Chapter – IV

Characterization of antibiofilm agents from marine bacteria against human fungal pathogens
The term infectious diseases will not be complete without the infections caused by fungi. In particular, the genus Candida accounts a lot among the fungal diseases. The genus Candida includes numerous human pathogens like *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. dubliniensis* and *C. rugosa*. The diseases caused by Candida are called in general as Candidiasis. *C. albicans* is the major pathogen among the genus causing numerous topical infections and systemic infections in immunocompromized individuals. *C. tropicalis* is a clinically important pathogen from Candida-non-albicans (CNA) group, next to *C. albicans* reported with numerous nosocomial infections especially the device associated infections (Wingard et al., 1979). Numerous studies have shown that biofilm formation confers multidrug resistance to *Candida* sp. which necessitates the identification of novel agents with antibiofilm potential against *Candida* sp. (Seneviratne et al., 2008; Leung et al., 2012).

*C. tropicalis* causes candidemia (systemic infection) in immune compromised individuals apart from its association with other topical infections. There are reports which show the association of *C. tropicalis* with meningitis, a rare phenomenon for the genus Candida (Goldani & Santos, 2010). *C. tropicalis* also causes diseases like endocarditis, acute disseminated candidosis, pyelonephritis, oesophagitis and vaginitis in immunocompromized individuals.

An epidemiological survey from Indian subcontinent showed that, CNA accounts for 67–90% of nosocomial candidemia with *C. tropicalis* as the dominant one (Wingard et al., 1979; Verma et al., 2003; Kothari & Sagar, 2009). *C. tropicalis* is found to be the major colonizer in catheter biofilms and are known to cause clinically relevant infections as stated above. Resistance to antibiotics and its ability to form biofilms attribute to the clinical significance of *C. tropicalis* (Crnich & Drinka, 2012). Even though the clinical relevance of *C. tropicalis* is much alike to that of *C. albicans*, studies about its virulence and control strategies are scanty (Chai et al., 2010). This prompted to study the possible control measures which could be useful in combating the device associated infections caused by *C. tropicalis*.

Similar to that of the Gram positive bacteria, there is no clear picture available representing the QS system of *Candida* sp. but there are certain genes identified to play a major role in pathogenesis like that of the secreted alkaline proteinases (SAP) genes. Similarly studies with mutants that lack the ability to produce hyphal growth showed increased susceptibility of the mutants towards antibiotics and reduced virulence.
delineating the importance of hyphal growth in pathogenesis of Candida. Hence marine bacteria were screened for antibiofilm agents with the potential to control the biofilms in *C. albicans* and *C. tropicalis* and also their ability to inhibit the hyphal growth and production of SAP has been studied.

This chapter explains the antibiofilm activity of marine isolates against *Candida* sp., with special reference to *C. tropicalis*, a fungal pathogen often reported in device associated infections nevertheless seldom studied.

**Results and discussion**

**4.1. Antibiofilm activity of marine isolates against *C. albicans***

Marine bacterial isolates from Karankadu coastal region were screened for antibiofilm activity against *C. albicans*. Cell free culture supernatant (CFCS) of CA4, 4ft3, G9d, G10b, G12.1, G19, G20, Sp4 and Sg12y were found to significantly inhibit the biofilm formation in *C. albicans* (Figure 4.1).

![Figure 4.1. Antibiofilm activity of Karankadu isolates against *C. albicans*](image)
Among the 9 isolates which showed antibiofilm activity against *C. albicans*, the seaweed associated actinomycetes (G9d – *Kocuria marina*) exhibited significant antibiofilm activity in terms of reducing the surface area covered by the biofilms and initial attachment of *C. albicans* to the substratum. In addition, G9d has significant antibiofilm potential against *S. epidermidis*, which prompted to proceed further with G9d.

### 4.2. Effect of G9d on the growth of *C. albicans*

G9d extract at varying concentrations ranging from 50 - 1000µg/ml was tested for antifungal activity against *C. albicans* MTCC3017 and a clinical isolate of *C. albicans* CA2 (Figure 4.2). The extract has no effect on the growth of *C. albicans* at any of the concentrations tested. Hence, the antibiofilm activity of G9d was tested further against the *C. albicans* MTCC3017 and the clinical isolate *C. albicans* CA2.

