# Isolation Of Pigmented Marine Bacteria Exiguobacterium Sp. From Peninsular Region Of India And A Study On Biological Activity Of Purified Pigment

Janani Balraj, Kiruthika Pannerselvam, Angayarkanni Jayaraman

**Abstract**: During the past two decades research on marine bacteria has highlighted the tremendous potential of these microorganisms as a source of new bioactive secondary metabolites and it is reported that most of the marine bacterial pigments exhibited antimicrobial activity. In the present study, Pigmented marine bacteria were isolated from water samples collected from Marina beach (Chennai), Rameshwaram, Tuticorin and Cochin which constitutes the South east (Bay of Bengal) and South west (Arabian Sea) coastal regions of India. Out of 19 distinct pigmented bacteria isolated, only one strain showed potent inhibitory activity against bacterial pathogens. Based on morphological, biochemical, physiological characteristics and 16s rRNA gene sequencing, it was conformed that the strain belonged to the genus Exiguobacterium sp. The pigment was extracted using diethyl ether and subjected to preparative HPLC for purification. Purified pigment exhibited antagonism towards Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas sp, Bacillus sp, Proteus sp, Klebseialla sp, Shigella sp, and Salmonella sp. In case of DPPH radical scavenging assay, the pigment showed higher radical scavenging activity with IC50 value 51.38µg/mL. The UV absorbance profile indicated that the pigment was probably a derivative of carotenoids. GC-MS analysis revealed that the pigment may be interlinked with methyl ester group.

**Keywords**: Exiguobacterium, Pigment, DPPH, antagonism, GC-MS, Preparative HPLC.

## 1 INTRODUCTION

The ocean covers 70% of the Earth's surface and contains about 97% of the Earth's water. Around 50-80% of all life on earth is found beneath the ocean surface. Approximately, less than 10% of the marine species has been identified, and the rests are yet to be explored. In the recent years, there is an immense interest among the researchers in tapping the potential of marine microorganisms for their novel secondary metabolites. Due to the complexity and dynamic system in ocean, the marine microorganisms have developed unique metabolic and physiologic capabilities that ensure survival in extreme variations in pressure, salinity and temperature. It also offers the potential in the production of metabolites, which may be different from terrestrial microorganisms [1], [2]. A main characteristic feature of marine bacteria is that a large proportion of them are pigmented [3]. It has been reported earlier that some pigments produced by microorganisms have the ability to prevent the growth of other bacteria.


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Few investigations have already been conducted to compare the antibiotic activities of marine bacteria isolated from different origins [4], [5], [6]. Besides antibiotic activity, the pigments may also possess antioxidant activity. Polyphenolic compounds are among the interesting antioxidant compounds isolated from marine resources [7]. In general, phenolic compounds are divided into ten types, based upon their structure. Among them, Phlorotannins and naphtoquinones have been reported to possess strong antioxidant activity. In addition to polyphenolic compounds, other interesting antioxidants, such as carotenoids, can be found in marine resources. Carotenoid pigments such as fucoxanthin, astaxanthin have been proven to possess strong antioxidative activities, and attracted greater attention due to their beneficial effects on human health such as their potential in the prevention of diseases such as cancer and cardiovascular complaints [8]. Hence the present investigation was focused on isolating antagonistic pigmented marine bacteria from different areas of southern peninsular regions of India and purification of pigment to analyze its antimicrobial and antioxidant potentials.

## 2. MATERIALS AND METHODS

### 2.1. Sampling

Sea water samples were collected from Marina beach (Chennai), Rameshwaram, Tuticorin and Cochin which constitutes the South east (Bay of Bengal) and South west (Arabian Sea) coastal regions of India. The water samples were collected in clean, sanitized and autoclaved bottles by adopting 'Scoop method' [9] and brought to laboratory in ice-cold containers. The subsequent isolation of the bacterial strains was carried out within 48h.

### 2.2. Isolation of pigmented bacteria

Samples were seeded onto Sea water yeast extract peptone medium (SYEP) and incubated at 28oC for 48h, after which all colonies with different pigmentation and others of frequent occurrence were chosen for isolation [10]. Isolated strains were maintained on SYEP agar slants at 4oC for further use.

### 2.3. Test microorganisms

The following microorganisms were procured from Institute of Medical Science, PSG hospitals, Coimbatore, Tamilnadu, India. Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Enterococcus sp, Pseudomonas sp, Bacillus sp, Proteus sp, Klebseialla sp, Shigella sp, and Salmonella sp. Stock cultures were maintained on nutrient agar at 40ºC, then subcultured in nutrient broth at 37ºC, prior to each test.

