Bioactive Materials 1 (2016) 140–150

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

# Molecular characterization and antibacterial effect of endophytic actinomycetes *Nocardiopsis* sp. GRG1 (KT235640) from brown algae against MDR strains of uropathogens

Govindan Rajivgandhi [a], Ramachandran Vijayan [a, b], Marikani Kannan [c], Malairaja Santhanakrishnan [d], Natesan Manoharan [a, *]

[a] Department of Marine Science, Bharathidasan University, Tiruchirappalli 24, Tamil Nadu, India
[b] School of Life Sciences, Jawaharlal Nehru University, New Delhi 67, India
[c] Department of Microbiology, VHNSN College, Virudunagar 01, Tamil Nadu, India
[d] Department of Marine and Coastal Studies, Madurai Kamaraj University, Madurai 21, Tamil Nadu, India

ARTICLE INFO

*Article history:*
Received 31 August 2016
Accepted 10 November 2016
Available online 25 November 2016

*Keywords:*
Endophytes
Multi-drug resistant strains
Urinary tract infections
Minimal inhibitory concentration

ABSTRACT

Our study is to evaluate the potential bioactive compound of *Nocardiopsis* sp. GRG1 (KT235640) and its antibacterial activity against multi drug resistant strains (MDRS) on urinary tract infections (UTIs). Two brown algae samples were collected and were subjected to isolation of endophytic actinomycetes. 100 strains of actinomycetes were isolated from algal samples based on observation of morphology and physiological characters. 40 strains were active in antagonistic activity against various clinical pathogens. Among the strains, 10 showed better antimicrobial activity against MDRS on UTIs. The secondary metabolite of *Nocardiopsis* sp. GRG1 (KT235640) has showed tremendous antibacterial activity against UTI pathogens compared to other strains. Influence of various growth parameters were used for synthesis of secondary metabolites, such as optimum pH 7, incubation time 5–7 days, temperature (30 °C), salinity (5%), fructose and mannitol as the suitable carbon and nitrogen sources. At 100 μg/ml concentration MIC of *Nocardiopsis* sp. GRG1 (KT235640) showed highest percentage of inhibition against *Proteus mirabilis (85%), and E.coli, Staphylococcus auerues, Psuedomonas aeroginasa, Enterobactor* sp *and Coagulinase negative staphylococci* 78–85% respectively.


## 1. Introduction

Urinary tract infections (UTIs) are the bacterial infection that affects of all age group to any part of the urinary tract. It is the second most common infectious presentation in community medical practice. It affects the group of all age people. 50 Million People are affected by UTI each year in all over the world [1]. The risk of developing UTI infection in diabetic patients is higher. Diabetic cystopathy and micro-vascular diseases may cause changes in host defense mechanism of kidney leads to higher incidence of UTI [2]. UTIs patients are susceptible to cause emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis. Bacteraemic patients have more chance to develop acute renal failure [3].The gram-negative bacteria play an important role in UTI and the most common causative agent is *Escherichia coli* (75–90%) [4,5]. The other gram negative bacterial pathogens causing UTI are *klebsiella* sp., *Proteus mirabilis* and *Pseudomonas aeruginosa*. However, the Enterococci and *coagulase negative Staphylococci* are the most predominant gram positive bacteria also present in UTI [6]. Multi drug resistant pathogens are the major issue in health care industry. In general, the natures of antibiotic susceptibility of UTI causing pathogens have been differing from various environmental conditions in both community and hospitals surroundings [7,8]. Rapidly growing drug resistances in pathogens are one of the major problems to treat diseases like malaria, tuberculosis, diarrheal diseases and UTI etc [9,10]. "Some new approaches [11,12] and challenges [13,14] related to drug resistant pathogens have been recently reported but further studies are much needed." In order to identify the novel potential

* Corresponding author. Department of Marine Science, Bharathidasan University, Trichirappalli 24, Tamil Nadu, India.
*E-mail address:* biomano21@yahoo.com (N. Manoharan).
Peer review under responsibility of KeAi Communications Co., Ltd.

http://dx.doi.org/10.1016/j.bioactmat.2016.11.002

inhibitor molecules against Multi drug resistant microbes are essential to eradicate the UTI causing pathogens.

The 70% of the earth surface are made of marine environment. At this condition, where the microorganisms are synthesized many different bioactive secondary metabolites. The ocean has an unexploited source for many potential drugs and secondary metabolites [15]. As marine environment has vast difference than terrestrial nature. Marine actinomycetes have the capability to synthesize various types of secondary metabolites at extreme salinity, stress and temperature. Only marine actinomycetes can able to synthesize active compounds against various diseases than terrestrial nature [16]. Actinomycetes are gram positive, filamentous have the ability to produce novel bioactive compounds such as antibiotics, vitamins, herbicides, pesticides, anti-parasitic and enzymes plays a major role in therapeutic applications and are active against many pathogenic microorganisms [17,18].

Today, the developing of effective antimicrobial agents is the major challenge to the health care industry, especially immunocompromised patients and multi drug resistant pathogens. Among the all known actinomycetes especially *Streptomyces* and *Nocardiopsis* species have been excellent bioactive secondary metabolites producer of antimicrobial, anti parasitic, antitumor and antiviral agents [19,20]. The isolation of new compounds by rotation from terrestrial sources is decreasing because of known compounds identification. Therefore, it is the urgent need to discover new group of bioactive metabolites from various marine sources. In particular, the endophytic actinomycetes from various marine plants and algae association and its secondary metabolites were not studied. Hence, our current study was focused on isolation and characterization of endophytic actinomycetes from brown algae for screening of antibacterial compounds against multidrug resistant uropathogens.

## 2. Materials methods

### 2.1. Collection of samples

The two young healthy brown macro algae *Turbinaria ornata, and Sargassum wightii* were collected from Gulf of Mannar region (Latitude 9°15'41.88″N, Longitude 79°04'05.81″E), Rameswaram, Southeast coast of Tamil Nadu, India. The collected algae samples were covered by sterile plastic bags to avoid contamination. The samples were kept in ice box and taken to the laboratory immediately. The collected samples were washed thoroughly with distilled water for removal of the free floating organisms and epiphytes and 70% ethanol was also used for surface sterilization. The samples were air dried and stored for further study.

### 2.2. Isolation of endophytic actinomycetes

Isolation and screening of endophytic actinomycetes were determined by selective media method. The algal samples were aseptically cut into small pieces (10 mm), and macerated with sterile distilled water by using mortar and pestle. The macerated samples were serially diluted up to $10^{-7}$. About 0.1 ml of the samples was spread on the sterile starch casein agar and actinomycetes Isolation Agar (AIA) (HiMedia laboratories Pvt. Mumbai, India). The plates were incubated at 28 ± 2 °C for 7–10 days. After incubation, the growth of endophytic actinomycetes were observed in the plates and stored in Starch Casein Agar (SCA) medium for further use [21].

### 2.3. Validation of endophytic actinomycetes

To prove the isolated actinomycetes were arisen from internal tissue of the host samples finger prints of the surface sterilized tissues were validated by using International Strptomyces Project Medium (ISP) agar plates and incubated for 28 °C. The tissues of algae samples were soaked in water for 2 min with continuous stirring after the three time distilled water sterilization. The last wash sample 0.1 ml was taken and inoculated in ISP 2 media. After incubation, if no microbial growth was observed on the agar plates, the sterilization was considered as very effective [22].

### 2.4. Test organisms

The multi drug resistant strains of uropathogens [23,24] such as *E.coli, Proteus mirabilis, Pseudomonas aeroginosa, Klebsiella pneumonia, Enterobacter* sp., *Staphylocoous aereus, Coagulase-negative Staphylococci, Candida albicanes* were obtained from Medical Microbiology unit, Department of Microbiology, Periyar University, Salem - 11. The resistances against fourth generation cepholosphorin strains were determined by disc diffusion method. The Collected uropathogens were maintained in glycerol stock and stored at −20 °C for future use.