![Antifungal activity of G9d against C. albicans at different concentrations](image)

**Figure 4.2.** Antifungal activity of G9d against *C. albicans* at different concentrations

### 4.3. Effect of G9d on *C. albicans* biofilm architecture

Biofilm inhibitory effect of G9d was studied microscopically. For microscopic studies, both the reference strain (*C. albicans* MTCC 3017) as well as a clinical isolate of *C. albicans* (CA2) was used. CLSM studies revealed the reduction in surface area colonized by *C. albicans* (Figure 4.3). CLSM observations also revealed the absence of hyphal growth in G9d treated surfaces that prove its ability as an anti-pathogenic
agent. There is no significant reduction in the thickness of the biofilms upon treatment and the possible reason could be the biofilm architecture itself (Table 4.1). Unlike bacterial biofilms which form as a mass, the Candida biofilms are found as a monolayer on the surface packed closely by their EPS and hyphae.

Table 4.1. Thickness of biofilms formed by *C. albicans* reference strain and its clinical isolate in the presence and absence of G9d extract

<table>
<thead>
<tr>
<th>Fungal pathogens</th>
<th>Thickness of biofilm (in µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>C. albicans</em> MTCC3017</td>
<td>19.3</td>
</tr>
<tr>
<td><em>C. albicans</em> CA2</td>
<td>19.6</td>
</tr>
</tbody>
</table>

Figure 4.3. CLSM’s 3D biofilm images of *C. albicans* reference strain and its clinical isolate grown in the presence and absence of G9d of extract
4.4. Antibiofilm activity of marine isolates against *C. tropicalis*

Since G9d exhibited significant antibiofilm activity against *C. albicans*, its activity was tested further against *C. tropicalis* which is a seldom studied pathogen irrespective of its clinical significance. The aim of this chapter is to emphasize the antibiofilm activity of G9d as a potential and possible strategy to control the device associated infections caused by *C. tropicalis*. Hence, the antibiofilm activity of G9d was tested against *C. tropicalis* at varying concentrations ranging from 50 to 200µg/ml and found that the biofilm inhibition was maximum at around 150 µg/ml concentration and the same has been used as its BIC for further studies (Figure 4.4). The antifungal effect of the extract at the concentrations showing antibiofilm activity was also tested and found that, the extract does not inhibit the growth of *C. tropicalis* upto the tested concentration of 200µg/ml (Figure 4.5).

![Figure 4.4. Antibiofilm activity of G9d extract at different concentrations against *C. tropicalis*](image)

![Figure 4.5. Effect of G9d extract on the growth of *C. tropicalis*](image)
4.5. Effect of G9d on *C. tropicalis* biofilm architecture

*C. tropicalis* biofilms grown on glass surfaces were observed under light microscope to study the surface colonization and by CLSM to study the effect of G9d on the 3D architecture of the biofilms. As observed with *S. epidermidis* and *C. albicans*, G9d significantly reduced the surface area colonized by *C. tropicalis* (Figure 4.6). Apart from reducing the surface colonization, G9d significantly reduced the hyphal growth in treated slide (Figure 4.6). CLSM’s Z-stack analysis revealed that there is no significant reduction in the biofilm thickness in G9d treated surface when compared to its untreated control as observed for *C. albicans* (Table 4.2). Biofilm formation as a monolayer could be the reason for the absence of reduction in biofilm thickness.

![Control vs G9d treated biofilms](image)

Figure 4.6. CLSM’s 3D images of *C. tropicalis* biofilms grown in the presence and absence of G9d extract

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>Thickness of biofilm (in µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>19.3</td>
</tr>
</tbody>
</table>

Table 4.2. Thickness of biofilms formed by *C. tropicalis* in the presence and absence of G9d extract
The capability of Candida to form biofilms on medical devices like catheters, endotracheal tubes, pacemakers and other prosthetic devices has made them the chief colonizers in nosocomial infections (Douglas, 2003; Ramage et al., 2005). Studies have provided evidence for interlace between biofilm formation and the virulence traits, dimorphism and/or phenotypic switching in Candida sp. (Baillie & Douglas, 1999; Chandra et al., 2001; Thompson et al., 2011; Brand, 2012). The ability of Candida to switch its phenotype conferred significant resistance against host immune machinery and antifungal drugs (Chandra et al., 2001).