### 2.4. Screening for antagonistic activity

The antagonistic activity of the pigmented bacterial strains against test pathogens was determined by adopting cross streak method [6]. Fresh cultures (24h old) were streaked across the diameter of SYEP agar plates and cultures of test pathogens were streaked at right angles across them. Plates were incubated at $30\pm2^0$ C for another 24h.

### 2.5. Phenotypic characterization and 16s rRNA gene sequencing

The phenotypic and biochemical characterization of the selected strain was done by using standard techniques according to the diagnostic table of Cowan and Steel [11] and Bergey's Manual of Determinative Bacteriology [12]. Chromosomal DNA isolation and purification was performed as described by Marmur (1961) [13]. The genomic DNA thus obtained was amplified using PCR primers f(5'-GCCCACGACCAGTTCGAC-3') and r(5'-CATCCCCCTCCCTATGAC-3'). Sequencing was outsourced at Synergy Research centre, Chennai, India. The 16s rRNA gene sequence was compared for similarity with the reference species of bacteria contained in genomic database banks using the NCBI BLAST. Multiple alignment of the sequence was performed using the ClustalW program [14], [15]. The phylogenetic tree was constructed by using treeing algorithms in PHYLIP software package [16]. The sequence was deposited in GenBank with the accession number HM119395.

### 2.6. Extraction of pigment

The pigment was extracted according to Meckel and Kester (1980) [17]. The selected pigmented bacterial isolate was cultured on SYEP broth medium, which were then centrifuged for bacterial pellet. A total pellets were taken and then were extracted using cold methanol with the aid of sonicator. After that the extract was evaporated vacuum below 30oC. The concentrate extract was used for further experiments.

### 2.7. Purification of pigment

#### 2.7.1. Analytical HPLC

The pigment was identified and analyzed by using HPLC Shimadzu LC-8A in reversed phase column AB with ODS, C18 column , diameter of 4mm×25 mm and a mobile phase of acetonitrile. Detection of pigment was performed at a wavelength of 300 to 650 nm with a flow rate of 0.5 mL/min at pressure 15 psi. [18].

#### 2.7.2. Preparative HPLC

Preparative HPLC was performed on Shimadzu HPLC system equipped and the mobile phase used was acetonitrile. The sample (5 ml) was injected at a flow rate of 85ml/min to C18 preparative column. The PDA detector was used to trigger fraction collection at various retention times. The fractions were evaporated to dryness and dissolved in minimum quantity of methanol.

### 2.8. Ultraviolet-visible spectroscopy

The fractions obtained from preparative HPLC was then analyzed using UV- visible spectrometry. UV/Visible scanning spectra of the acetonitrile extract containing the pigment were recorded between 200 to1100 nm. Acetonitrile was used as blank.

### 2.9. Gas Chromatography-Mass Spectrometry

The molecular weight was measured by GC-MS (THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II). GC-MS was performed by the electron ionization and direct injection mode. When only mass spectrometry was used, the sample was injected into the ionization chamber and the temperature was raised from 100 - 250°C, ramped at $8^0$ C/min, holding time for 10 min at 250°C. The carrier gas used was Helium, at a flow rate of 1 mL/min and column used was TR 5 - MS capillary standard non - polar column. The mass spectrum was analyzed with a CLASS-5000 Analysis System & Software [19].

### 2.10. Antimicrobial activity of purified pigment

The antimicrobial activity of the pigment dissolved in methanol was tested by agar well diffusion method [20]. Chloramphenicol and methanol were used as positive and negative control. The plates were incubated at $28^0$ C for 24h after which activity was evidenced by the presence of zone of inhibition surrounding the well. The experiment was done in triplicates, zone of inhibition was measured, and the antibacterial activity was expressed as the mean diameter of zone inhibition (mm).

### 2.11. DPPH free radical scavenging activity

The antioxidant activity of the purified pigment was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical. From the stock solution, various concentrations 5-100µg/mL of the extracts was added to 1mL of 3mM ethanolic DPPH free radical solutions and incubated for 30min. After which, the absorbance of the preparations were taken at 517nm in a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid solution The percentage of radical scavenging activity was calculated using the formula, % radical scavenging activity = [(absorbance of blank-absorbance of sample)/ (Absorbance of blank)] x 100 From the calibration curves obtained from different concentration of the extracts, the IC50 (inhibitory concentration 50%) was determined. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [21].