### 2.5. Primary screening and antagonistic activity

The Primary screening of antimicrobial activity was determined by conventional cross streak method [24].The isolated strains were streaked across the diameter on Muller Hinton Agar (MHA) plates. The plates were incubated at 28 °C for 3–4 days. After observing the fine growth of the strain, the 24 h cultures of uropathogens were streaked perpendicular to the angle of central strip of the actinomycetes culture. All plates were incubated at 37 °C for 24 h. After 24 h, the antagonistic activities of the highly potential strains were observed based on the zone of inhibition. The broad spectrum activity of highly potential *Nocardiopsis* sp. GRG1 (KT235640) culture filtrate was added with equal volume of five different solvents (alcohol, dichloromethane, ethyl acetate, chloroform, and methanol) and shaken for 1hr.The antimicrobial activity of extracted filtrates was performed against test pathogens using well-diffusion method [25].

### 2.6. Extraction of bioactive compounds from Nocardiopsis sp. GRG1 (KT235640)

The antimicrobial compounds of *Nocardiopsis* sp. GRG1 (KT235640) was recovered from the filtrate by active solvent of ethyl acetate extraction method followed by Ref. [26]. The *Nocardiopsis* sp. GRG1 (KT235640) was inoculated with starch casein nitrate (SCN-B) broth (50% seawater and 50% distilled water), and the broth was incubated at 28 °C for 7–15 days. After fermentation, the broth was centrifuged at 10,000 rpm for 10 min and the supernatant was collected and filtered by Whattman No.1 filter paper. The pellet and cell free supernatant were collected separately for further use. Ethyl acetate was added with filtrate of supernatant in the ratio of 1:1(w/v) and shaken vigorously 1hr for complete liquid-liquid extraction. The organic phase was separated from aqueous phase were collected using separating funnel and evaporated with water bath at 40–50 °C. After evaporation, the dried crude compounds were collected and determined the antimicrobial activity against MDRS of UTI pathogens by agar well diffusion method.

### 2.7. Secondary screening

The antimicrobial activity of *Nocardiopsis* sp. GRG1 (KT235640) were performed by against test pathogens [24] at regular intervals (24 h, 48 h, 96 h) using well-diffusion method [25]. The isolated [23] UTIs pathogens were spread on MHA plates using sterilized

cotton swab. Each of the plates were cut the well by using gel borer and five different concentrations viz. 15, 25, 50, 75,100 μl of the extract was added separately into each well. The ethyl acetate extract act as a control. The plates were incubated at 37 °C for 24 h. After 24 h all the plates were observed for the zone of inhibition around the wells.

### 2.8. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of antimicrobial compound of *Nocardiopsis* sp. GRG1 (KT235640) was determined against MDRs of UTIs pathogen (24) by micro broth dilution method [27] and the results of MIC was determined by spectrophotometer using microtiter plate. The various concentration (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/ml) of ethyl acetate extract of *Nocardiopsis* sp. GRG1 (KT235640) was used to identified the MIC value against MDRS of UTI pathogens. The solution was transferred into the first well of a 96-well plate (Himedia laboratory, India) before filled with 100 μl of tryptone soya broth. The two fold serial dilution was done in to 11 following wells. 95 μl of sterilized fresh tryptone soya broth and 5 μl of 24 h bacterial culture were added. The final volume of each well contained100 μl. The without extract in the test pathogen of control wells were also prepared. Each plate was mixed well and then the plates were incubated at 37 °C for 24 h. After 24 h, no visible growth was observed in the plate. Lowest concentrations of the extract were indicated as MIC [28] and the plates were read with UV spectrophotometer at 570 nm and percentage of inhibition was calculated by using the following formula

Percentage of inhibition:

$$\frac{(\text{Control OD 570 nm} - \text{Test OD 570 nm})}{\text{Control OD 570 nm}} \times 100$$

### 2.9. Biochemical characterization of endophytic actinomycetes isolates

The biochemical characterization of Indole, MR, VP, Citrate, $H_2S$ Urease, Oxidase test with *Nocardiopsis* sp. GRG1 (KT235640) was performed by various broth and slant cultures (ST 1). The procedure was followed by Ref. [34].

### 2.10. Genomic studies of potential strain

The isolation of Genomic DNA from endophytic actinomycetes was followed by Ref. [29]. Amplification of 16S rDNA by using Universal primers. (Actino specific forward Primer -5'-GCCTAACACATGCAAGTCGA-3' and Actino Specific reverse primer - 5'-CGTATTACCGCGGCTGCTGG-5') followed by Ref. [30]. 35 cycles was performed. Detection of PCR amplification using agarose gel electrophoresis after ethidium bromide staining. Then PCR product was sent to sequencing by automated sequenced method (Eurofins Genomics India Pvt Ltd). The same primers as reported above were used for sequencing. Further, NCBI-BLAST [www.ncbi.nlm.nih.gov/blast] was used to compare the sequence similarity of isolated endophytic actinomycete strain with reference actinomycetes strains. The 16S rDNA a sequence of actinomycetes was deposited in NCBI and the sequences accession number was obtained. Reference sequence was downloaded from the Genbank Database (http://www.ncbi.nlm.nih.gov/genbank). Both the sequences were aligned by using the multiple sequence alignment program CLUSTAL W [31]. The gaps were identified manually from the aligned sequences and arranged in a block of 250bp in each row and as an input format in software MEGA V 2.1. The pair wise evolutionary distances were performed using the Kimura 2-parameter model [32]. In order to obtain the confidence values for bootstrap analysis, the original data set was re-sampled toll, 1000 times by using the bootstrap program of Phylogeny. The bootstrapped data set was used to build the phylogenetic tree by using the MEGA software. The resulted multiple distance matrixes was then used to construct phylogenetic tree using Neighbour Joining method [33].

### 2.11. Phenotypic characterization

The morphology and biochemical observation of isolated colonies are important for taxonomy of actinomycetes. Gram staining, biochemical characterization, aerial mass color, reverse side pigment, melanoid pigments, spore chain morphology, and some minerals such as carbon, nitrogen sources [35,36] were performed to determine the taxonomy of actinomycetes.

#### 2.11.1. Aerial mass color

The aerial mycelium is one of the important characters for identification of isolated endophytic actinomycetes. The isolated strains were inoculated on the starch casein nitrate agar (SCN) plates and the plates were incubated at 28 °C for 6–7 days. After incubation the nature of the actimycetes studies were observed. Basically, color of the matured spore forming aerial mycelium is white, red, grey, blue and violet. Sometimes the aerial mycelium is also present in combination of two colors. So, both the colors were also recorded. Sometimes aerial mass color of a strain showed intermediate tints, and then also, both the color series should be noted.

#### 2.11.2. Reverse side pigments

The strains were classified into the following two categories based on their ability to produce characteristic of pigments on the reverse side of the colony known as distinctive (Positive) and not distinctive or none (Negative). Pale yellow of chroma and yellowish brown color of the growth were recorded as positive (P) and no color of the plates were recorded as negative (N).

#### 2.11.3. Melanoid pigments

The isolated colonies were inoculated on the ISP-5 plates and the plates were incubated at 28 °C for 4–5 days for identification of the melonoid pigmentation. After the incubation period, the positive strains of the cultures showed greenish brown, brown to black diffusible pigment or a distinct brown pigment modified by other color are recorded as positive (P). The absence of the pigment plates were recorded as negative (N).

### 2.12. Stress tolerance of endophytic actinomycetes isolates

The identification of stress tolerance observation is most important for the studies of native strains of actinomycetes. The ability to check the various stress tolerance (Different concentration of NaCl, pH, and temperature) of isolated strains (ST 2) were studied by Ref. [37].

#### 2.12.1. Effect of salinity

Various concentrations of (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50%) NaCl solutions were added to the starch casein broth. The actinomycetes strains were inoculated into the broth and incubated at 28 °C for 7–15 days. After incubation, the positive and negative growth of the broth was observed and the antimicrobial activity of the positive growth of the extract was tested against uropathoges.

*2.12.2. Effect of temperature*

The actinomycetes strains were streaked over the actinomycetes isolating agar plates and the plates were incubated at various temperatures at 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C for 7–15 days. After incubation the positive and negative growth of the plates were identified. Based on the temperature, the potential activities of the extracts were performed against uropathogens by agar well diffusion method.

