

Original Article

Isolation of Pigment producing bacteria from Marine environment and its anti-microbial activity

Shigee K

Teacher, Senior school Biology and Environmental systems and Societies, Indus International School, Bangalore.

Abstract:

Background: Marine organisms have a potential resource and unexplored area to study the diversity of organisms. Currently many researchers are showing interest in the identification of novel organism from the marine environment. Therefore, the present study was carried out to isolate the pigment producing microorganism from the marine source.

Methodology: Sea water was collected from the different place of Tamilnadu and the bacteria was isolated using serial dilution method. From the isolated pure colonies, pigment was extracted and the antimicrobial activity was performed using disc diffusion method.

Results: From the spread plate, 11 yellow, 6 orange and 2 peach isolates were obtained. A preliminary well diffusion method showed that, methanol extract of yellow pigment was found inhibitory to *Bacillus cereus*, *Enterococcus sp.*, and *Escherichia coli* with 19, 15 and 14 mm respectively and less inhibitory to *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Conclusion: The present study showed the antimicrobial activity of the pigment isolated from the marine organism. Further study has to be carried out to explore the antimicrobial activity against the resistant microorganism.

Keywords: *Antimicrobial activity, pigment, disc diffusion method.*

Introduction:

Marine ecosystem inhabits the wide range of organisms which are yet to be explored to study for the biological properties. Comparing with the terrestrial ecosystem, marine ecosystem is more complex with diversity of microorganism¹. Exploring the marine organism is still in early phase and research in this area will be able to discover new compounds which may be a promising source for pharmaceutical industry². Microorganism remains a significant source of untapped scientific potential till date. Every year, more microorganisms are separated from the huge ocean regions and the number of new organisms

is increasing every year. Therefore, natural products isolated from microorganisms living in habitats other than terrestrial regions are a desirable study tool for pharmacologists, clinicians, and biochemists.

For centuries, microorganisms have been utilized to produce a wide range of compounds, including antibiotics, enzymes, vitamins, texturizing agents, and so forth. The usage of natural ingredients is becoming increasingly popular in the food sector. When ingredients, including colours, come from biological sources like plants or microbes, they are regarded as natural. The fish business already uses microbial colours, for instance to

intensify the pink hue of farmed salmon few pink producing lactic acid bacteria were used³. At present, 13 marine microorganism-isolated natural products are being examined in various stages of clinical trials, while many more are in preclinical research⁴. Among various pigments, Carotenoid is the most common pigment which is the main reason for red and yellow colouration in plant and animals. Since 1930 after the first discovery of carotenoid, 750 naturally occurring carotenoid compound have been discovered and merely 250 compounds are from marine source. Marine carotenoids have potent antioxidant, wound healing, antiproliferative, and anti-inflammatory properties. These pigments are employed as nutraceutical or cosmeceutical components to prevent oxidative stress-related disorders⁵. Therefore, the present study focuses on the isolation and identification of pigment producing microorganism from marine source. The study also aimed to check the therapeutic properties including antimicrobial and anti-oxidant property.

Methodology:

Collection of sea water and isolation of Bacteria:

Sea water was collected from Rameshwaram, Tuticorin and Chennai district from Tamilnadu and Kochi district from Kerala. Serial dilution method was used to isolate the pigment producing bacteria. 10^{-2} and 10^{-3} dilutions were evenly spread in the plate to isolate the single colony of producing bacteria.

Isolation of Pure colony:

Pigmented colonies were picked randomly and streaked using quadrant plate method on Sea water Yeast extract Peptone medium (SYEP) agar plate and Zobells marine agar plates.

Biochemical identification of bacteria:

Identification of bacteria was done using Catalase test and oxidase test.

Pigment extraction

The pigment producing bacteria was grown in the SYEP medium. The culture was incubated for 72 hours at 28°C. the culture was centrifuged and the cell pellet was collected. The pellet was washed with high saline water. The cell pellet was mixed with methanol and sonicated to break the cell wall. After sonication, the mixture was centrifuged and the supernatant was collected. To this supernatant, methanol was added and solvent solvent extraction was carried out to isolate the pigments. The solvent was evaporated and the pigment was stored at -4°C.

Antibacterial activity of extracted pigment:

Antibacterial activity was studied using disc diffusion method using various microorganisms including *Staphylococcus aureus*, *Enterococci sp*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus cereus*.

Results and discussion:

The investigation of novel microbial products with antibacterial, antiviral, anticancer, anticoagulant, and cardioactive activities has recently placed a greater emphasis on marine microorganisms. These active substances could be used as model systems to find novel medications. Though many antibiotics are available in the market, and 70 years since the discovery of first antibiotic, infectious disease are still worrying with huge lose of death per year⁶. The search for new antibiotics will be continuing to combat the resistance developed by the bacteria over the period of time⁷. Therefore, the present study was carried out to identify the pigment producing bacteria.