## 3. RESULTS AND DISCUSSION

### 3.1. Isolation of bacterial isolates

Morphologically distinct bacterial strains were isolated from seawater sample whose sampling conditions are presented in Table1. Among 61 bacterial colonies isolated 19 (31%) strains were pigmented. Out of 19 pigmented strains isolated 11 (58%) of them were yellow pigmented, 6 (32%) of them produced orange colored pigment and the remaining 2 (10%) isolates were peach in color (Fig.1). ZoBell and Feltham (1934) examined thousands of colonies growing on agar inoculated with sea water or marine mud and found that 69.4% were chromogenic [22]. Out of which 31.3% were yellow, 15.2% were orange, 9.9% were brown and 5.4% were red or pink. Later ZoBell and Upham (1944) described 60 new marine species 19 of which were yellow, 5 brown, 5 pink or salmon colored, 4 orange and 1 red [23]. Similar to the earlier reports, the marine organisms isolated in the present study were yellow coloured, orange and peach coloured.

### 3.2. Screening for antagonistic activity

The antagonistic activity of the pigmented marine bacteria was carried out in the present study. Earlier studies have demonstrated that most of the antibiotics producing marine bacteria were pigmented [6]. Out of 19 pigmented bacteria, 5 strains showed inhibitory activity towards the test pathogens. Among the strains YM3, YM6, YM10, OM2 and PM1, PM1 showed potent inhibitory activity towards all the test pathogens. Marine bacteria are known to produce inhibitory substances in the marine environment, though they are not specific antibiotic producers. Marine bacteria showing antibacterial activities have been isolated from various biotopes such as surface or deep sediments, seaweeds and other substrates. The bacteria identified so far mostly belong to the species of *Bacillus, Micrococcus, Pseudomonas, Vibrio, Flavobacterium, Alcaligens, Xanthomonas* and *Achromobacter.* Some of these antagnositic bacteria show antibacterial activity against bacterial pathogens [24], [25], [26]. Among the isolates, PM1 showed maximum antibacterial activity with ten of organisms. Hence PM1 was chosen for further studies.

### 3.3. Phenotypic characterization and 16s rRNA gene sequencing

The colony morphology of the strain PM1 was punctiform, flat, appeared shiny, translucent and smooth. Gram staining of the isolate revealed that it was Gram positive cocci. Gram positive bacteria collected from coastal sand dune vegetation showed a predominance of pigmented isolates [27]. The physiological and biochemical characterization were shown in Table 2. The strain showed high catalase activity. The organism was able to tolerate upto 8% NaCl with an optimum concentration of 5% NaCl. The genomic DNA of the strain PM1 was isolated and subjected to 16s rRNA gene amplification and sequencing. The 16S rDNA PCR amplification was performed with 80ng DNA. The size of amplified product was around 1.5kb (Fig.2). The BLAST search with homologous sequences in NCBI database revealed that PM1 strain showed maximum (93%) identity with *Exiguobacterium sp* (Table 3). The phylogenetic tree was presented in Fig. 3.

### 3.4. Extraction of pigments

The pigment production by the isolate was observed after about 72 h of incubation as the culture appeared peach in colour. The pigment was extracted by methanol followed by sonication for 30 min. The extracted pigment appeared orange colour and it was condensed using rotary vaccum evaporator for further analysis. Similarly Godinho and Bhosle (2008) extracted orange colour pigment by sonication for 10 minutes at 3sec interval [27].

### 3.5. Purification

#### 3.5.1. Analytical HPLC

At 450nm best resolution of peak was obtained (Fig.4). Totally seven peaks were obtained in which seventh peak was major with the area percent of 63.24%. Goswami *et al.,* (2010) observed HPLC peak at 437nm and it was reported that the pigment might belong to the family of carotenoid [18]. Similar to this result in our present study our pigment showed maximum peak at 450nm. So the pigment probably may belong to carotenoid family.

#### 3.5.2. Preparative HPLC

The elution profile of organic solvent, soluble pigment in analytical HPLC showed seven peaks and it was eluted at various retention times by preparative HPLC. At the retention time of 14.848, one major peak (seventh peak) was obtained which was considered to be carotenoid and it was subjected to further analysis. Stafsnes (2010) have reported 6 peaks in preparative HPLC and the maximum peak was obtained at the retention time of 14.634 [28]. The author reported that the major peak might be a carotenoid.

### 3.6. UV- visible spectroscopy

The UV-visible absorption spectrum of $7^{th}$ fraction corresponding to the major peak obtained in spectrum of preparative HPLC was measured to determine the presence of carotenoid pigment. The pigment showed multiple absorption peaks between 400 to 600nm. The UV-visible absorption spectra of the pigment showed absorption maxima at 321nm, 450nm and 500nm. The maximum absorption peak for the purified pigment was shown at 450nm. Godinho and Bhosle (2008) studied UV-visible spectroscopy of carotenoid pigment and reported absorption maxima at 533nm, 468nm and 341nm which were similar to the results of our present study [27]. The clear three band shape of the absorption spectrum of the pigment is the characteristic feature of carotenoid and reflects its purity.