*2.12.3. Effect of pH*

The pH of the SC broth was adjusted to (4, 5, 6, 7, 8 and 9) with 0.1N NaOH/0.1 N HCl. The entire flask were inoculated with various strains of endophytic actinomycetes culture and incubated at 28 °C for 7 days. After incubation the positive and negative indication of the culture were determined. The active strains of the culture were performed the antimicrobial activity against uropathogens by agar well diffusion method.

*2.12.4. Assimilation of carbon sources*

The utilization of various carbon sources of different endophytic actinomycetes strains were performed by the method was followed by Ref. [38] and recommended in International *Streptomyces* Project Medium (ISP2). The stock solution containing the 10× concentration of different carbon sources i.e., xylose, inositol, sucrose, raffinose, fructose, rhamnose and mannitol were prepared on double distilled water and filtered by using 0.22 μm pore size membrane filter and stored at 4 °C for further use. The strains were streaked with 1% of carbon sources (1%) containing sterile ISP2 medium and the plates were incubated at 28 °C for 7–15 days. The growth of the actinomycetes were identified depending on the utilization of carbon sources and positive growth of the results were called as positive (P), if no growth occurs it is referred as negative (N). The influence of active carbon compounds growth of the active strains was used to check their antimicrobial activity by well diffusion method.

*2.12.5. Assimilation of nitrogen sources*

The utilization of various nitrogen sources of different endophytic actinomycetes strains were studied by the method was followed by Ref. [39] and recommended in International *Streptomyces* Project medium (ISP2). The stock solution containing the 10× concentration of various nitrogen sources i.e., L-Argine, L-Coralline, L-Histidine, L-Glysine, L-Lysine and L-Proline were prepared with double distilled water and filtered by using 0.22 μm pore size membrane filter at 4 °C for future use. Strains were streaked with 1% of the nitrogen sources containing sterile ISP2 medium at 28 °C for 7–15 days. The growth of the actinomycetes were identified depending on the uptake of nitrogen sources and the results were called as positive reactions (P), if no growth occurred they were referred as negative reaction(N). The influences of active nitrogen compounds growth of strains were used to check antimicrobial activity by well diffusion method.

## 3. Statistical analysis

The experiments were carried out independently in triplicate with pooled samples of biological replicates. Statistical analysis was performed using SPSS. Values were expressed as mean + SD. A Duncan-ANOVA test with a p-value of 0.001 being highly significant and to compare the parameters between the groups [40].

## 4. Results

*4.1. Isolation and identification of endophytic actinomycetes*

The healthy leaves of two brown algae (Fig. 1) were collected from Gulf of Mannar region Rameswaram, Tamil Nadu, South East coast of India. In validation, no microbial colonies were observed in the ISP 2 plates and the result noticed that the sterilization was good. After validation, the 100 pure ribbons like powdery white color colonies of endophytic actinomycete were isolated from the two algal samples grow on SCA medium and AIA medium respectively. The isolated strains were recorded in (Fig. 2). Approximately 40% (40 isolates) of the endophytic actinomycetes strains were observed with good antimicrobial activity against various clinical pathogens. These active strains were further studied for the production of bioactive compounds and the strains were identified by gram staining, biochemical, physiological characterization, and genomic studies. From validation, the result proved the isolated actinomycetes were recovered from internal tissues of the algae (Data not Shown).

*4.2. Primary screening and antagonistic activity of isolated endophytic actinomycetes against MDRS of UTI infection*

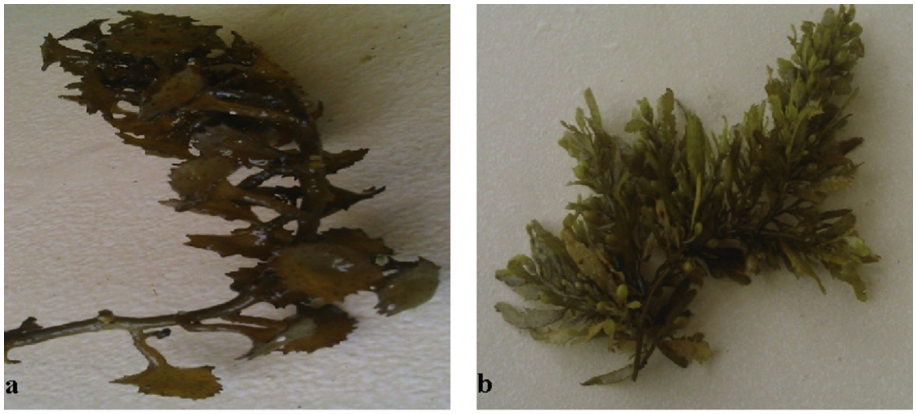
The multi drug resistant effect of pathogens was screened against fourth generation of cephalosporin (Ceftazidime) and the result confirms the pathogens were multidrug resistant using disc diffusion agar well diffusion method (SF. 1). In the primary screening, the antagonistic activities of 40 isolated strains were determined for antimicrobial activity against various multi drug resistant uropathogens. Among the 40 strains, 10 strains (first five strains from *Turbinaria ornata,* and second five strains from (*Sargassum wightii*)) were showed comparatively better antagonistic activity (Table 1). They also showed minor discrepancy in relation to different strains and test organisms. Interestingly, *Nocardiopsis* sp. *GRG1* (KT235640) showed relatively better antibacterial activity against all isolated UTI pathogens (20) than other nine strains and this *Nocardiopsis* sp. *GRG1* (KT235640) strain was chosen for further studies.

*4.3. Extraction and antimicrobial activity of secondary metabolites from Nocardiopsis sp. GRG1 (KT235640)*

The potential strain of *Nocardiopsis* sp. GRG1 (KT235640) showed excellent antibacterial activity was selected and inoculated into starch casein broth for 4–7 days at 28 °C. After 7days, the secondary metabolites were extracted by different polarity solvents and the extracts were further screened for antimicrobial activity against multi drug resistant strains of UTI pathogens. However, only ethyl acetate extract (SF. 2) of the *Nocardiopsis* sp. GRG1 (KT235640) has shown good activity against all the test pathogens except *CoN. Staphylococci.* The zone of inhibition was18 mm for *E. coli,* 15 mm for *P. aeroginosa, 14 mm* for *K. pneumonia,* 13 mm for *Enterobacter,* 15 mm for *S. aereus* and 30 mm for *P. mirabilis* were observed (Fig. 3A). No zone of inhibition was observed in the control well. When compared with other solvents, ethyl acetate extract of *Nocardiopsis* sp. GRG1 (KT235640) showed good activity and the zone of inhibition were presented in (Table 2).

*4.4. Minimum inhibitory concentration (MIC)*

Minimum Inhibitory Concentration (MIC) is referred as the highest dilution or least concentration of the extract that inhibit growth of organisms. MIC is an important parameter that helps to determine the activity of newly discovered compounds against




a) *Turbinaria ornata*, b) *Sargassum wightii*

**Fig. 1.** Collection of brown Algae from Gulf of Mannar Region.

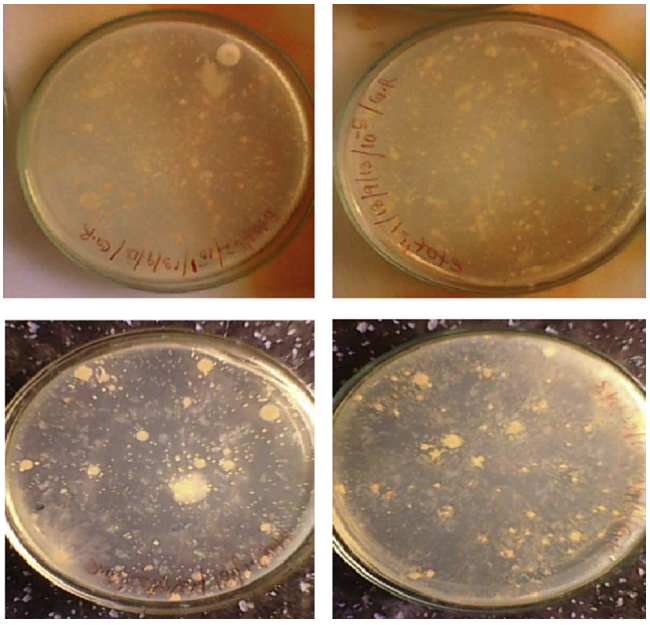


**Fig. 2.** Isolation of endophytic actinomycetes.

various types of pathogens. The ethyl acetate extract of the *Nocardiopsis* sp. GRG1 (KT235640) showed 80% inhibition against MDR strains of *P.mirabilis, E.coli, P.aeruginosa, S.aereus, Enterococcus* at and 73% inhibition against *K.pneumonia and* 77% inhibition against *CoN. Staphylococci* were observed at a concentration of 100 μg/ml. The treatment of *Nocardiopsis* sp. GRG1 (KT235640) was decreased the pathogenic effect in MDRS by concentration-dependent. This extract revealed a maximum inhibition (78 and 80%) against most of the uropathogens (Fig. 3B) at the same concentration (100 μg/ml). Hence, 100 μg/ml was chosen for further study. The statistical analysis [ST3] reveals that the extract was very efficient against uropathogens by comparing between test pathogens and control.

