges are among the macroorganisms investigated for their microbial inhabitants (Tan *et al.* 2004, Vikineswary, 2004, Vikineswary *et al.* 2005). Among the macroorganisms studied, marine sponges were rich (45.8%) in actinomycetes, followed by seaweeds (29.4%) and soft corals (16.7%). Further, marine sponges have been reported to be the most prolific producers of many compounds with diverse antagonistic properties. The ability to produce a diverse assemblage of compounds may be due to the fact that 40% of the sponge biomass harbors a diverse assemblage of microorganisms, such as the proteo-bacteria, cyanobacteria, fungi, microalgae and actinomycetes (Webster *et al.* 2001).

Why marine actinomycetes?

Terrestrial actinomycetes are no aliens to the pharmaceutical discovery industry. Their fame as prolific producers of structurally and functionally diverse compounds is indisputable. To date, several novel metabolites with antibacterial, antifungal and especially antitumor properties have also been discovered from the marine-derived actinomycetes isolated from marine sediments and invertebrates. The breakthrough came with the discovery of salinsporins. The discovery of *Salinospora* spp has opened the Pandora's box - true marine actinomycetes do exist contrary to the belief that all actinomycetes were casual inhabitants (marine-derived).

Why are marine actinomycetes successful? In order to survive in the harsh and extreme environment where food is scarce, competition for space and defense against predators are critical. The marine actinomycetes would have to adapt biochemical pathways to survive and this has resulted in many of these producing chemically unique and different compounds compared to terrestrial actinomycetes. Microbes living on the surface of marine sponges were reported to have biofouling activity, which protects the sponges from predators and other pathogenic microbes.

Isolation and characterization of actinomycetes from sponges

Sponges were collected at 10-15 metres deep from three sampling sites in Tioman Island including the Marine Parks on the East Coast of Malaysia and later identified and processed. The isolation of actinomycetes from the sponges was carried out using standard protocols (Tan *et al.* 2004). Putative actinomycetes were recovered from marine sponges on various isolation media. The putative actinomycetes were characterized culturally, morphologically, chemotaxonomically and physiologically up to genus level.

407 putatuive actinomycetes isolates were recovered from 39 marine sponges collected from nine sites in Tioman Island using a selective isolation protocol

developed in this study to increase the recovery of rare actinomycetes (non-streptomycetes) isolates (Fg. 1). All isolates were then assigned into 15 color groups and 106 morphologically different representatives. One hundred and six isolates were dereplicated to 64 physiologically different isolates, chemotaxonomically, 21 (32.8%) were streptomycetes and 43 (67.2%) were non-streptomycetes isolates from the Actinoplanetes, *Verrucosispora* spp., *Micromonospora* spp., *Saccharomonospora* spp., *Pseudonocardia* spp., Nocadioform and Maduromycetes groups (Fig. 2, 3 and 4).

The diversity of actinomycetes from marine sponges is low, probably due to its naturally low occurrence in the marine environment (Tan *et al.* 2004). However, in this study it was observed that *Plakortis nigra*, a common black sponge and other marine sponges of Tioman Island, may be potential sources of novel actinomycetes and bioactive compounds, as marine-derived actinomycetes may have a different biochemical metabolism to adapt to the harsh marine environment. Further molecular characterization is necessary to assess the actual diversity of actinomycetes within the marine sponges.

Although *Streptomyces* spp. are the dominant isolates in marine sponges, a higher percentage of non-streptomycetes were recovered. This was in accordance to a report by Sponga *et al.* (1999). *Actinoplanes* spp. and *Micromonospora* spp.

