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Bacterial diversity and distribution in Soft Corals and Sponges in the Red Sea

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ABSTRACT

The Red Sea has high biodiversity, with at least 266 coral and sponge species. The coral/Sponge holobiont is comprised of the coral/sponge animal and its associated microorganisms consisting of bacteria, archaea, fungi and viruses. It has been hypothesized that this interaction plays a role in coral/ sponge defense. This study aims to study the bio-diversity of some collected types of sponge and soft coral from Red Sea and to isolate associated bacteria from them. The result includes five types of sponges; *Ircinia strobilina*, *Callyspongia viridis*, *Suberea* spp., *Spongia officinalis* and *Biemna ehrenbergi* and five types of soft corals; *Heteroxenia fuscescens*, *Didemnum moseleyi*, *Lobophytum pauciflorum*, *Sarcophyton trocheliophorum* and *Sinularia* spp. Twenty two bacterial isolates were isolated from the sponges with 41 % and thirty two bacterial isolates were isolated from soft coral samples with 59 % collected from different Red Sea areas.

INTRODUCTION

Coral reefs are well developed along Red Sea which has a coast extend to 2270 Km (Kotb *et al.,* 2008). It contains biologically diverse sponges and corals and considered to be among the most diverse in the world species (Loya, 1972). These corals and sponges show contrasts in their biology and environmental attributes (Sheppard *et al.*, 1992). They are considered economically valuable, providing shelter, food and breeding sites for plants and animals living in oceans (Rinkevich, 2005). Sponges and coral reefs are considered to be natural ecosystem and provide an excellent habitat for marine organisms due to their structure and high retention of nutrients (Stoeckl *et al.,* 2014). Bacteria and other microorganisms are ubiquitous in the marine environment are taxonomically diverse and biologically active (Rheinheimer, 1992). Marine organisms such as corals and sponges are mostly colonized by bacteria and the surface of living corals is covered by mucus which is colonized by bacteria, allowing for the establishment of diverse bacterial community, some of these bacteria can be pathogenic and some of them can be beneficial (Ritchie *et al.,* 2017). So this study aims to study the bio-diversity of some sponge and soft coral collected from Red Sea.

MATERIALS AND METHODS

Soft coral/Sponge collection and cultivation condition

From three different areas in Red Sea, Egypt, in depth of ± 5-8m, sponge and soft corals sampled were collected and transferred into a sterilized plastic bag under the water itself and stored in the ice box and transferred to the laboratory for identification and isolation of the associated bacteria.

Isolation of sponge and soft coral associated bacteria (Chen *et al*., 2012)

Sponge and soft corals samples were washed with autoclaved filtered sea water. Sponges and soft corals were cut into small pieces and were homogenized by grinding using sterile mortar and sterile sea water.

Table 1: biochemical test used for bacterial identification using VITEK 2.

Gram Negative tests	Gram Positive testes
Ala-Phe-Pro-ARYLAMIDASE	D-AMYGDALIN
ADONITOL	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C
L-Pyrrolydonyl-ARYLAMIDASE	D-XYLOSE
L-ARABITOL	ARGININE DIHYDROLASE 1
D-CELLOBIOSE	BETA-GALACTOSIDASE
BETA-GALACTOSIDASE	ALPHA-GLUCOSIDASE
H2S PRODUCTION	Ala-Phe-Pro ARYLAMIDASE
BETA-N-ACETYL-GLUCOSAMINIDASE	CYCLODEXTRIN
Glutamyl Arylamidase pNA	L-Aspartate ARYLAMIDASE
D-GLUCOSE	BETA GALACTOPYRANOSIDASE
GAMMA-GLUTAMYL-TRANSFERASE	ALPHA-MANNOSIDASE
FERMENTATION/ GLUCOSE	PHOSPHATASE
BETA-GLUCOSIDASE	Leucine ARYLAMIDASE
D-MALTOSE	L-Proline ARYLAMIDASE
D-MANNITOL	BETA GLUCURONIDASE
D-MANNOSE	ALPHA-GALACTOSIDASE
BETA-XYLOSIDASE	L-Pyrrolydonyl-ARYLAMIDASE
BETA-Alanine arylamidase	BETA-GLUCURONIDASE
L-Proline ARYLAMIDASE	Alanine ARYLAMIDASE
LIPASE	Tyrosine ARYLAMIDASE
PALATINOSE	D-SORBITOL
Tyrosine ARYLAMIDASE	UREASE
UREASE URE	POLYMIXIN B RESISTANCE
D-SORBITOL	D-GALACTOSE
SACCHAROSE/SUCROSE	D-RIBOSE
D-TAGATOSE	L-LACTATE alkalinization
D-TREHALOSE	LACTOSE
CITRATE (SODIUM)	N-ACETYL-D-GLUCOSAMINE
MALONATE	D-MALTOSE
5-KETO-D-GLUCONATE	BACITRACIN RESISTANCE
L-LACTATE alkalinization	NOVOBIOCIN RESISTANCE NOVO
ALPHA-GLUCOSIDASE	GROWTH IN 6.5% NaCl
SUCCINATE alkalinization	D-MANNITOL
Beta-N-ACETYL-GALACTOSAMINIDASE	D-MANNOSE
ALPHA-GALACTOSIDASE	METHYL-B-D-GLUCOPYRANOSIDE
PHOSPHATASE	PULLULAN PUL
Glycine ARYLAMIDASE	D-RAFFINOSE
ORNITHINE DECARBOXYLASE	O/129 RESISTANCE (comp.vibrio.)
LYSINE DECARBOXYLASE	SALICIN
DECARBOXYLASE BASE	SACCHAROSE/SUCROSE
L-HISTIDINE assimilation	D-TREHALOSE
COUMARATE CMT	ARGININE DIHYDROLASE 2
BETA-GLUCURONIDASE	OPTOCHIN RESISTANCE
O/129 RESISTANCE (comp.vibrio.)	
Glu-Gly-Arg-ARYLAMIDASE	
L-MALATE assimilation	
ELLMAN	
L-LACTATE	

Serial dilution for the soft coral and sponge homogenates to 10^{-6}, then 0.1 ml were streaked on six general bacterial media (Marine Agar (Oxoid), R2A Agar (Difco Lab), Starch casein Agar (HIMEDIA), ISP2 Medium (Difco Lab), Actinomycetes Medium (HIMEDIA) and M1 Agar (Oxoid) and incubated at 25˚C for 7 days, starting from the third day to the seventh day colonies with different characters were picked up and re-streaked again to obtain pure cultures (Wilson *et al*., 2010).