Sea water samples were collected from four different locations such as Marina, Kochi, Tuticorin and Rameshwaram. From the spread plate, 11 yellow, 6 orange and 2 peach isolates were obtained. Out of the 11 yellow isolates, 2 were isolated from Tuticorin [designated as YT-1, YT-2], 3 from Rameshwaram [YR-1, YR-2, YR-3], 3 from Kochi [YK-1, YK-2, YK-3] and 3 from Marina [YM-1, YM-2, YM-3]. Out of the 6 orange isolates, 2 were isolated from Tuticorin [OT-1, OT-2], 2 from Marina [OM-1, OM-2] and one each from Rameshwaram and Kochi [OR-1, OK-1]. Both peach isolates were obtained from Marina samples [PM-1, PM-2]. Majority of the isolates showed positive reactions for catalase and oxidase tests. The YM-1, PM-2 and OM-1 isolates from Marina sea water showed high catalase activity by unusual bubble formation on reacting with hydrogen peroxide. YM-1 isolate showed negative reaction for oxidase test whereas PM-2 and OM-1 showed positive reaction by formation of purple colour on oxidase disc. The results were tabulated in Table.1.

PLATE 1: Isolation of pigmented marine bacteria by spread plate technique



Marina sample



YM - 1

OM - 1

PM - 1

Fig 1: isolation of bacteria from marine samples and the pure colony of the isolated colonies

Table.1: Biochemical characterization of isolates showing catalase activity and oxidase activity

Isolates	Catalase Activity	Oxidase Activity
YT-1	+	+
YT-2	++	+
YR-1	+	+
YR-2	+	+
YR-3	+	+
YK-1	++	+
YK-2	+	+
YK-3	+	+
YM-1	+++	-
YM-2	++	+
YM-3	++	-
PM-1	++	+
PM-2	+++	+
OT-1	+	+
OT-2	+	+
OR-1	++	-
OK-1	+	-
OM-1	+++	+
OM-2	++	+

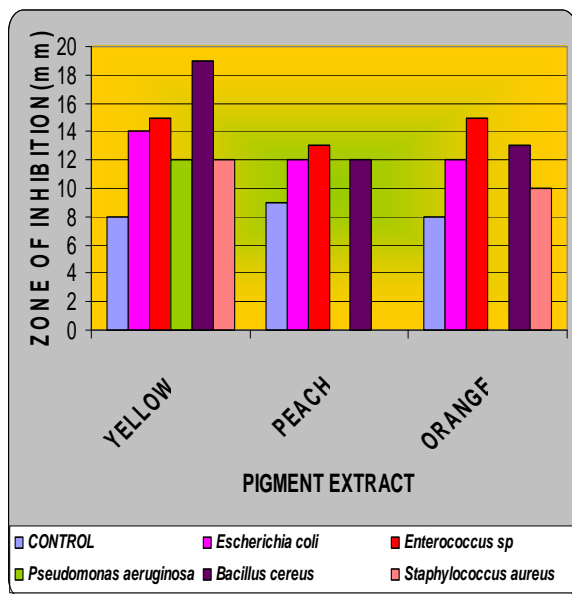
The antibacterial activity of different pigment extracts of yellow [YM-1], peach [PM-2] and orange [OM-1] bacteria was tabulated in table - 2, fig- 1. A preliminary well diffusion method showed that, methanol extract of yellow pigment was found inhibitory to *Bacillus cereus*, *Enterococcus sp.*, and *Escherichia coli* with 19, 15 and 14 mm respectively and less inhibitory to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Whereas, the crude extract of yellow bacteria doesn't exhibit any inhibitory action towards test organisms. The methanol extract of peach pigment was less inhibitory to

Table 2: Antibacterial Activity of Marine Pigment Extracts

Sl. No	Pigment Extract	Zone of inhibition (Diameter in mm)					
		Control	<i>Escherichia coli</i>	<i>Enterococcus sp</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>
1	Yellow	8	14	15	12	19	12
2	Peach	9	12	13	-	12	-
3	Orange	8	12	15	-	13	10

Enterococcus sp., *Escherichia coli* and *Bacillus cereus*. But *Pseudomonas aeruginosa* and *Staphylococcus aureus* were completely resistant to pigment extract. The methanol extract of orange pigment exhibited inhibition to *Enterococcus sp.*, with 15 mm and showed less inhibition to *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*, where as *Pseudomonas aeruginosa* was completely resistant to pigment extract. Thus further study was warranted to do the antibacterial activity.

Figure 2: Antibacterial Activity of Marine Pigment Extracts



Conclusion:

Marine environment are the greatest resource for the identification of lead compound. The present study showed the

antimicrobial activity of the pigment isolated from the marine organism. Future research will focus on the application of these pigment for various industrial applications.

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