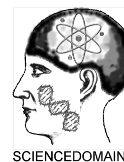
British Microbiology Research Journal
6(6): 348-357, 2015, Article no.BMRJ.2015.086
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org



Isolation and Characterization of a Marine Bacterium from Sundarbans, Bangladesh

Ashish Kumar Sarker[1*], Md. Anwarul Haque[1], Mohammad Sayful Islam[2], Md. Ajijur Rahman[2] and Md. Anwar Ul Islam[2]

[1]Department of Pharmacy, Pabna University of Science and Technology, Pabna-6600, Bangladesh.
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author AKS did most of the experiments, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors MAH, MSI and MAR managed the analyses of the study and literature searches. Author MAI designed the study, proofread the manuscript and gave critical comments. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/15907
Editor(s):
(1) Rashedul Islam, Department of Biological Sciences, Inha University, South.
Reviewers:
(1) Anonymous, South Africa.
(2) Ilham Zahir, Laboratory of Microbial Biotechnology, Department of Biology, University Sidi Mohamed Ben Abdellah, Morocco.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=832&id=8&aid=8076

Original Research Article

Received 24th December 2014
Accepted 31st January 2015
Published 7th February 2015

ABSTRACT

Aims: Marine environment is a vast but represents a largely untapped source for isolation of new microorganism having ability to produce novel bioactive compound(s). The present study was designed with an aim to discover and identify marine bacterial strain producing antibacterial metabolites from marine soil samples of Sundarbans, Bangladesh.
Study Design: We used serial dilution technique for isolation of marine bacteria, streak plate technique for screeninig of priliminery antibacterial activity, cover slip culture method for morphological characterization and the 16S rDNA sequencing approach for phylogenetic characterization.
Place and Duration of Study: Pharmaceutical Microbiology Research Laboratory, Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh between August 2010 and July 2011.
Methodology: A total of 39 bacterial colonies were isolated from the marine soils collected from different locations of Sundarbans, a mangrove forest of Bangladesh. Among them ANAM-39 was selected on the basis of morphological, cultural, physiological, biochemical and taxonomic

**Corresponding author: E-mail: ashish_phar@yahoo.com;*

characterization, and it exhibited the highest antibacterial activity against a series of test organisms.
Results: The 16S rDNA sequence of the strain ANAM-39 showed close similarity with *Smithella propionica* (95.99%), *Syntrophus aciditrophicus* (93.63%) and *Syntrophus gentianae* (93.17%). However, the cultural, morphological, physiological and biochemical characteristics of the strain and *S. propionica* were completely different. So, the strain ANAM-39 may not be assigned within the genus *Smithella*.
Conclusion: To confirm the taxonomic position of the strain further studies are needed in respect to the DNA relatedness studies, DNA-DNA hybridization, small subunit (SSU) sequences, cell wall composition and other characterization.

Keywords: Characterization; phylogeny; marine bacteria; sundarbans.

1. INTRODUCTION

Marine microbial biodiversity has opened up unexpected new horizons for finding novel organisms for trapping their potential resources. More than 70% of our planet's surface is covered by oceans and life on earth originated from the sea. Marine sediment is an inexhaustible resource that has not been properly exploited. It is estimated that less than 1% of potentially useful chemicals from marine environment has been screened so far, with microbial products representing approximately 1% of the total number [1]. Marine ecosystems and coastal regions are particularly promising, because of the rightly adopted species found in this harsh environment. Studies have shown that microorganism isolated from the marine environment are metabolically active and have adapted to life in the sea [2]. So, the exploration of microbial secondary metabolites from marine environment has led to the discovery of hundreds of biologically active compounds.

Marine environments have been a potential source for unique microorganism producing different types of bioactive compounds [3]. Marine microorganisms have become important resource of novel microbial products exhibiting antibacterial, anticancer, antiviral, antitumor as well as anticoagulant and cardioactive properties [4-6].

Antibiotic resistance arising mainly through antibiotic misuse is now recognized as a global health problem. The situation is exacerbated by the fact that no novel antibiotics have been discovered for last 20 years. Although many pre-existing antibiotics have been modified to yield new derivatives, bacteria have the potential to develop new resistance mechanisms to combat these molecules [7-8]. The need for new, safe and more effective antibiotic is a major challenge to the pharmaceutical industry today, especially with the increase in antibiotic resistance.

As the frequency of novel bioactive compounds discovered from terrestrial microorganism decreases with time, much attention has been focused on screening of microorganism from diverse environments. As marine environmental conditions are extremely different from terrestrial ones, it is summarized that marine microorganism have different characteristics from those of terrestrial counterparts and therefore might produce different types of bioactive compounds [9]. Among marine microorganisms, actinomycetes, particularly the genus *Streptomyces*, are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities [10].