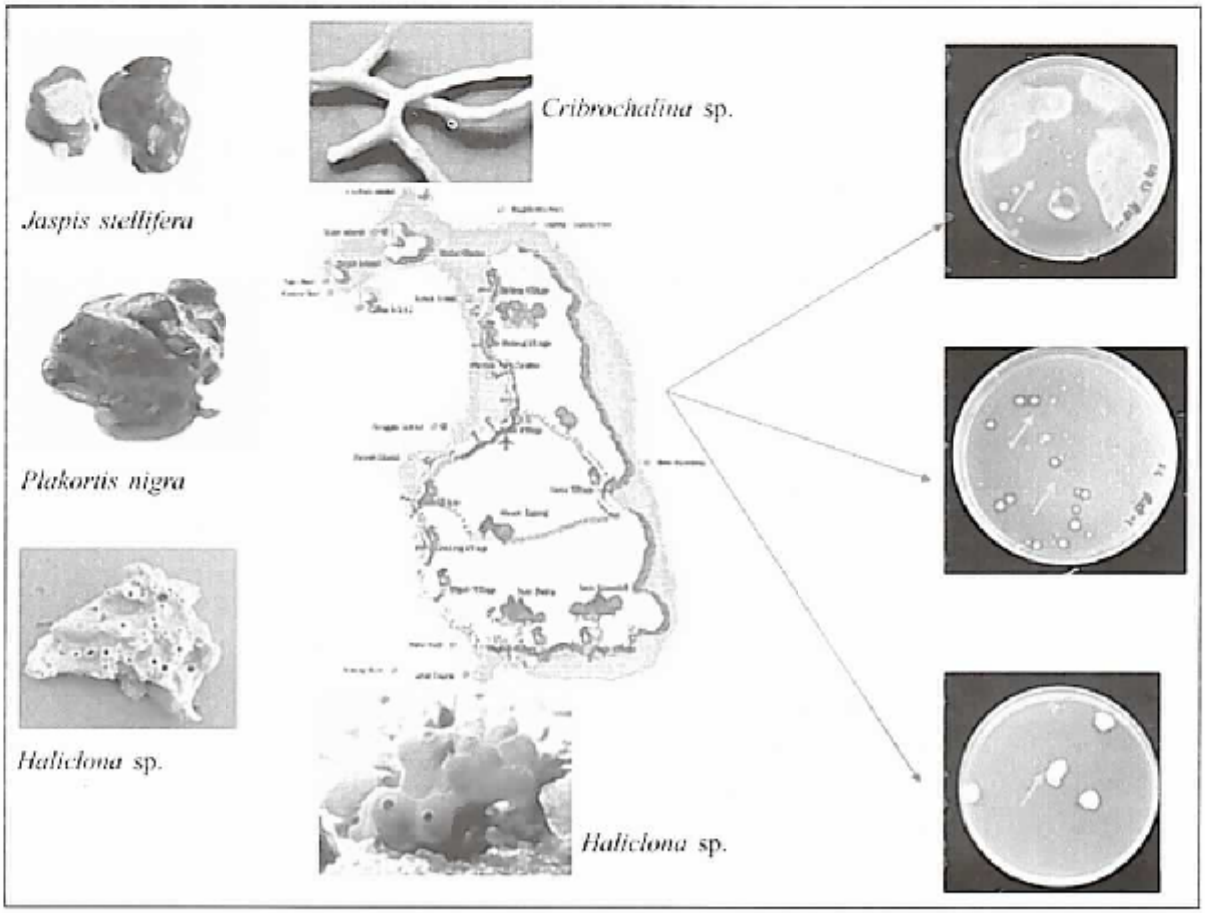

Figure 1: Selected marine sponges collected from Tioman Island and the isolation of actinomycetes (arrows).