Hyphal or mycelial mode of growth promotes the virulence of Candida sp. by one of the following means: a) hyphae can invade host cells facilitating their penetration and growth at deeper layers (Kumamoto & Vinces, 2005); b) hyphae are competent to infringe the endothelial barrier facilitating systemic infection (Zink et al., 1996; Jong et al., 2001; Kumamoto & Vinces, 2005); and c) hyphae could provide resistance to phagocytes by killing them (Lo et al., 1997; Korting et al., 2003). In contrast to the imperative role of hyphal development and biofilm formation, mutants that lack the ability to form hyphae were shown to form biofilms, and such biofilms are easily detachable and susceptible to antibiofilm agents (Baillie & Douglas, 1999).

Hence, agents that effectively control the biofilm formation and/or phenotype switching i.e. hyphal development could be a promising drug candidate to treat infections caused by Candida. In this context, the active principle from K. marina with the ability to inhibit both the biofilm formation and hyphal development could be a novel candidate to combat nosocomial infections caused by Candida.

4.6. Effect of G9d on EPS production and cell surface morphology

Fine structure analysis of C. tropicalis cell surface morphology grown in the presence and absence of G9d extracts was done by observation under SEM. Electron micrographs clearly revealed the absence of secreted EPS around the cells in case of G9d treated samples, thus confirming the ability of G9d to inhibit the EPS production in C. tropicalis. Apart from interfering with the production of EPS, G9d prevented the elongation of C. tropicalis, a phenomenon prelude to the development of hyphal growth (Figure 4.7). EPS acts as a barrier preventing the influx of antibiotics and also as ion-exchange resins that bind to charged antibiotics preventing its movement to the
actual site of action (Hoyle et al., 1990; Gristina et al., 1994). Hence inhibition of EPS production by the active fraction of G9d highlights its potential as a candidate for anti-pathogenic agent.

Figure 4.7. Scanning electron micrographs of *C. tropicalis* biofilms grown in the presence and absence of G9d extract.

4.7. Purification and characterization of the active principle

It is evident from various analyses that the crude extract of G9d possesses significant antibiofilm activity against *C. albicans* and *C. tropicalis*. Hence, the ethyl acetate extract was purified further to identify the active principle responsible for antibiofilm activity. Partial purification of the extract was done through TLC as stated for *S. epidermidis* under the section 1.2.6. The bands separated by TLC was scraped and collected as three different fractions as orange (O), above orange (AO) and below orange (BO) (Figure 4.8). The scraped fractions with silica were extracted again with ethyl acetate and the extract was dried. Dried extract was then dissolved in methanol and the antibiofilm activity of the fractions was then assessed against test pathogens.
4.8. Antifungal and antibiofilm activity of G9d partially purified fractions

The antifungal and antibiofilm activity of TLC fractions were assessed against *C. tropicalis*. The fractions of G9d do not have any antifungal activity against *C. tropicalis* and it is obvious as the crude extract itself has no activity on the growth of the pathogen (Figure 4.9). On the other hand, the fraction AO significantly inhibited the biofilm formation to about 90% compared to its untreated control (Figure 4.10). The antibiofilm activity of the fraction AO is equivalent to that of its crude extract.

Figure 4.9. Antifungal activities of G9d fractions (AO, BO and O) along with the crude extract
Figure 4.10. Antibiofilm activities of G9d fractions (AO, BO and O) along with the crude extract

4.9. Anti-pathogenic potential of G9d

Since the fraction AO significantly reduced the biofilm formation in C. tropicalis, the fraction was further tested for its ability to reduce the production of a virulence factor, protease secretion. The production of protease was assessed by using bovine serum albumin (BSA) as substrate in agar plates. Significant reduction of secreted protease was observed in C. tropicalis treated with the active fraction (AO) (Figure 4.11).