### 4.5. Biochemical characterization of endophytic actinomycetes isolates

The highly active principle of the *Nocardiopsis* sp. GRG1 (KT235640) was characterized by various biochemical tests with broth and slant cultures. After 24 h incubation, the MR, citrate, urease, catalase, oxidase were observed as positive as well as Indole, VP, and $H_2S$ were observed as negative. [ST4].

### 4.6. Genomic studies of potential strains

The 16S rDNA sequence of the *Nocardiopsis* sp. GRG1 was processed (GC content 57%) and deposited in the Genbank (NCBI) with the Accession number: KT235640. The phylogenetic tree analysis showed that the 347bp sequence has the highest homology (98.5% identity) with the *Nocardiopsis* sp. GRG1 (KT235640) (Fig. 4).

### 4.7. Phenotypic characterization

The phenotypic characterizations of 10 active strains of endophytic actinomycetes strains were studied with aerial mass color, reverse side pigments, melanoid pigments, carbon, nitrogen

**Table 1**
Identification of endophytic actinomycetes.

| S.No | Strains | Name of the organisms and accession number | Antagonistic activity |
|---|---|---|---|
| 1 | GRG1 | *Nocardiopsis* sp. GRG1 (KT235640) | Good Activity |
| 2 | GRG 2 | *Nocardiopsis* sp. GRG 2 (KT235641) | Good Activity |
| 3 | GRG3 | *Nocardiopsis* sp. GRG 3 (KT235642) | No Activity |
| 4 | GRG 4 | Submitted | No Activity |
| 5 | GRG 5 | Submitted | Poor Activity |
| 6 | GRG 6 | Submitted | No activity |
| 7 | GRG 7 | Submitted | Good Activity |
| 8 | GRG 8 | Submitted | Poor Activity |
| 9 | GRG 9 | Submitted | No Activity |
| 10 | GRG 10 | Submitted | No Activity |

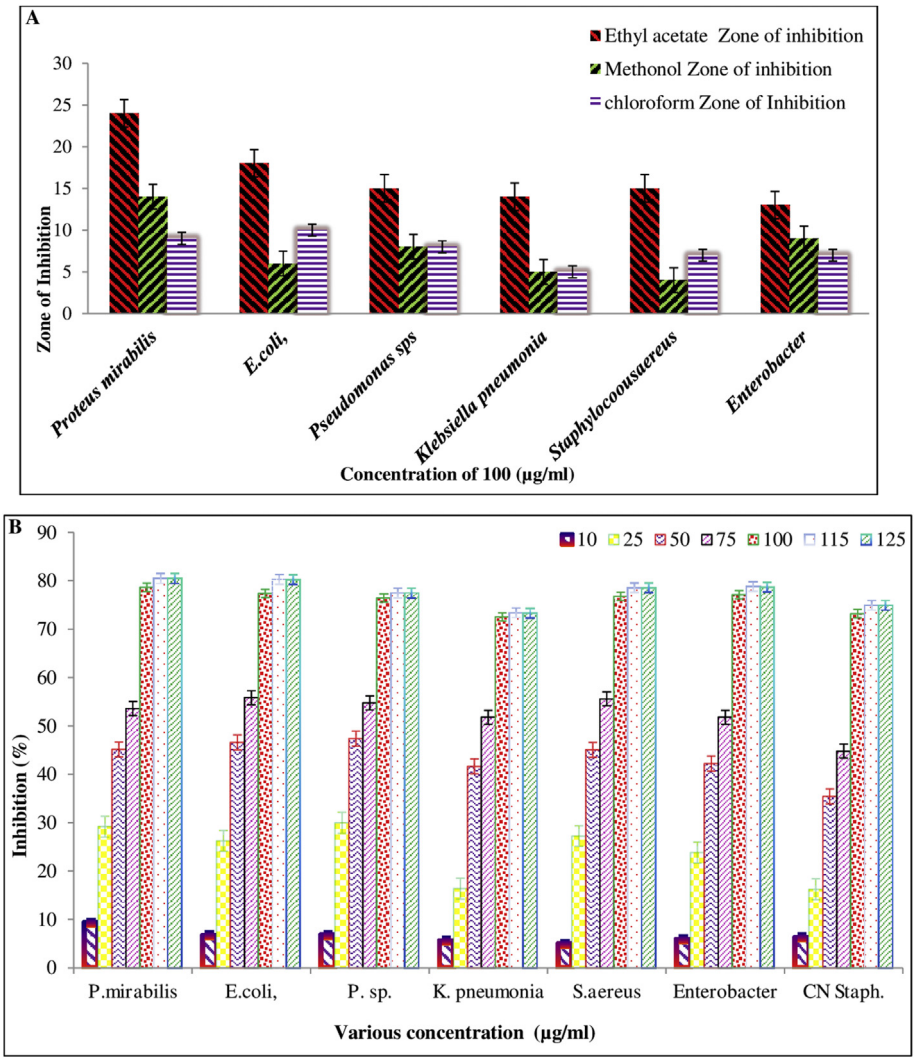



**Fig. 3.** (A). Antimicrobial activity of *Nocardiopsis* sp. GRG1 (KT235640) against MDRS of uropathogens. (B). Percentage of inhibiton by Minimum Inhibition Concentration (MIC) against Multi drug resistant strains (MDRs) of Uropathogens.

**Table 2**
Antimicrobial activity of *Nocardiopsis* sp. GRG1 (KT235640) against UTIs.

| S.No | Pathogens | Ethyl acetate extract | Methanol extract | Chloroform extract | Control (Ethyl acetate) |
|---|---|---|---|---|---|
| | | Zone of inhibition (mm) | Zone of inhibition (mm) | Zone of inhibition (mm) | Zone of inhibition (mm) |
| 1 | E.coli | 18 | 6 | 10 | – |
| 2 | *Proteus mirabilis,* | 24 | 14 | 9 | – |
| 3 | *Pseudomonas aeruginosa* | 15 | 8 | 8 | – |
| 4 | *Klebsiella pneumoniae* | 14 | 5 | 5 | – |
| 5 | *Staphylocoous aureus* | 15 | 4 | 7 | – |
| 6 | *Enterobacter* | 13 | 9 | 7 | – |

sources and spore chain morphology. The identification of colors (Fig. 5B- a.f) was recorded in (Table 3).

*4.7.1. Aerial mass color*

After 5–7 days of incubation of 10 active strains, the white and grey color colonies were observed in all strains of the SCA plates and the heavy spores of the mycelia growth was also observed in all the SCA plates. These results demonstrate the common characteristics of actinomycetes (Fig. 5A a.e).

*4.7.2. Reverse side pigments*

The isolated endophytic actinomycetes strain ofGRG2, GEG3, GRG6 and GRG9 (Fig. 5B c.e) were produced yellow color pigmentation and the growth was also called as a positive or distinctive character. The other plates did not produce any pigmentation in their growth and were called as a non-distinctive or negative character.