Actinomycetes populations are relatively lower than other soil microbes and contain a predominance of *Streptomyces* that are tolerant to acid conditions [11]. *Streptomyces* is the largest genus of the Actinobacteria and are Gram positive bacterium with high GC content [12]. *Streptomyces* includes filamentous bacteria which produce well developed vegetative hyphae (between 0.5-2.0 µm in diameter) with branches. Members of *Streptomyces* are found almost everywhere, and the largest reservoir of these microorganisms is soil. *Streptomyces* covers around 80% of total antibiotic product, with other genera trailing. *Streptomyces* are widely recognized as sources of antibiotics and other important novel metabolites, including antifungal agents [13], antitumor agents [14], antihelminthic agents [15] and herbicides [16]. Although thousands of antibiotics have been isolated from *Streptomyces,* only a small fraction of the repertoire of bioactive compounds was produced [17,18].

In this study, we explored the presence of antibiotic producing microorganism in soils of Sundarbans, the largest tidal halophytic mangrove forest in the world. Marine bacteria producing antibacterial compounds were isolated from Sundarbans, Bangladesh and were characterized. This study demonstrated the diversity of the Sundarbans as a rich source of bioactive molecules producing new and potential marine bacteria species.

2. MATERIALS AND METHODS

2.1 Isolation and Selection of Organism

A total of nine marine soil samples were collected carefully from the various depth of the earth, ranging from layers just beneath the upper surface to 1.5 meter depth from different location of Sundarbans, Bangladesh (08-16 August, 2010). The isolation of the marine bacteria from these soil samples were carried out by spread plate technique [19]. A total of 39 strains [ANAM-1 to ANAM-39] were isolated as pure culture as their colonies showed *Streptomyces* like appearance under light microscope. The purified isolates were preserved on yeast-extract-glucose-agar slants at 4ºC. Then all the pure isolates were preliminary screened for antibacterial activity though the use of streak plating technique [20] on yeast-extract-glucose-agar medium. Due to the highest antibacterial activity in preliminary screening, visibility of the metabolite as well as excellent growth properties, the isolate ANAM-39, a brownish yellow colored microorganism was chosen for further characterization.

2.2 Morphological Characterization

Morphology had played a major role in distinguishing bacteria and in the characterization of bacteria species. The morphological characteristics of the strain ANAM-39 was determined in accordance with the method described by Shirling and Gottlieb [21]. Microscopic characterization was done by cover slip culture method. In this method, four sterile cover slips were inserted aseptically into the sterile solidified agar medium in a petri dish at an angle of 45° to 60° by a sterile forceps and the strain was inoculated by streaking method, at the free space of the plate. The hyphae of the strain grew and spread on the cover slip and produced spores during incubation period (at 37.5°C for 10 days). After this period, the cover slips were then taken out smoothly by using sterile forceps, mounted on slides and observed under microscope, using lactophenol cotton blue [22]. Colonies was identified on the basis of their colony morphology and color [21]. The characteristics of the spore-bearing hyphae and spore chains were determined by direct microscopic examination of the culture surface on opened dishes. To establish the presence or absence of chains of spores 100x - 700 x magnifications were used.

2.3 Cultural Characterization

The cultural characteristic of the strain was also determined according to the method described by Shirling and Gottlieb [21]. Cultural characteristics are best made on a variety of standard cultivation media such as International *Streptomyces* Project (ISP) 2 (yeast-extract-malt extract agar); ISP 3 (oatmeal agar); ISP 4 (inorganic salts-starch agar); and ISP 5 (glycerol-asparagine agar), ISP 1 (Trypton –yeast extract agar), ISP 7 (Tyrosine agar) and yeast-extract – glucose agar (YEGA). A National Bureau of Standards Color Chart was used to determine the color of the substrate mycelia, aerial mycelia, and spore mass and pigment production [23].

2.4 Biochemical and Physiological Characterization

The biochemical and physiological characteristics of the strain were determined as described by Shirling and Gottlieb [21]. The growth of the strain ANAM-39 was determined on different carbon sources like D-glucose, sucrose, D-fructose, L-rhamnose, D-galactose, xylose, rhamnose, maltose, lactose, starch and mannitol. Here, basal mineral salts agar (ISP medium 9) and different carbon sources were used for the medium of the test. The optimization of nitrogen sources such as yeast extract, peptone, ammonium chloride, ammonium sulphate, $NaNO_3$, casein, beef extract, KNO_3, sodium nitrate and L-asparagine were carried out by adding nitrogen source (0.2%) to the basal medium containing glucose (3%). The pH of the medium was adjusted using hydrochloric acid (1M) and sodium hydroxide (1M). For the optimization of temperature, after sterilizing and inoculating with spores the flasks were incubated at different temperatures (25-47ºC).