### 3.7. Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis of pigment showed numerous peaks at various retention times, where six peaks at different retention times were showing higher relative abundance (Fig.5). The mass spectrum patterns were screened against the NIST library. A peak with higher relative abundance and area was obtained at the retention time of 17.76 and thus were selected and identified. Weber *et al.,* (2004) reported an unusual complex and polyunsaturated fatty acid substituted with one hydroxyl and one aldehyde group has been described as a new polyene pigment, laetiporic acid, in the wood-rotting basidiomycete *Laetiporussulphureus* [19].

Similarly the compound of our interest may be interlinked with the methyl ester group. In the present study, when compared the GC-MS and UV-Visible spectra, it was concluded that the pigment may be identified as a derivative of Caretenoids, bearing an unprecedented methyl ester group as part of its chromophore.

### 3.8. Antimicrobial activity of purified pigment

The antimicrobial activity of the purified pigment (1mg/mL) was checked against common human pathogens. The purified pigment showed antimicrobial activity against 10 human pathogens except *Enterococcus* sp. The *Enterococcus* sp. showed no zone of inhibition. The most of the antibiotic producing marine bacteria were pigmented [6]. Jayanth and her coworkers (2002) studied antagonistic activity of marine bacteria and they tested their ability to inhibit the growth of test organisms [5]. The author found that out of 62 antagonistic marine bacteria 18 (29.03%) had the ability to inhibit atleast any one of the test organism. This was similar to our results. In the present study, the maximum zone of inhibition was obtained against *Shigella*, *Klebsiella* sp and *Staphylococcus aureus* (Fig.6). The results indicated that the purified pigment contains antimicrobial substances, and it possess the ability to inhibit the growth of human pathogens.

### 3.9. DPPH- free radical scavenging activity

DPPH test which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-518nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The $IC_{50}$ values of the pigment extract and ascorbic acid was noted (Table 4). The $IC_{50}$ value of peach coloured pigment was 51.38µg/mL as against ascorbic acid 8.8µg/mL. The peach coloured pigment had a better antioxidant potential. The generation of free radicals has been suggested to play a major key role in progression of a wide range of pathological disturbances such as diabetes [29], cancer [30]. Hence therapy using free radical scavenging antioxidants has potential to prevent, delay or ameliorate many of these disorders [31]. More than 700 carotenoids have been isolated from natural sources [32] and evaluated for their pharmaceutical potentials in this field. Only a small number of Carotenoids were proven to possess strong antioxidative activity.

## 4. CONCLUSION

The results of the present study highlights that sea water is a rich source of pigmented bacteria. This study shows that the marine Exiguobacterium sp. is a prominent pigment producer along with efficient antibacterial activity as well as antioxidative potential. The future work is aimed at purification, characterization and analyzing the biological activity of the pigment.

## 5. ACKNOWLEDGMENT

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## 6. REFERENCES

[1] Fenical, W. and Jensen, P.R. Marine Microorganisms: A New Biomedical Resource. Marine Biotechnology. Volume 1, Pharmaceutical and Bioactive Natural Products. (Attaway DH, Zaborsky OR, ed), New York, 1993, pp: 419-459.

[2] Fenical, W. Chemical studies of marine bacteria: developing a new resource. Chem Rev, 1993, 93:1673–1683.

[3] Zobell, C.E. Marine Microbiology. ChronicaBotanica Co., Waltham, Mass, 1946, pp:1-240.

[4] Burgess, J.G., Miyashita, H., Sudo, H. and Matsunaga, T. Antibiotic production by the marine photosynthetic bacterium chromatiumpurpuratum NKPB 031704: Localization of activity to the chromatophores. FEMS Microbiol Lett, 1991, 84: 301-306.

[5] Jayanth, K., Jeyasekaran, G. and Jeya shakily, R. Isolation of marine bacteria, antagonistic to human pathogens. Indian journal of marine sciences, 2002, 30(1): 39-44.

[6] Lemos, M.L., Toranzo, A.E. and Barja, J.L. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microbial Ecol, 1985, 11: 149-163.

[7] Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. Nutrition Reviews, 1998, 56(11): 317-333.

[8] Wollgast, J. and Anklam, E. Polyphenols in chocolate: is there a contribution to human health? Food Research International, 2000, 33: 449-459.

[9] U.S. Environmental Protection Agency. Microbiological methods for monitoring the environment. EPA-600/8-78-017. U.S. Environmental Protection Agency, Cincinnati, OH, 1978, pp: 337.