Figure 4.11. BSA agar plate depicting the production of protease (Zone around control colony) by C. tropicalis

Thus the result clearly demonstrates the ability of G9d to reduce the pathogenicity of C. tropicalis through reduction of virulence factors like protease and
biofilm formation. Since the proteolytic activity is predominantly due to the action of secreted aspartate proteinase (SAP) enzymes (Kantarcioglu & Yucel, 2002), it is believed that the metabolite of *K. marina* has the ability to reduce the expression of SAP genes that plays a decisive role in the virulome of *C. tropicalis*. Inhibition of SAP activity by actinomycete was reported from *Streptomyces* sp. by its hexapeptide pepstatin A (Fallon et al., 1997). Since the SAP enzymes play key role in *C. albicans* as well as in other non-*Candida albicans* Candida species (*C. glabata*, *C. lipolytica*, *C. parapsilosis, C. tropicalis*) (Kantarcioglu & Yucel, 2002; Silva et al., 2011), the active metabolite from *K. marina* could be used to control the infections caused by the members of genus Candida.

### 4.10. Identification of the active principle

The active fraction (AO) and the crude extract were analyzed through HPLC using silica C\(_{18}\) column. HPLC analysis revealed that the crude extract of G9d has been partially purified (Figure 4.12).

![HPLC chromatogram of the active fraction AO](image)

**Figure 4.12.** HPLC chromatogram of the a) G9d crude extract and b) active fraction AO
The constituents of the active fraction (AO) were identified by GC-MS analysis (Figure 4.13).

![GCMS chromatogram of the active fraction AO](image)

**Figure 4.13. GCMS chromatogram of the active fraction AO**

Analysis of the major peaks (by comparison with the NIST 2005 database), revealed the presence of 6 compounds with 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione as the major one that constitutes to about 40 %. Details of the peaks and corresponding compounds identified through GC-MS are tabulated (Table 4.3).

**Table 4.3. Volatile constituents of the active fraction (AO) of K. marina**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Retention time</th>
<th>Peak area (%)</th>
<th>Compound name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.1</td>
<td>11.9</td>
<td>N-(1-cyclohexylethyl)-propanamide</td>
</tr>
<tr>
<td>2.</td>
<td>14.1</td>
<td>7.5</td>
<td>2,7,10-trimethyl-dodecane (or) N-amidinophenylphthalimide</td>
</tr>
<tr>
<td>3.</td>
<td>16.9</td>
<td>6.8</td>
<td>3,5-dimethoxy-phenol</td>
</tr>
<tr>
<td>4.</td>
<td>18.3</td>
<td>5.4</td>
<td>3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Same as Sl. No. 6)</td>
</tr>
<tr>
<td>5.</td>
<td>18.9</td>
<td>23.72</td>
<td>N- cyclopropyl carbonyl-butylester-l-leucine</td>
</tr>
<tr>
<td>6.</td>
<td>19.3</td>
<td>37.23</td>
<td>Gancidin W (or) 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione</td>
</tr>
<tr>
<td>7.</td>
<td>20.5</td>
<td>7.45</td>
<td>Hexadecanoic, methyl ester</td>
</tr>
</tbody>
</table>

Among the 6 compounds (Sl. No. 4 & 6 are same), Gancidin W or 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione is the only compound with known
biological activity (Figure 4.14). The compound Gancidin W has been patented as a QS inhibitor with the ability to interfere with the bacterial cell to cell communication (Patent No.: US2010305182; US7642285). Hence, it is supposed that the antibiofilm and anti-pathogenic properties of G9d were due to the presence of Gancidin W alike compound.

![Structure of Gancidin W](image)

**Figure 4.14. Structure of Gancidin W**

Further purification and structure elucidation of the active fraction are warranted to come out with a novel agent with antibiofilm properties against *Candida* sp.