2.5 Phylogenetic Characterization

Phylogenetic analysis were also performed with the strain ANAM-39. To prepare the genomic

DNA, isolated colony from the agar plate was dispersed in 500 µl of saline-EDTA buffer (NaCl 150 mM; EDTA 10 mM; pH 8.2) and incubated for 1h at 37ºC. Then, 10 µl of lysozyme solution (5 mg/ml), 5 µl of proteinase K solution (15 mg/ml) and 10 µl SDS solution (25%) were added and incubated for 30 minutes at 55ºC. Lysate was extracted, purified and dissolved in nano pure sterile water (60 µl). The 16S rDNA was amplified by the PCR in reaction mixture containing KOD FX buffer with 200 µM dNTP, 100 ng genomic DNA and 0.5 µg forward (5'-AGAGTTTGATCCTGGCTCAG -3') and 0.5 µg reverse (5'- GGTTACCTTGTTACGACTT -3') primers. Thermal cycle was performed with a model 22331 eppendrof (Germany). The samples were subjected to an initial denaturing step consisting of 2 minutes 98ºC, after which 2U of Taq polymerase was added to each sample at 90ºC. The thermal profile used was 30 cycle consisting of 1 min annealing at 52ºC, 2 min extension at 72ºC and 1 min denaturation at 94ºC. A final extension step consisting of 4 min at 65ºC. PCR amplificants were detected by agarose gel electrophoresis and visualized in Dolphin WaveTech. The band was excised and isolated using TAE buffer. Purified by phenol chloroform extraction followed by ethanol precipitation and dissolved in water. The resultant PCR solution was used in sequencing reaction with Pre-Mix and primer. The reaction mixture was applied to thermal cycles in 30 cycles consisting of 10 sec at 98ºC, 30 sec at 47ºC and 2 min at 68ºC. DNA was purified and by using Prep-A-Gene Kit (Bio-Rad). The liquid was evaporated at reduced temperature and using vacuum pressure. The dried DNA was dissolved in 20 µl of polyacrylamide sequencing gel and sequenced by Applied Biosystems automated DNA sequencer. The resulting sequences were analyzed with DDBJ/EMBL/GenBank database using analysis softwares- BLAST and BIBI (Bioinformatics Bacterial Identification Tool) [24]. BIBI software is available online (hptt/umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibiexecx.cgi). The rooted phylogenetic tree was constructed by using BIBI.

3. RESULTS AND DISCUSSION

The isolate ANAM-39, a brownish yellow colored microorganism, was chosen for further characterization due to the highest preliminary antibacterial activity (Fig. 1.), visibility of the metabolite as well as excellent growth properties of the strain.

The isolate ANAM-39 grew very slowly at first and gave white colonies only after 24 h (Fig. 2.) After 48 h, it gradually turned into velvety cream colonies.

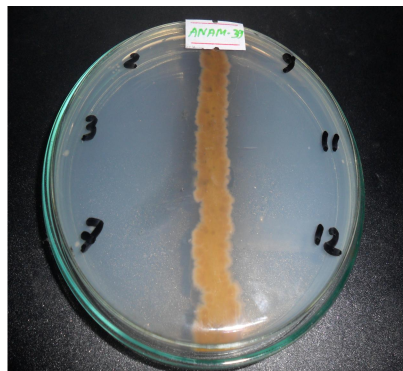


Fig. 1. Antibacterial activity of ANAM-39 isolate. The perpendicular lines marked with numerical digits are the test organism- 2. *Streptococcus agalactiae*, 3. *Bacillus cereus*, 7. *Pseudomonas aeruginosa*, 9. *Escherichia coli*, 11. *Shigella dysenteriae,* 12. *Shigella sonnei*

Then it appeared to grow very fast and after 72 h ANAM-39 starts to release a soluble brown pigment through the medium. It became light brown after 96 h and gave brownish yellow colonies after 120 h. As the time went, the aerial mycelium became more powdery as well as deep brown due to sporulation of the microorganism. Under microscope, the marine bacteria ANAM-39 produced well developed substrate mycelium and aerial hyphae which were differentiated into long and extended filamentous chains with cylindrical, smooth spores occurring in chains of more than 7. Spores are observed after five days. The isolate ANAM-39 exhibited morphology of typical *Streptomyces;* the colonies were slow growing, aerobic, glabrous or chalky, folded, and with aerial and substrate mycelia [24]. In addition, this isolate possessed an earthy odor and fitted to the description of genus *Streptomyces* in Bergey's Manual of Systemic Bacteriology.