*4.7.3. Melanoid pigments*

The strains of GRG1 and GRG2 were observed with greenish

brown to black diffusible pigment production and the strains were also called as positive producer. Rest of the other strains did not produce any pigment (Fig. 5B a.b) in their nature and they were called as negative producer. The positive and negative growth of the substrate mycelium and aerial mycelium were also studied with all the strains and the results were recorded in above.

### 4.8. Optimization of endophytic actinomyctes

The effect of salinity, temperature and pH of the isolated strains were evaluated by using various concentrations (0 μg/ml, 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml, 25 μg/ml, 30 μg/ml, 35 μg/ml, 40 μg/ml, 45 μg/ml, 50 μg/ml, 55 μg/ml, 60 μg/ml, 65 μg/ml, 70 μg/ml, 75 μg/ml) respectively.

#### 4.8.1. Effect of salinity

After 6–15 days of incubation, the isolated strains of GRG1, GRG2, GRG4, GRG6, and GRG9 showed increase in growth and turbidity at 5% concentration. Some strains were also grown in the same concentration and moderate level at 7.5% concentration. None of the strains were exhibited growth in minimum concentration of 5–7%. Among these isolates, the active strain of GRG1 showed better antimicrobial activity against uropathogen than other isolates at the concentration of 5% [ST5].The other concentrations of this strain did not produce better activity (Fig. 6A).

#### 4.8.2. Effect of temperature

The effect of temperature is one of the most important factors used for the identification of actinomycetes. A different type of endophytic actinomycetes was isolated depends on the temperature level. Among the10 isolates, all the strains were easily grown in the temperature of 25°C-45 °C (SF. 3A) and some strains were able to grow at 55 °C. The strains were observed with moderate and fair level of the growth at 40°C-50 °C. Few strains (GRG8, GRG9) were did not grow in the temperature of 55 °C [ST6]. The excellent activity of *Nocardiopsis* sp. GRG1 (KT235640) extract was observed at 30 °C and other temperatures were not perfect for production of potential antimicrobial compounds the picture was shown in (Fig. 6B).

#### 4.8.3. Effect of pH

All the isolated strains were identified using various pH levels (4–9). Almost all the strains were easily grown at the level of pH (5–8) (SF. 3B). Few strains were not grown at pH 4. Hence, the potential antimicrobial compounds of *Nocardiopsis* sp. GRG1 (KT235640) against uropathogens were synthesized at pH7 [ST7] and the acetic pH has not able to produce any active metabolites. The picture was shown in (Fig. 6C).

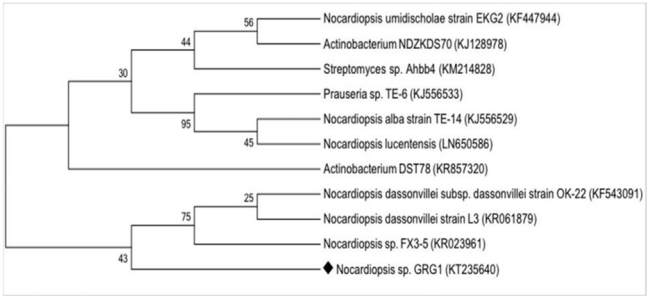



**Fig. 4.** Phylogenetic analysis of *Nocardiopsis* sp GRG1 (KT235640).

#### 4.8.4. Assimilation of carbon sources by the endophytic actinomycetes isolates

The utilization of carbon compounds indicates a good source of energy for all strains of endophytic actinomycetes. After 5 days of incubation, the fructose was determined as a major carbon compound for all the strains of endophytic actinomycetes. Because, all the strains were able to grown in fructose and absence in mannitol were observed (SF. 4A). The results were compared with positive as well as negative result of carbon utilization ability, the fructose showed highly positive to all isolates and the mannitol showed negative to all isolates [ST8]. Hence, the excellent activity of *Nocardiopsis* sp. GRG1 (KT235640) extract was observed in fructose containing broth. The image was shown in (Fig. 6D).

#### 4.8.5. Assimilation of nitrogen sources by the endophytic actinomycetes isolates

The ability to utilize to various nitrogen compounds is a source of energy for the isolated strains of endophytic actinomycetes and the isolates were performed by ISP-2. After 5 days of incubation, the L-Aspergine was determined as a major nitrogen compound for all the strains of endophytic actinomycetes (SF. 4B). Because, almost all isolated strains were grown well in L-Aspergine, and L-Proline was not suitable for all the strains and the compound was identified as low level. The growth were compared with positive and negative control, the L-Arginine was observed as highly positive to all the isolates and L-Proline was observed as negative for all the isolates and the results were recorded in [ST9]. Hence, the excellent activity of *Nocardiopsis* sp. GRG1 (KT235640) extract was determined in L-Aspargine containing broth. The picture was shown in (Fig. 6E).

Furthermore, the standard deviation of MIC, Temperature, pH, Carbon assimilation, Nitrogen assimilation were clearly indicates the statistically significant ($p < 0.001$) of endophytic actinomycetes and the value of correlation of MIC was noticed in table [ST 10].

## 5. Discussion

In our study, a total of 100 strains of endophytic actinomycetes were isolated from two brown algae. Among the 100 isolates, 40 isolates were more effective against clinical pathogens and 10 active strains were sequenced based on the respective order of broad spectrum of antimicrobial activity against uropathogens.

The antagonistic activities of GRG1, GRG2, GRG3, GRG4, GRG5, GRG6, GRG7, GRG8, GRG9 and GRG10 were identified in SC agar plates. Among these 10 strains, *Nocardiopsis* sp. GRG1 (KT235640) showed very good activity against gram negative bacteria than gram positive bacteria of UTI pathogens. The ethyl acetate extract of *Nocardiopsis* sp. GRG1 (KT235640) showed excellent activity and high zone of inhibition against *P. mirabilis* (30 mm), *P.aeroginosa* (15 mm), *E.coli* (18 mm), S. *aereus* (15 mm), *K. pneumonia* (14 mm) and the minimum zone of inhibition against *Enterobacter* sp (13 mm) were determined. The methanol extract showed the activity against *P. mirabilis* (14 mm), *P. aeroginosa* (8 mm), *E.coli* (6 mm), *S.s aereus* (4 mm), *K. pneumonia* (5 mm) and *Enterobacter* sp (6 mm) and Chloroform extract showed the activity against *P. mirabilis* (9 mm), *P. aeroginosa* (8 mm), *E.coli* (10 mm), *S. aereus* (7 mm), *K. pneumonia* (5 mm) and *Enterobacter* sp (7 mm) were observed [39]. Hence, the ethyl acetate extract was observed with better activity compared with other solvent extract. Our results were in accordance with the earlier findings of [20,41] and reported that *Nocardia brasiliensis* PTCC 1422 has showed significant antimicrobial activity against *P.mirabilis* (9 mm), *P. aeroginosa* (12 mm), E.coli (17 mm), K. pnemoniae (15 mm).