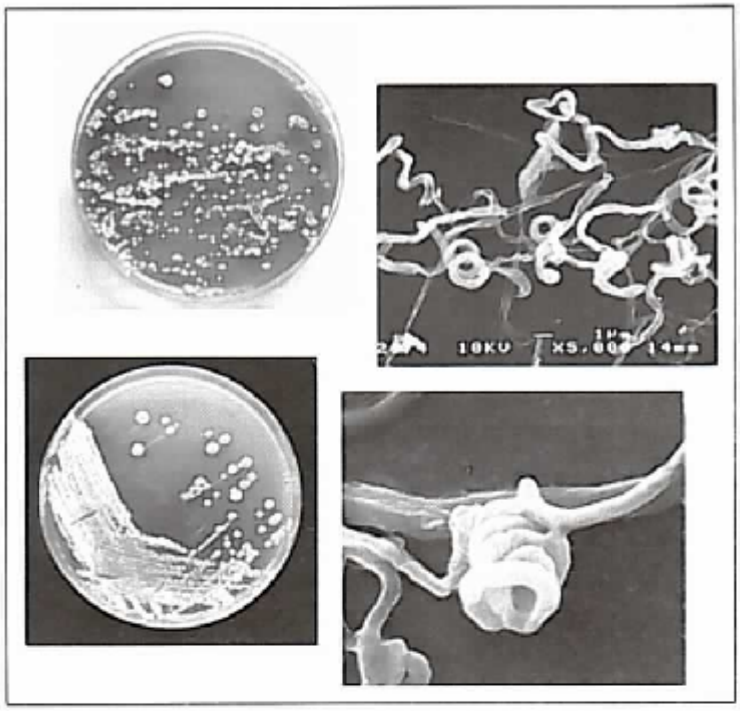

Figure 2: Culture plates of *Streptomyces* spp. *and SEM micrographs.*

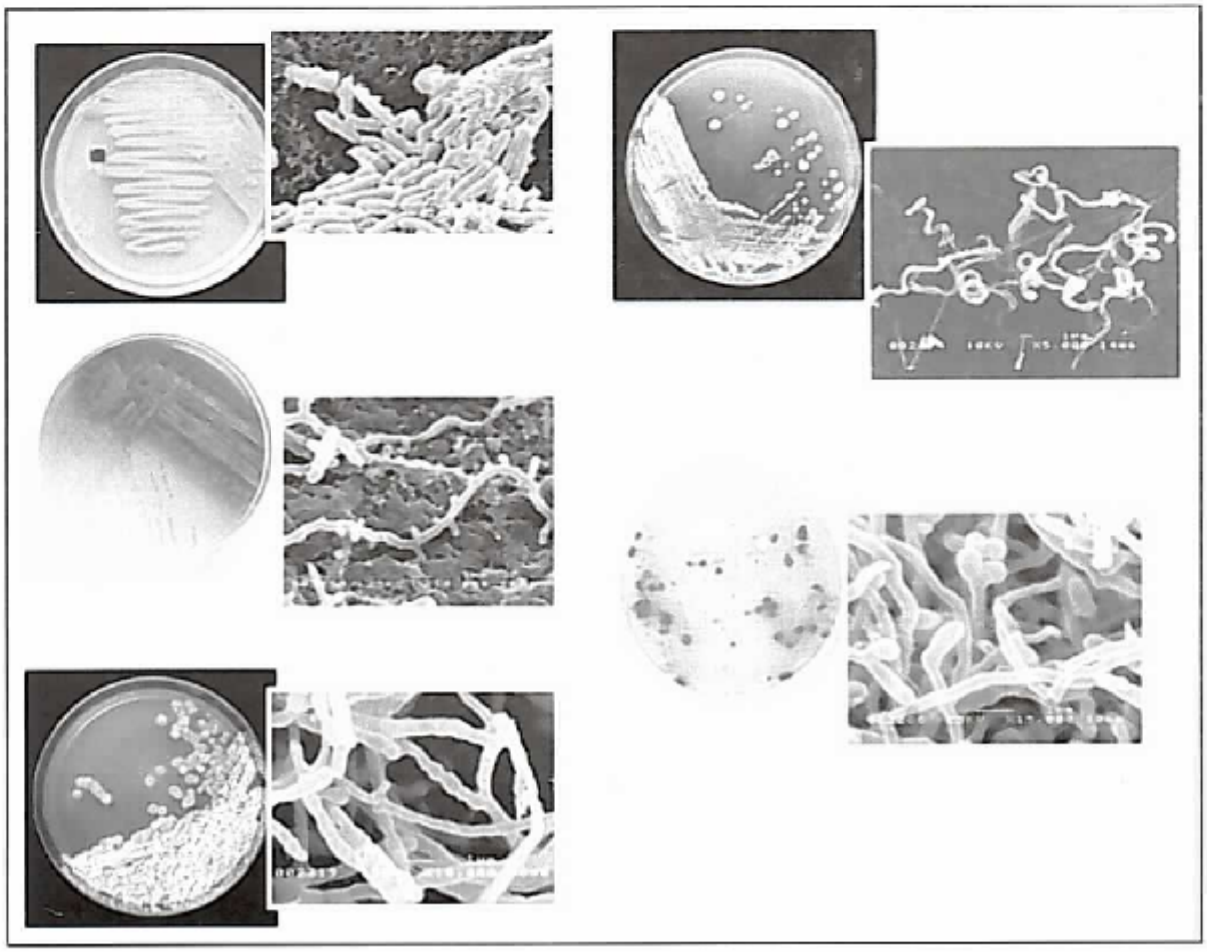

Figure 3: Culture plates of actinomycetes and SEM micrographs (Bar=1μm).