[10] Schneider, J. and Rheinheimer, G. Isolation Methods. In: Methods in Aquatic Bacteriology, Austin (Ed.). John Miley and Sons, New York, 1988, pp: 73–94.

[11] Cowan, S.T. and Steel, K.J. Manual for the identification of medical bacteria. 3rd edn. Cambridge University Press, London, 1998.

[12] Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley. J.T. and Williams, S.T. Bergey's Manual of Determinative Bacteriology. 9th edn, Baltimore: Williams & Wilkins, 1994, pp: 605-667.

[13] Marmur, J. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J MolBiol, 1961, 3: 208-218.

[14] Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J MolEvol, 1980, 16(2):111-20.

[15] Thompson, J.D., Higgins, D.G. and Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res, 1994, 22:4673–4680.

[16] Felsenstein, J. Philip (Phylogeny Inference Package) version 3.5c. Seattle: Department of Genetics, University of Washington, 1993.

[17] Meckel, R.A. and Kester, A.S. Extractability of carotenoid pigments from nonphotosynthetic bacteria with solvents and detergents: Implications for the location and binding of the pigments. J Gen Microbiol, 1980, 120: 111–116.

[18] Goswami, G., Chaudhuri, S. and Dutta, D. Effect of pH and temperature on pigment production from an isolated bacterium. Chemical Engineering Transactions, 2010, 20:127-132.doi: 10.3303/CET 1020022

[19] Weber, A.P., Oesterhelt, C., Gross, W., Bräutigam, A., Imboden, L.A., Krassovskaya, I., Linka, N., Truchina, J., Schneidereit, J., Voll, H., Voll, L.M., Zimmermann, M., Jamai, A., Riekhof, W.R., Yu, B., Garavito, R.M. and Benning, C. EST-analysis of the thermo-acidophilic red microalga Galdieriasulphuraria reveals potential for lipid A biosynthesis and unveils the pathway of carbon export from rhodoplasts. Plant MolBiol, 2004, 55: 17–32.

[20] Grammer, A. Antibiotic sensitivity and Assay test. In. Microbiological methods, Collins, C. H and Lyne, P.M. (Eds), Butterworths, London, 1976, pp: 235.

[21] Gupta, M., Mazumdar, U.K., Sivahkumar, T., Vamsi, M.L.M., Karki, S., Sambathkumar, R. and Manikandar, L. Antioxidant and anti-inflammatory activities of Acalyphafruticosa. Nig J Nat Prod Med, 2003, 7:25-29.

[22] Zobell, C.E. and Feltham, C.B. Preliminary studies on the distribution and characteristics of marine bacteria. Bull. Scripps Inst. Oceanog. Tech. Ser, 1934, 3: 279-296.

[23] Zobell, C.E. and Upham, H.C. A list of marine bacteria including descriptions of sixty new species. Bull.Scripps Inst. Oceanogr, 1944, 5:239-292.

[24] Austin, B. Novel pharmaceutical compounds from marine bacteria. J Appl Bacteriol, 1989, 67(5):461-470.

[25] Bernan, V.S., Greenstein, M. and Maiese, W.M. Marine microorganisms as a source of new natural products. Adv Appl Microbiol, 1997, 43:57-90.

[26] Gauthier, M.J., Shewan, J.M., Gibson, D.M. and Lee, J.V. Taxonomic position and seasonal variations in marine neritic environment of some Gram-negative antibiotic-producing bacteria. J Gen Microbiol, 1975, 87: 211-218.

[27] Godinho, A. and Bhosle, S. Carotenes produced by alkaliphilic orange-pigmented strain of microbacteriumarborescens-AGSB isolated from coastal sand dunes. . Indian journal of marine sciences, 2008, 37(3):307-312.

[28] Stafsnes, M.H., Josefsen, K.D., Kildahl-Andersen, G., Valla, S., Ellingsen, T.E. and Bruheim, P. Isolation and characterization of marine pigmented bacteria from Norwegian coastal waters and screening for carotenoids with UVA-blue light absorbing properties. J Microbiol, 2010, 48(1):16-23. doi: 10.1007/s12275-009-0118-6.

[29] Sabu, M.C. and Kuttan, R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethanopharmacol, 2002, 81: 155-160.

[30] Tsao, H., Goel, V., Wu, H., Yang, G. and Haluska, F.G. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. J Invest Dermatol, 2004, 122: 337–341.

[31] Delanty, N. and Dichter, M.A. Antioxidant therapy in neurologic diseases. Arch Neurol, 2000, 57: 1265-1270.