**Summary**

The potential of marine actinomycete to inhibit the biofilms of Candida species viz. *C. albicans* and *C. tropicalis* has been proven through the studies described in this chapter. An epiphytic actinomycete *Kocuria marina* G9d, associated with the seaweed *Gracilaria gracilis* was assessed for its antibiofilm activity against the human fungal pathogens *C. albicans* and *C. tropicalis* and found to have significant (more than 90%) antibiofilm activity against them. Apart from inhibiting the formation of biofilms, G9d reduced various other virulence traits like secretion of EPS and protease in *C. tropicalis*. Several studies have shown the clinical relevance of hyphal growth of pathogenic yeasts with disease onset and the ability of G9d to inhibit the hyphal growth increases its potential as an anti-pathogenic agent. Being resistant to various antibiotics, *C. albicans* and *C. tropicalis* are difficult to treat crafting them as a major threat in nosocomial infections. Application of novel therapeutics like antibiofilm/anti-pathogenic agents could be valuable in controlling the infections caused by these fungal pathogens.
Summary
At the end of 19th and start of 20th centuries, microbes were considered as beneficial organisms even though there are instances which reported their association with diseases. Even then, viruses were believed as life threatening pathogens compared to the bacterial, fungal and other protozoan pathogens. In the later part of 20th century numerous studies have revealed the severity of infectious diseases caused by bacterial and fungal pathogens. Developing countries like India have suffered more due to these infectious diseases and still these countries stand apart in disease management compared to the developed nations. Lack of knowledge, inaccurate diagnosis, abuse and overuse of drugs, self-medication and negligence are few reasons which paved way for these infectious diseases as life threatening ones in developing countries. Development of resistance is another predicament that aids in the transformation of an infection to a life threatening disease and in such instance as well, the developing countries are at the top as victims.

Efflux pumps, drug inactivating enzymes, target modifications, etc. are the phenotypes known for decades as classical reasons for drug resistance. But the development of advanced tools have revealed various other factors like the biofilm formation and population dependent cell to cell communication (quorum sensing, QS) in these pathogens that largely contribute to the development of resistance. Hence the interference of the phenotypes like biofilm formation and QS will be an attractive as well as handy strategy to control the diseases caused by resistant pathogens.

Though there are few antibiotics available today to control and cure the infections caused by bacterial and fungal pathogens, the need for novel drugs to combat drug resistance in a long run is huge. Knowing the importance of discovering novel drugs against multidrug resistant pathogens, numerous studies have been initiated to look for possible alternatives for antibiotics. With this backdrop, marine sources were explored for the identification of novel antibiofilm and anti-pathogenic agents as potential alternatives for antibiotics to combat infections caused by drug resistant pathogens. The study has been designed to screen various bacteria associated with diverse marine ecosystem like coral, sea water, seaweed, sea grass, marine sediment, mangrove rhizosphere soil, etc. for antibiofilm agents against Gram positive and fungal human pathogens and anti-QS agents against Gram negative human pathogens.

Diverse sources from synthetic agents to natural agents were studied with varying success rates. Natural sources have been of interest for centuries as they are
easily available, renewable and cost effective when compared to the synthetic agents. Marine bacteria gain more attention than their terrestrial counterparts as the rate of discovery of novel products from the later has declined significantly. Above that the information about marine microbes known today is as less as 0.1% that explains the room for in-depth studies. Epibiotic bacteria associated with higher marine organisms like corals and seaweeds were studied in particular as they were believed to play a key role in host defense against invading pathogens.

Bacteria associated with coral mucus were taken from the Department’s culture collection repository, Biobank. Sea water, seaweed, sea grass, marine sediment and mangrove rhizosphere soil were collected from the Karankadu mangrove forest region of Palk Bay. A total of 19 morphologically different coral associated bacteria and 149 bacteria (isolated from other marine samples) were screened primarily for anti-QS activity against the indicator organism *C. violaceum* ATCC12472. Nine bacteria from Gulf of Mannar and 37 from Palk Bay exhibited anti-QS activity against *C. violaceum* that is evident from the overlay assay where the QS regulated pigment, violacein’s production was found inhibited.

The positive isolates were further tested for their antibiofilm and anti-QS activities against individual human pathogens like *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *S. marcescens*, *C. albicans* and *C. tropicalis*. Coral associated actinomycete CAA-3 was tested for its antibiofilm activity against *S. aureus* and its drug resistant counterpart (MRSA) along with few other clinical isolates isolated from pharynx of pharyngitis patients. Various quantitative and qualitative experiments were done which revealed the antibiofilm potential of CAA-3 extract at concentrations as less as 100 µg/ml. Apart from reducing the surface area covered by the biofilms, CAA-3 also reduced the thickness of biofilms to about 50%. PCR amplification and sequencing of the 16S rRNA gene from CAA-3 revealed its identity as an actinomycete, *Streptomyces akiyoshiensis*.