Identification of associated bacteria (Moehario *et al*., 2019).

Identification of bacterial isolates after Gram staining was performed by using VITEK 2 (bioMerieux). It is an automated full system used for bacterial identification. It works by evaluating each biochemical reaction present in microbial identification cards by using optical signals. Suspension with unknown bacteria is inoculated and then incubated with identification cards.

RESULTS

Collection of Soft oral and sponge:

Sponge samples:

Eight samples collected from 5 sponges species were collected from red sea as shown in Table 2.

Table 2: Sponge classification

Name	Type	Number of Samples
Ircinia strobilina	Sponge	1
Callyspongia viridis	Sponge	1
Suberea spp.	Sponge	2
Spongia officinalis	Sponge	2
Biemna ehrenbergi	Sponge	2
Total		8

Soft coral Isolation

Twelve samples collected from 5 soft corals species were collected and as shown in Table 3.

Table 3: Soft corals classification

Name	**Type**	**Number of samples**
Heteroxenia fuscescens	Soft Coral	2
Didemnum moseleyi	Soft Coral	2
Lobophytum pauciflorum	Soft Coral	4
Sarcophyton trocheliophorum	Soft Coral	3
***Sinularia* spp.**	Soft Coral	1
Total		12

Isolation of Soft coral and sponge associated bacteria

Soft Coral associated bacteria

Thirty two bacterial isolates were isolated and identified from soft coral samples as identified in Table 4

Table 4: Bacterial isolates from soft corals

Soft Coral species	**Number of Soft coral samples**	**Bacterial species**	**No. of Isolates**
Didemnum Moseleyi	2	*Aerococcus viridans*	1
		Alloiococcus spp	3
		Staphylococcus hominis	1
Heteroxenia Fuscescens	2	*Vibrio* spp.	2
		Helcococcus kunzii	3
Lobophytum Pauciflorum	4	*Vibrio* spp.	1
		Staphylococcus epidermidis	5
		Aerococcus viridans	3
		Staphylococcus gallinarum	1
		Staphylococcus saprophyticus	3
Sarcophyton Trocheliophorum	3	*Enterococcus faecalis*	2
		Granulicatella Elegans	2
		Staphylococcus vitulinus	2
***Sinularia* spp.**	1	*Lactococcus garvieae*	2
		Arthrobacter creatinolyticus	1
Total	12		32
Percent from the total 54 isolates			59 %

Sponge associated bacteria

Twenty two bacterial isolates were isolated and identified from Soft Coral samples as identified in Table 5

Table 5: bacterial isolates from Sponges.

Sponge species	Number of sponge samples	Bacterial species	No. of Isolates
Biemna ehrenbergi	2	*Kocuria rosea*	5
		Kocuria kristinae	2
Callyspongia viridis	1	*Kocuria rosea*	4
Spongia officinalis	2	*Bacillus pumilus*	2
		Staphylococcus cohnii	1
		Staphylococcus xylosus	2
S*uberea* spp.	2	*Kocuria rosea*	3
Ircinia strobilina	1	*Kocuria kristinae*	3
Total	8		22
Percent from the total 54 isolates			41 %

DISCUSSION

Marine environment is considered one of the most diverse environments (Choudhary *et al.*, 2018). The Red Sea is one of these global hotspots for biodiversity contain numerous types of coral and sponges (DiBattista *et al.*, 2016). This study was intended to show the bio diversity for the Marine Macro and Micro-organism. Soft corals were classified as; *Heteroxenia fuscescens, didemnum moseleyi, Lobophytum pauciflorum, Sarcophyton trocheliophorum and Sinularia* spp. which was reported by Ibrahim *et al.* (2014), Afifi *et al.* (2016), Hassan *et al.* (2016), Tarek *et al.* (2016) and Zubair *et al.* (2016), . Sponges were classified to: *Ircinia strobilina*, *Callyspongia viridis*, *Suberea* spp, *Spongia officinalis* and *Biemna ehrenbergi*, this was reported by Ilan *et al.* (2004), El-Ganainy *et al.* (2005), Mohamed *et al.* (2014) and Shaala and Almohammadi (2017) (2014) . In this study bacterial isolated had been isolated from soft coral and sponges as following, *Aerococcus viridans, Alloiococcus* spp., *Enterococcus faecalis, Vibrio* spp., *Granulicatella elegans, Helcococcus kunzii, Kocuria kristinae, Kocuria rosea, Lactococcus garvieae, Bacillus pumilus, Arthrobacter creatinolyticus, Staphylococcus cohnii, Staphylococcus lentus, Staphylococcus xylosus Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus saprophyticus* and *Staphylococcus vitulinus.* Generally bacterial isolates from Soft coral was thirty two isolated with 59 % which was higher than sponge which was twenty two isolates with 41 %.

That was hypothesized to that sponge is more aggressive than soft corals which make corals develop defense mechanism by having more types of bacteria to grow (Aerts and Rob, 1997).

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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