[32] Britton, G., Liaaen-Jensen, S. and Pfander H. Carotenoids Hand - book. BirkhäuserVerlag, Basel, 2004

**Table 1.** Sampling condition during collection of seawater

| S. No. | Characteristics | Marina water Sample | Tuticorin water sample | Rameshwaram water sample | Kochi water sample |
|---|---|---|---|---|---|
| 1. | Temperature($^{o}$C) | 26 | 27 | 26 | 24 |
| 2. | Turbidity | Clear | Clear | Turbid | Clear |
| 3. | Weather | Hot and Cloudy | Hot | Cloudy | Humid |
| 4. | Human Activity | High | Low | Medium | High |
| 5. | Depth(Feet) | 2 | 2 | 2 | 2 |
| 6. | Distance From Shore(Feet) | 20 | 22 | 25 | 20 |

**Table 2.** Biochemical and physiological characteristics of PM-1

| Character | PM-1 |
|---|---|
| **Morphological characteristics** | |
| Pigment | Peach |
| Cell shape | Cocci |
| Gram staining | Positive |
| Acid fast staining | Negative |
| **Physiological characteristics** | |
| Range of temperature for growth | 25-30$^{0}$ C |
| Optimum temperature | 28$^{0}$ C |
| Range of pH for growth | 6.5-7.5 |
| Optimum pH | 7.0 |
| NaCl tolerance | 7% |
| **Biochemical characteristics** | |
| Indole production | - |
| Methyl red | - |
| Vogesproskauer | - |
| Citrate utilization | - |
| Urease | - |
| Oxidase | + |
| Catalase | + |
| $H_2S$ production and carbohydrate fermentation | Alkaline slant and alkaline butt |
| Nitrate reduction | - |
| Starch hydrolysis | - |
| Gelatin hydrolysis | - |

**Table 3.** Partial sequence analysis of 16s rRNA of strains PM1

| **Isolate PM-1; *Exiguobacterium* sp. by BLAST analysis; Accession number HM119395 (NCBI GenBank, USA)** |
|---|
| TCGGCGGCTGGCTCCCTAAGGTTACCTCAACGACTTCGGGTGTTACAAACTCTCGT<br>GGTGTGACGGGCGGTGTGTACAAGGACCCGGGAACGTATTCACCGCAGTATGCTG<br>ACCTGCGATTACTAGCGATTCCGACTTCATGCAGGCGAGTTGCAGACTGCAATCCG<br>AACTGAGAACGGATTTCTGGGATTGGCTCCACCTCGCGGCTTCGCTGCCCTTTGTA<br>CCGTCCATTGTAGCACGTGTGTAGCCCAACTCATAAGGGGCATGATGATTTGACGT<br>CATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCAACTGAAT<br>GGTGGCAACTAAGGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA<br>CGACACGAGCTGACGACAACCATGCACCACCTGTCACTCCTTGCCCCCGAAGGGG<br>AACGCTACATCCTCTGGTACTGGTCAAAGGGAGGATGTCAAGCAGGTGGAAAGAG<br>TGCTTCTTCGCGTCTCTTCAATATATAAAACCATATGCTACCAGCGTCTTGTGCGGG<br>CTCCCGCGTACAATTTCCTTGTGAGTTTAGAGCCCGTGACGA |

**Table 4.** DPPH free radical scavenging activity

| **S.No** | **Concentration of extract / standard µg/ml** | ***Exiguobacterium* sp.-PM1** | **$IC_{50}$ µg/ml** | **Ascorbic acid** | **$IC_{50}$ µg/ml** |
|---|---|---|---|---|---|
| 1 | 5 | 10.83 | 51.38 | 35.70 | 8.80 |
| 2 | 10 | 15.63 | | 56.84 | |
| 3 | 15 | 17.94 | | 62.34 | |
| 4 | 20 | 20.43 | | 72.82 | |
| 5 | 25 | 24.33 | | 77.62 | |