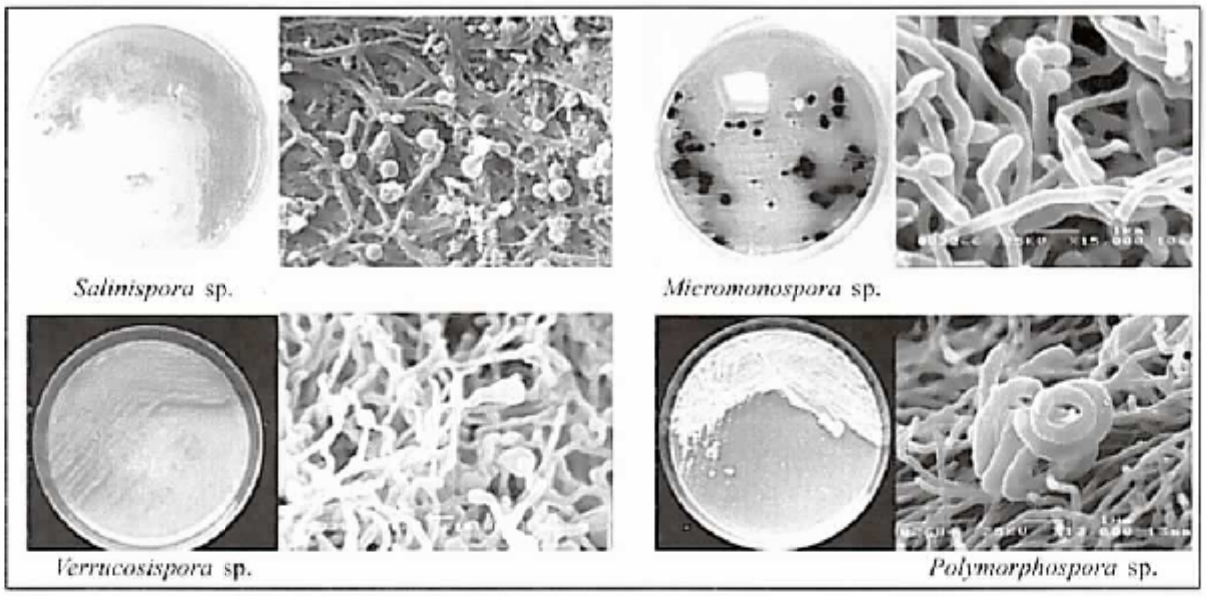

Figure 4: Culture plates of selected rare actinomycetes and SEM micrographs.

were dominant compared to other genus, which was commonly reported in the marine environment. The other rare actinomycetes *Saccharomonospora, Pseudonocardia, Actinomadura* and *Rhodococcus* were also inhabitants of the marine sponges of Tioman Island.

In primary screening trials, 20 of the 53 representative putative actinomycetes exhibited a broad spectrum of antibacterial and antifungal activity against human pathogenic fungi. In the secondary screening, 34 out of the 38 isolates gave positive activity against at least one of the test organisms while nine isolates exhibited broad spectrum bioactivity against bacteria and fungi. *Streptomyces, Salinispora, Micromonospora* and *Pseudonocardia* spp. are among the potential strains.

In this study, the first discovery of the *Salinospora* spp. from Malaysian marine sponges is reported. The discovery of *Salinospora* spp. proved that the Malaysian waters may harbour a gene pool of novel actinomycetes yet to be explored and exploited for the biotechnological and pharmaceutical industries (Christabel *et al.* 2007).

Future of marine and marine-derived actinomycetes

The discovery of *Salinospora*, a truly marine actinomycete in tropical and subtropical marine sediments by the Scripps Institute in California has been a breakthrough, as such a diversified assemblage of novel compounds with potent antitumor properties isolated from a single strain of actinomycete (Fehling *et al.* 2003) has never before been reported. This new group of obligate marine actinomycetes are widely distributed in oceanic sediments (Jensen *et al.* 2003). These strains were reported to have vast potential as producers of bioactive compounds against cancer cells such as breast cancer, cervical cancer and colon cancer and thus promising a vital importance in the production of antibiotics and

other commercially valuable therapeutic compounds. To date, the research on *Salinispora* spp by Fenical *et al.* (2002) has yielded a strong bioactive compound, Salinosporamide A. Salinosporamide A is an extremely potent inhibitor of the chymotrypsin-like proteolytic activity of the mammalian 20S proteasome, an important target in cancer chemotherapy (Fehling *et al.* 2003).

With the increasing threats of drug-resistant pathogens, emerging and reemerging pathogens and the limited successes in new drug discovery from land, more and more studies are turning to the oceans and seas. The recent years have seen an explosion of information in the field of novel bioactive compounds that have been isolated from marine microbes. It is critical that new groups of microbes, especially the actinomycetes from unexplored habitats be pursued as sources of novel antibiotics and other therapeutic agents. The developments of improved methods of isolation including molecular tools and targeted bioassay systems to evaluate and assess the microbial diversity will undoubtly provide a continued supply of novel microorganisms that can be incorporated into the biotechnological and pharmaceutical industries.

Conclusion

The oceans and seas are often erroneously perceived as dark, gloomy and lifeless. There are, however, in the deep waters and sediments vibrant activity of life forms, colors, and a huge diversity of marine organisms and microbes in very exclusive niches. These microbes hold a huge potential for the discovery of a cure for cancer and infectious diseases that are emerging or reemerging and threatening our very existence on this planet. In Malaysia, the study of marine actinomycetes as a unique niche remains virtually unexplored and is a promising resource for biotechnological applications including drug discovery.

Acknowledgements

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