At the3rd day of incubation, the maximum and minimum antimicrobial activity of *Nocardiopsis* sp. GRG1 (KT235640) showed better antimicrobial activity against *Proteus mirabilis* (16 mm),

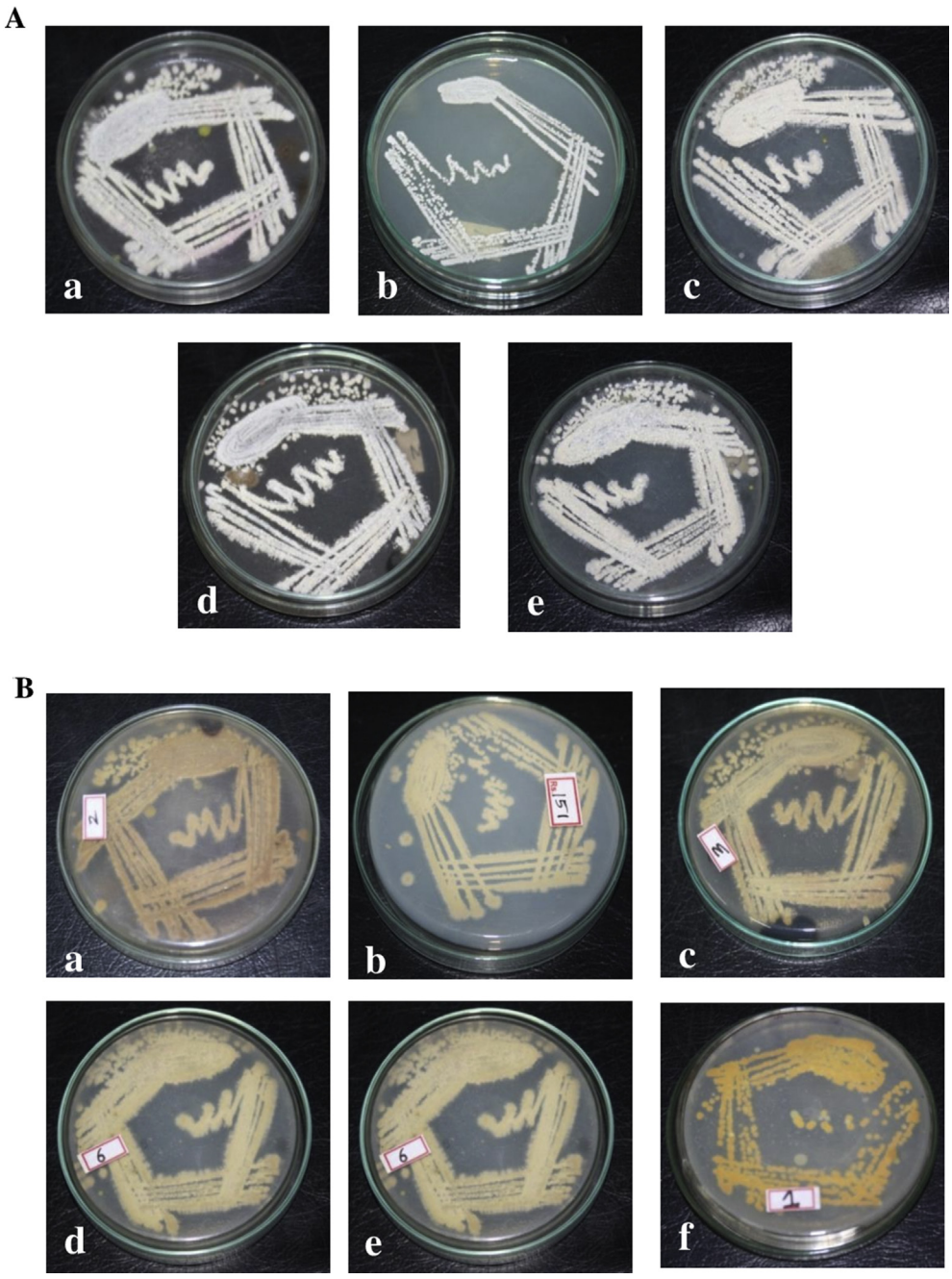



**Fig. 5.** (a). Aerial mass culture of endophytic actinomycetes strains (a.e). (b). Phenotypic characterizations of isolated endophytic actinomycetes strains (a–e).

**Table 3**
Phenotypic characterization of endophytic actinomycetes isolates.

| S.No | Isolates | Aerial Mass color | Melanoid pigments | Reverse side pigments | Aerial mycelium | Substrate mycelium |
|---|---|---|---|---|---|---|
| 1 | *Nocardiopsis* sp. GRG1 (KT235640) | W | N | p | P | P |
| 2 | *Nocardiopsis* sp. GRG 2 (KT235641) | W | P | P | P | P |
| 3 | *Nocardiopsis* sp. GRG 3 (KT235642) | G | P | P | P | P |
| 4 | GRG4 | G | N | N | P | P |
| 5 | GRG5 | W | N | N | P | P |
| 6 | GRG6 | W | P | N | P | P |
| 7 | GRG7 | W | P | P | P | P |
| 8 | GRG8 | G | N | N | P | P |
| 9 | GRG9 | G | P | N | P | P |
| 10 | GRG10 | W | N | P | P | P |

P: Positive Growth, N: Negative Growth, W: White, G: Grey.

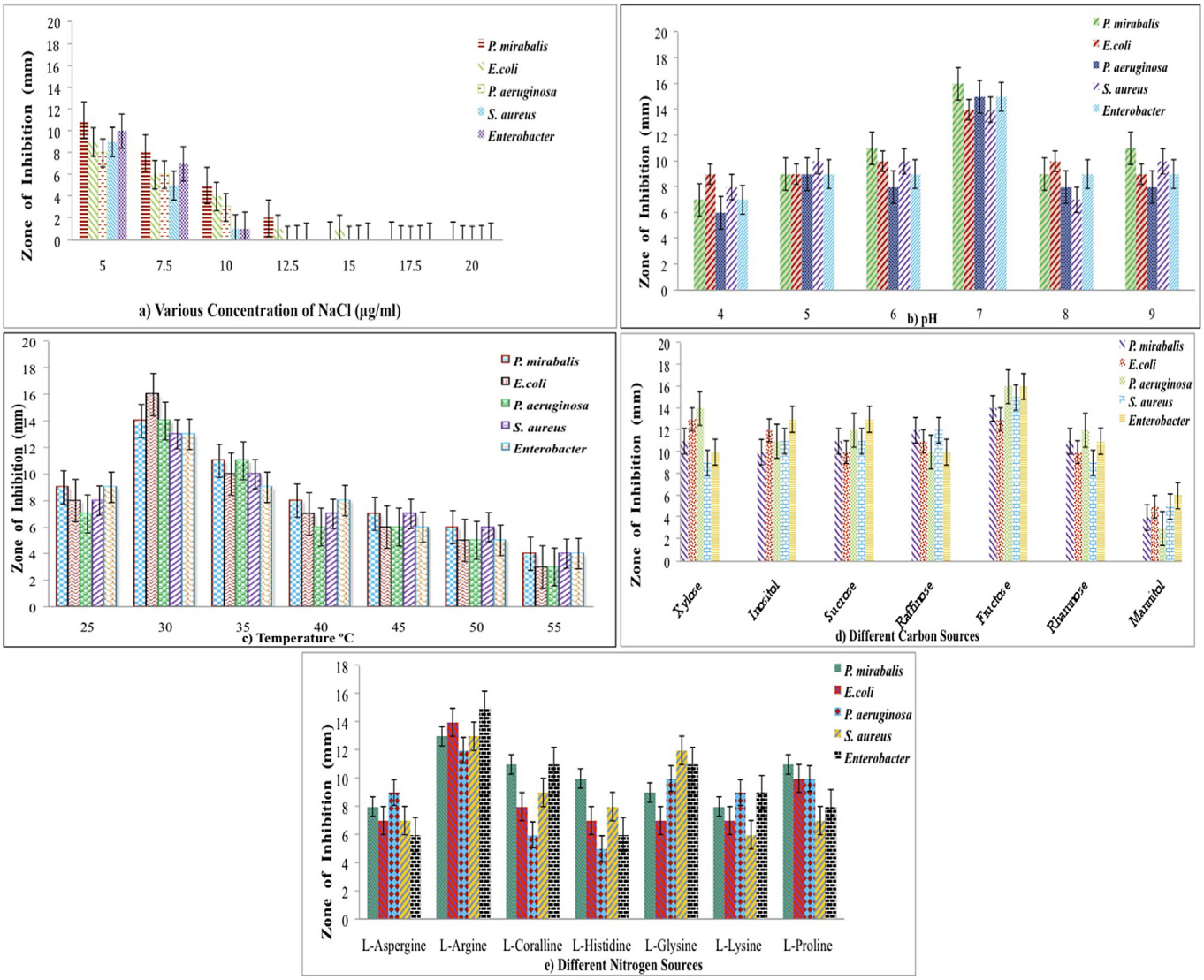



**Fig. 6.** (a). Sodium Chloride Tolerance on various endophytic actinomycetes Growth. (b). Effect of Different Temperature on various endophytic actinomycetes growth. (c). Effect of Different pH level on various endophytic actinomycetes growth. (d). Carbon utilization of isolated endophytic actinomycetes. (e). Nitrogen utilization of isolated endophytic actinomycetes.