**Fig.1.** Bacteria isolated from various locations

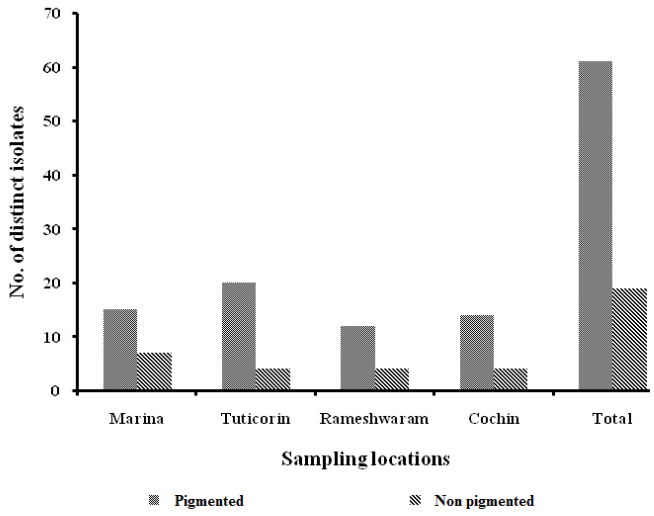

**Fig.2.** 16S rRNA Gene amplification of isolate PM1

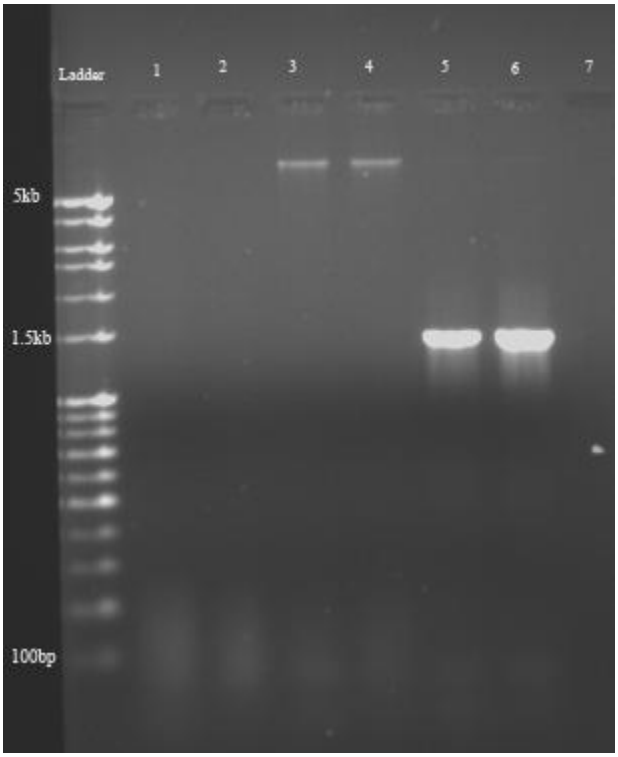


**Fig. 3.** Phyloenetic tree

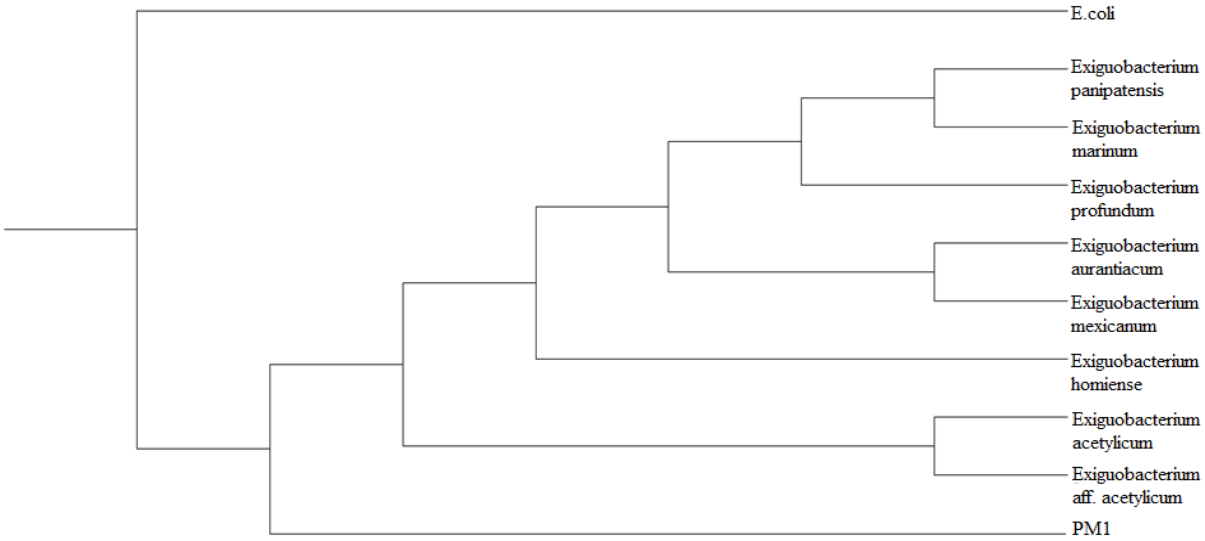