During the screening process, a bacterium associated with the seaweed *G. gracilis* with unique pigmentation was observed. It produced a brown coloured highly diffusible pigment that fluoresces in orange under UV light. The bacterium was later identified as *Kocuria marina*, an actinomycete. The growth conditions were optimized for maximum pigment production and it was found that the production is independent of the nutritional source but highly dependent on temperature. Maximum
production was observed at temperatures around 30 ºC and incubation for 3-4 days. Further, the antibiofilm activity of the pigment was tested against *S. epidermidis* a pathogen associated with device associated nosocomial infections. The pigment reduced the biofilm as well as the production of EPS in *S. epidermidis* at a concentration of 150 µg/ml. Purification and structure analysis of the active principle containing the pigment revealed the presence of pterin-6-carboxylic acid and Perfluoroeicosane as the major components apart from the fatty acids which commonly found during GC-MS analysis. Till date there is no report available to explain the biological activity of pterin-6-carboxylic acid and perfluoroeicosane. The structure of perfluoroeicosane resembles that of the structure of autoinducer-2, the furanosyl borate diester leaving a hint that it could mimic the signalling molecule thereby preventing biofilm formation and the expression of other QS controlled genes.

The two coral associated bacteria CAB23 and CAB41 which displayed significant anti-QS activity were tested further against the Gram negative nosocomial pathogens *P. aeruginosa* and *S. marcescens*. The extracts of CAB23 and 41 were found to inhibit the formation of biofilms in both the pathogens, relatively at higher concentrations at around 1.5 – 2 mg/ml. Purification and assessing the activity of the pure compound could significantly reduce the required concentration. Biofilm thickness was also found to get reduced upon treatment with CAB extracts. Anti-QS activity of CAB extracts was evident from their inhibitory action on the production of QS regulated virulence traits like LasA Staphylolytic protease, LasB elastase, total protease, pyocyanin and pyoverdin in *P. aeruginosa*, and prodigiosin, protease, lipase and swarming motility in *S. marcescens*. The inert effect of CAB extracts on the cell surface hydrophobicity of the pathogens suggested that the biofilm inhibition could be because of their anti-QS activity and not by any other action like that of detergents and biosurfactants. PCR amplification and sequencing of the 16S rRNA gene from the CAB isolates revealed their identity as *Delftia tsuruhatensis* and *Stenotrophomonas maltophilia*. This is the first study to disclose the anti-QS potential of *D. tsuruhatensis* and *S. maltophilia*.

The potential of *K. marina* to inhibit the biofilm in *Candida* sp. viz. *C. albicans* and *C. tropicalis* has also been studied and found that the extracts of *K. marina* significantly (to about 90%) inhibit the biofilm formation in both the pathogens. Further, the extract of *K. marina* exhibited an unique potential of inhibiting
the hyphal development which is considered as a key phenotypic adjustment of Candida during infections in host. Fine structure analysis of the biofilm grown in the presence and absence of the extract using SEM revealed the inhibitory role of *K. marina* on EPS production. Further, the extract inhibited the production of protease, which is a major virulence factor produced by Candida that aids in host tissue damage and to combat the pathogocytes. Analysis of the active principle through GC-MS revealed the presence of a compound named Gancidin W which has been patented for its ability to interfere with the bacterial cell to cell communication.

Further studies to reveal the exact identity of the active compound are warranted for the development of these active compounds as drug candidates. *In vivo* testing in animal models is also indispensible to prove their efficacy in higher animals to proceed further with development and trials. However, this study on the whole highlights the potential of marine bacteria with special reference to actinomycetes as potential source for antibiofilm and anti-QS agents that are effective even against the drug resistant human pathogens. This study also unveils numerous leads from marine bacteria which could be further explored for many bioactive as well as industrially important agents.