*E. coli* (15 mm) and *P. aeroginosa* (14 mm). At the 7th day of incubation, the growth was increased and reached with excellent activity against these pathogens were observed. *Nocardiopsis* sp. GRG1 (KT235640) and *Nocardia brasiliensis* PTCC 1422 showed similar activity was observed at the 7th day of incubation. Our results confirm the study of [42].

MIC was used to determine the inhibition ranges of pathogen at various concentrations. MIC of a *Nocardiopsis* sp. GRG1 (KT235640) extracts has reduced the inhibition up to 77%–80% against *P. mirabilis, E.coli, S.aureus, Enterococcus and K.Pneumonia, CoN Staphylococcus* respectively. Our result confirms that the antimicrobial activity at the concentration of 100 µg/ml has showed better activity against UTI pathogens. Hence, 100 µg/ml was used for further analysis [43].

The aerial mass of the strains were observed with white color powdery growth (GRG1, GRG2, GRG5, GRG6, GRG7 and GRG10) in almost all the strains and few strains were showed whitest grey color (GRG3, GRG4, GRG8, and GRG9). These results were correlated with their finding [44,45].

Further, we have studied the effects of salinity by using various concentrations of NaCl with isolated strains. Almost all the strains were observed as negative at the concentration of 5–25%. Very few strains were shown as positive at the concentration of 5–7.5% because of their salt nature (low salt condition). Our results were similar to their work of [45,46].

The production of potential antimicrobial metabolites were depends on the temperature. Here, the determination of *Nocardiopsis* sp. GRG1 (KT235640) has showed excellent activity at 30 °C and no activity was observed at 5°C-10 °C. The isolated strain showed excellent activity against *P.mirabilis* (14 mm), *E.coli* (16 mm), *P. aeroginosa* (14 mm), *S. aureus* (13 mm) at 30 °C. Our results confirm the study of (48), that *Streptomyces afghaniensis VPTS3-1* was observed as highly active against *P.Valgaris* (20 mm) and *B.subtilis* (12 mm) at 30 °C.

Studying various levels of pH is one of the important parameter was used to synthesize the new secondary metabolites and antibiotic production [47].

In our study, at optimum pH-7, the potential secondary metabolites has produced excellent zone of inhibition against *P.mirabilis* (16 mm), *E.coli* (14 mm), *P. aeroginosa* (15 mm), *S.aureus* (14)

and *Enterobacter* (15 mm). Because, acidic condition is not suitable for production of potential antimicrobial compounds. The *Nocardiopsis* spp. TE1 and APA1 were grown well and produced potential antimicrobial compounds in optimum pH7. Our results were correlated with their findings of [19,48].

The carbon and nitrogen compounds utilization was one of the most important factors for identification of endophytic actinomycetes. Out of 10 active isolates, we have determined only fructose and raffinose are the main sources utilized by the strains GRG1, GRG2, GRG3, GRG5, GRG6, GRG7 and GRG10 as highly positive. GRG4, GRG8, GRG9 were observed as negative and Mannitol was found negative in most of the strains. For nitrogen utilization, the isolated strains were easily grown with L-Aspargin and most of the strains were not grown with L-proline. The similar results were observed with the nitrogen utilization ability were recorded [49]. But, the results were in comparison with [44], which was totally different and their work on the mannitol was the most assimilated carbon sources by all strains of the actinomycetes and the arabinose was least carbon sources utilized by all strains of actinomycetes [38].

## 6. Conclusion

Our findings, *Nocardiopsis* sp. GRG1 (KT235640) act as a major source of novel antibiotics against various types of pathogens mainly UTIs. Therefore, isolation, characterization and study of *Nocardiopsis* sp. GRG1 (KT235640) has been useful in discovery of novel compounds. The nature of marine environment, low salinity, optimum pH, high temperature and carbon-nitrogen content influences *Nocardiopsis* sp. GRG1 (KT235640) revealed tremendous antimicrobial activity against *Proteus mirabilis* and *Pseudomonas aeroginosa and* minimum zone of inhibition against *E.coli, Staphylococcus aereus, Kiebsiella pneumonia*. This study helps in designing drugs against multi drug resistant strains of urinary tract infections.

## Acknowledgment

The authors would like to thank for Mr. T.Santhanakrishnan, Geological and Biostatistical Laboratory, Department of Marine Science, Bharathidasan University for statistical analysis discussion. We gratitude to the Bharathidasan University, Tiruchirappalli-24 for University Research Fellowship (URF) (Ref. No. 05441/URF/K7/2013).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bioactmat.2016.11.002.