**Fig.4.** Chromatogram obtained from analytical HPLC

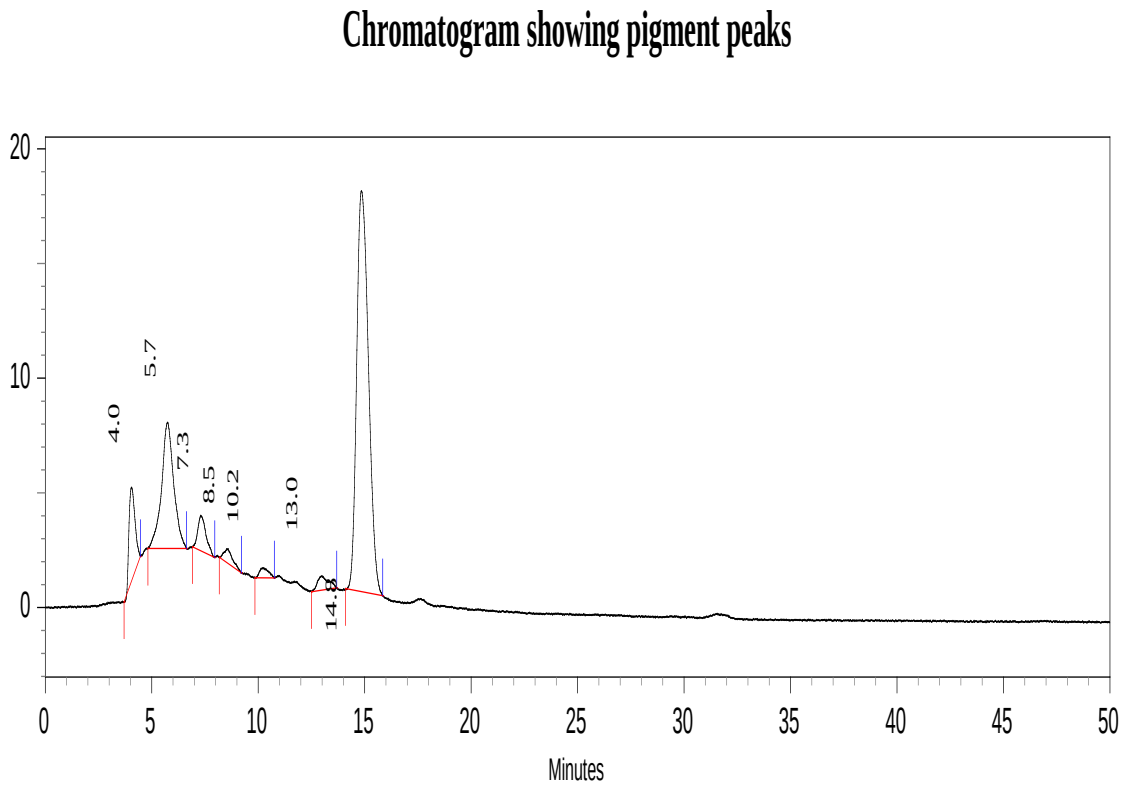


**Fig. 5.** GC-MS spectrum of the purified pigment

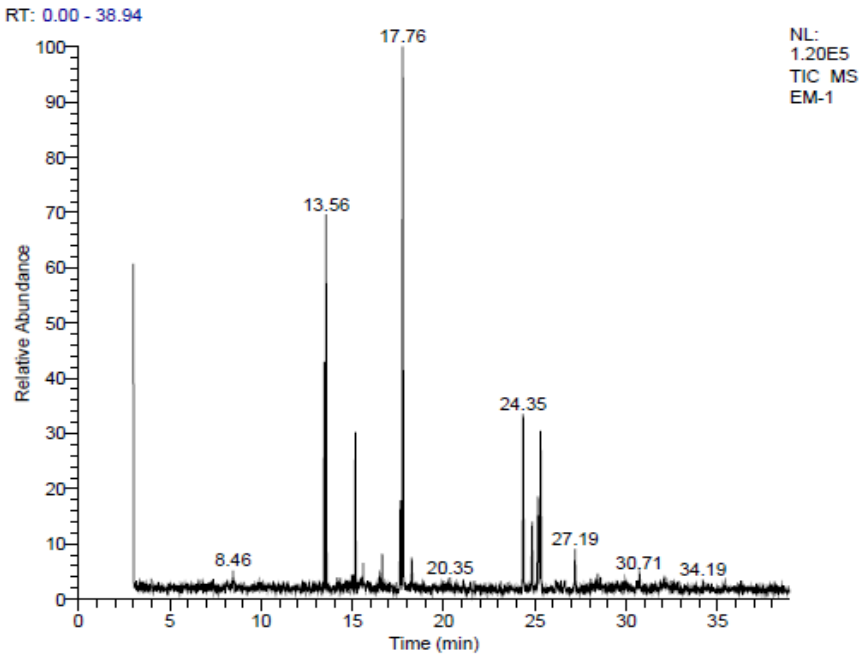

**Fig. 6.** Antimicrobial activity of purified pigment