## References

[1] K. Guptha, T.M. Hoton, W.E. Stamm, Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections, Int. J. Antimicrob. Agents 135 (2001) 41–50.
[2] C. Sridhar, S. Anjana, J. Mathew, Acute infections, in: Text Book of Diabetes Mellitus, vol. 34, RSSDI, Hyderabad, India, 2002, pp. 471–478.
[3] A. Ronald, E. Ludwig, Urinary tract infections in adults with diabetes, Int. J. Antimicrob. Agents 17 (2001) 287–292.
[4] J.A. Karlowsky, L.J. Kelly, C. Thornsberry, M.E. Jones, D.F. Sahm, Trends in antimicrobial resistance among urinary tract infection isolates of Escherichia coli from female outpatients in the United States, Antimicrob. Agents Chemother. 46 (2002) 2540–2545.
[5] F.A. Nakhjavani, A. Mirsalehian, M. Hamidian, B. Kazemi, M. Mirafshar, F. Jabalameti, Antimicrobial susceptibility testing for Escherichia coli strains to fluoroquinolones in urinary tract infections, Iran. J. Public Health 36 (2007) 89–92.
[6] O. Omigie, L. Okoror, P. Umolu, G. Ikuuh, Increasing resistance to quinolones: a four-year prospective study of urinary tract infection pathogens, Int. J. Gen. Med. 2 (2009) 171–175.
[7] R.N. Gruneberg, Antibiotic sensitivities of urinary pathogens, J. Clin. Pathol. 33 (1980) 853–856.
[8] M.J. Saffar, A.A. Enayti, I.A. Abdolla, M.S. Razai, H. Saffer, Antibacterial susceptibility of uropathogens in 3 hospitals, Sari, Islamic Republic of Iran, 2002–2003, East Mediterr. Health. J. 14 (2008) 556–563.
[9] S. Manikandan, S. Ganesapandian, M. Singh, A.K. Kumaraguru, Antimicrobial Susceptibility pattern of urinary tract infection causing human pathogenic bacteria, Asian J. Med. Sci. 3 (2011) 56–60.
[10] J.R. Kerr, Antibiotic treatment and susceptibility testing, J. Clin. Pathol 58 (2005) 786–787.
[11] B. Li, B. Jiang, B.M. Boyce, B.A. Lindsey, Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections, Biomaterials 30 (2009) 2552–2558.
[12] B.M. Boyce, B.A. Lindsey, N.B. Clovis, E.S. Smith, G.R. Hobbs, D.F. Hubbard, S.E. Emery, J.B. Barnett, B. Li, Additive effects of exogenous IL-12 supplementation and antibiotic treatment in infection prophylaxis, J. Orthop. Res. 30 (2) (2012) 196–202.
[13] A.L. Armstead, B. Li, Nanomedicine as an emerging approach against intracellular pathogens, Int. J. Nanomed. 6 (2011) 3281–3293.
[14] T. Hamza, M. Dietz, D. Pham, N. Clovis, S. Danley, B. Li, Intra-cellular Staphylococcus aureus alone causes infection in vivo, Eur. Cells Mater. 25 (2013) 341–350.
[15] F.M. Rashad, H.M. Fathy, A.S. El-Zayat, A.M. Elghonaim, Isolation and characterization of multi functional Streptomyces species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt, Microbiol. Res. 175 (2015) 34–47.
[16] A. Kavitha, M. Vijayalakshmi, P. Sudhakar, G. Narasimha, Screening of Actinomycete strains for the production of antifungal metabolites, Afr. J. Microbiol. Res. 4 (2011) 27–32.
[17] J. Usha Rakshanya, N. Hema Shenpagam, D. Kanchana Devi, Antagonistic activity of actinomycetes isolates against human pathogen, J. Microbiol. Biotech. Res. 1 (2011) 74–79.
[18] V. Rambabu, S. Suba, S. Vijayakumar, Antimicrobial and anti proliferative prospective of kosinostatin — a secondary metabolite isolated from Streptomyces sp, J. Pharm. Anal. 5 (2015) 378–382.
[19] M.A. Atta, M.S. Ahmad, Antimycin - a antibiotic biosynthesis produced by Streptomyces Sp. AZ-AR-262: taxonomy, fermentation, purification and biological activities, Austral. J. Basic Appl. Sci. 3 (2009) 126–135.
[20] H.K. Jalali, A. Salamatzadeh, A.K. Jalali, H.H. Kashani, S.A. Asbchin, K. Issazadeh, Antagonistic Activity of Nocardia brasiliensis PTCC 1422 Against Isolated Enterobacteriaceae from Urinary Tract Infections, Probiotics Antimicrob Proteins 8 (1) (2016) 41–45, http://dx.doi.org/10.1007/s12602-016-9207-0.
[21] S. Krishnakumar, Isolation and Standardization of Antimicrobial Compounds from Sponge Associated Antagonistic Actinomycetes, Ph.D. thesis, Manonmaniam Sundaranar University, India, 2005.
[22] A.K. Passari, V.K. Mishra, R. Saikia, V.K. Gupta, B.P. Singh, Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in-vitro* antimicrobial biosynthetic potential, Front. Microbiol. 6 (2015) 273.
[23] G. Rajivgandhi, J. Vijayarani, M. Kannan, N. Manoharan, Optimization and inhibitory effect of various anti-swarming agents against biofilm forming Proteus mirabilis strain on urinary tract infection, Int. J. Adv. Life. Sci. 9 (2016) 1–13.
[24] G. Rajivgandhi, J. Vijayarani, M. Kannan, A. Murugan, R. Vijayan, N. Manoharan, Isolation and identification of biofilm forming uropathogens from urinary tract infection and its antimicrobial susceptibility pattern, Int. J. Adv. Life. Sci. 7 (2014) 352–363.
[25] D. Dhanasekaran, N. Thajuddin, A. Panneerselvam, Antifungal compound: 4'phenyl-1 napthyl – phenyl acetamide from Streptomyces sp, DPTB16 Facta Univ. Ser. Med. Biol. 15 (2008) 7–12.
[26] C.M. Liu, J.W. Westley, T.E. Herman, B.L. Prosser, N. Palleroni, R.H. Evans, P.A. Miller, Novel polyether antibiotics. X- 14873 A, G and H produced by streptomyces. Taxonomy of the producing culture, fermentation, biological and ionospheres properties of antibiotics, J. Antibio. 39 (1986) 1712–1718.
[27] P.I. Alade, O.N. Irobi, Antimicrobial activity of crude leaf extract of *Acalypha wilesiana*, J. Ethnopharmacol. 39 (1993) 171–174.
[28] G. Thulasi, V. Amsaveni, Antibacterial activity of *Cassia auriculata* against ESBL producing *E. coli* from UTI patients, Int. J. Microbio. Res. 3 (2012) 24–29.
[29] H.A. Lechevalier, A Practical Guide to Generic Identification of Actinomycetes, in: Bergey's Manual of Systematic Bacteriology, vol. 4, Williams & Wilkins Company, Baltimore, 1989, pp. 2344–2347.
[30] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, Current Protocols in Molecular Biology, Greene Publishing and John Wiley & Sons, New York, 1994, pp. 1–7.
[31] W.B. Nilsson, M.S. Strom, Detection and identification of bacterial pathogens of fish in kidney tissue using terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes, Dis. Aquat. Org. 48 (2002) 175–185.
[32] D.G. Higgins, A.T. Bleasby, R. Fuchs, W. Clustal, improved software for multiple sequences alignment, CABIOS 8 (1992) 189–191.
[33] M.A. Kimura, Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences, J. Mol. Evol. 16 (1980) 111–120.
[34] N. Saitou, M. Nei, The neighbour joining method: a new method for reconstructing phylogenetic trees from evolutionary distance data, Mol. Biol. Evol. 4

(1987) 406–425.
[35] E.B. Shirling, D. Gottlieb, Methods for characterization of *Streptomyces* species, Int. J. Syst. Bacteriol. 16 (1966) 313–340.
[36] Y.S.Y.V. Jagan mohan, B. Sirisha, R. Haritha, T. Ramana, Selective screening, isolation and characterization of antimicrobial agents from marine actinomycetes, Int. J. Pharm. Pharm. Sci. 4 (2013) 443–449.
[37] M.O. Ilori, C.J. Amobi, A.C. Odocha, Factors affecting bio surfactant production by oil degrading Aeromonas spp. isolated from a tropical environment, Chemosphere 61 (2005) 985–992.
[38] T.G. Pridham, D. Gottlieb, The utilization of carbon compounds by some actinomycetales as an aid for species determination, J. Bacteriol. 56 (1948) 107–114.
[39] R. Vijayakumar, K. Panneerselvam, C. Muthukumar, N. Thajuddin, A. Panneer selvam, R. Saravanamuthu, Optimization of antimicrobial production by a marine actinomycete streptomyces afghaniensis VPTS3-1 isolated from palk strait, east coast of India, Ind. J. Microbiol. 52 (2012) 230–239.
[40] K.A. Gomez, A.A. Gome, A Statistical Procedure for Agricultural Research, John Willyand Sons, New York, NY, 1984.
[41] C. Lin, C. Lu, Y. Shen, Three new 2-pyranone derivatives from mangrove endophytic actinomycete strain *Nocardiopsis* sp. A00203, Rec. Nat. Prod. 4 (2010) 176–179.
[42] K. Kathiresan, R. Balagurunathan, M. Masilamani Selvam, Fungicidal activity of marine actinomycetes against phytopatho- genic fungi, Ind. J. Biotechnol. 4 (2005) 271–276.
[43] J. Selvin, S. Shanmughapriya, R. Gandhimathi, G. Seghal Kiran, T. Rajeetha Ravji, K. Natarajaseenivasan, T.A. Hema, Optimization and production of novel antimicrobial agents from sponge associated marine actinomycetes *Nocardiopsis* dasson villei MAD08, Appl. Microbiol. Biotechnol. 83 (2009) 435–445.
[44] K. Sathiyaseelan, D. Stella, Isolation, Identification and Antagonistic activity of marine actinomycetes isolated from the mutupettai mangrove environment, Int. J. Pharm. Biolo. Arch. 2 (2011) 1464–1468.
[45] S. Ravikumar, S.J. Inbaneson, M. Uthiraselvam, R. Kaleeswari, A. Ramu, M.B. Banerjee, J. Rajasekar, Antibacterial activity of heterotrophic endophytes from karangkadu mangrove ecosystem, India, J. Pharm. Res. 4 (2011) 195–198.
[46] C.R. Kokare, K.R. Mahadik, S.S. Kadam, Isolation of bioactive marine actinomycetes from sediments isolated from Goa and Maharastra Coastlines (west coast of India), Ind. J. Mar. Sci. 33 (2004) 248–256.
[47] M. Jami, M. Ghanbari, W. Kneifel, K.J. Doming, Phylogenetic diversity and biological activity of culturable action bacteria isolated from freshwater fish gut microbiota, Microbiol. Res. 175 (2015) 6–15.
[48] R. Balagurunathan, M. Radhakrishnan, S.T. Somasundaram, L-glutaminase producin actinomycetes from marine sediments – selective isolation, semi quantitative assay and characterization of potential strain, Aus. J. Basic Appl. Sci. 4 (2010) 698–705.
[49] P. Gayathri, V. Muralikrishnan, Isolation and characterization of Endophytic actinomycetes from mangrove plant for antimicrobial activity, Int. J. Curr. Microbiol. App. Sci. (2013) 78–89.