Cultural characteristics of the strain ANAM-39 in different cultural media are presented in Table 1, showing morphology and properties of typical *Streptomyces.* The growth and pigmentation of the strain was very low in ISP 5 medium compared with both ISP 2 and ISP 4 medium where the growth of the strain was very high, the spore mass was profuse in these medium and pigmentation was very high. In ISP 1, ISP 3, ISP

7 and YEGA medium the growth of the strain was moderate. The color of the substrate mycelium was tended to either yellow, pinkish yellow, dark white or white. Whereas, aerial mycelium found to be either brownish yellow, deep brown, light brown, yellowish brown, or light yellow. The strain produces yellowish brown to brown pigment or a distinct brown pigment modified by other color on ISP 1 and ISP 3 medium.

Utilization of carbon and nitrogen sources by the strain ANAM-39 is presented in Table 2. The production of antibiotic by various *Streptomyces* species was greatly influenced by suitable carbon and nitrogen sources [25]. The growth and production of yellow pigment was good by the strain in the presence of glucose, lactose and sucrose as carbon sources in the medium. In the presence of D-xylose as a sole carbon source in the medium, no growth was observed. Utilization and metabolite production by the strain in the presence of inositol and mannitol was doubtful. In the absence of any carbon source (negative control) the strain could not grow. Therefore, it may be concluded that, proper carbon source is needed for optimum growth of the strain and for optimum production of pigment. In this present study, the strain was found to produce high amount of metabolites in the medium supplemented with glucose, lactose and sucrose as sole carbon source. In case of *Streptomyces* species, with regards to carbon sources species specific variation may occur for cell growth and secondary metabolites production [26]. The strain used different inorganic and organic nitrogen sources differently and exhibited differences in metabolites production. The growth of the strain as well as metabolite was very good in the medium supplemented with yeast extract and casein as nitrogen sources. In the presence of peptone, ammonium chloride, ammonium sulphate, $NaNO_3$ and beef extract as nitrogen sources the growth and metabolites production of the strain were moderate.

Fig. 2. White and brown colonies of ANAM-39

Table 1. Cultural characteristics of strain ANAM-39

Medium	Growth	Spore mass	Aerial mycelium	Reverse side substrate mycelium	Pigmentation
Trypton –yeast extract agar (ISP 1)	++	Very low	Light brown	Dark white	+
Yeast-extract-malt extract agar (ISP 2)	+++	Abundant	Brownish yellow	Pinkish yellow	++
Oatmeal agar (ISP 3)	++	Moderate	Brownish yellow	Yellow	++
Inorganic salt-starch agar (ISP 4)	+++	Abundant	Deep brown	Yellow	+++
Glycerol-asparagine agar (ISP 5)	+	Very low	Light yellow	Yellow	-
Tyrosine agar (ISP 7)	++	Moderate	Yellowish brown	Dusky yellow	+
Yeast-extract glucose agar (YEGA)	++	Low	Brownish yellow	White	+

The sign '++' indicates strongly positive utilization, '+' represents positive utilization and '-' indicates negative utilization

The growth and pigmentation was doubtful for the strain in the medium containing KNO_3 and sodium nitrate. The growth of the strain was doubtful and metabolite production was negative in the medium containing l-asparagine. Different types of carbon and nitrogen sources were found to have significant effect on growth and secondary metabolites production by various *Streptomyces* species [27]. On this respect, the strain ANAM-39 showed similar characteristics with *Streptomyces* species. The growth of the strain was very high and production of high levels of antibiotic when culture medium incubated at 30°C and pH 6.0. Extreme pH and temperature were unfavorable for antibiotic production. So, the morphological, cultural, biochemical and physiological properties of the strain ANAM-39 exhibited consistency with its assignment to the genus *Streptomyces sp.*

On identifying isolates at the species level, incongruities were observed. The 16S rDNA sequence of strain ANAM-39 was determined and compared to those deposited in GenBank using the BLAST and BIBI, and Fig. 3 shows a phylogenetic tree derived from these sequences. It was observed that the sequence was actually similar to those sequences of more than one species (Table 3). Species identification was based on maximum score, identity and coverage values. The genetically related species were *Smithella propionica*, *Syntrophus aciditrophicus*, *Syntrophus gentianae*, *Syntrophus buswellii*, *Pelobacter propionicus*, *Algidimarina propionica* and *Algorimarina butyrica*. But, this strain showed is most closely related (95.99 % sequence similarity) to strain *S. propionica*, an anaerobic, syntrophic, propionate-oxidizing bacteria whose sequence was determined in the laboratory of David R. Boone [28]. This strain also closely related to *Syntrophus aciditrophicus* (93.93% sequence similarity) and *Syntrophus buswellii* (92.52%), but these similarities were not sufficient to indicate that strain ANAM-39 should be classified within the genus *Syntrophous.* According to Drancourt et al. [29], >99% and >97% sequence similarities were used as the cut-offs for species and genus identification, respectively, depending on the bacterial genus under investigation. On the other hand, Janda and Abbott in their recommended guidelines suggested that a minimum of >99%, and ideally >99.5%, sequence similarity be used as the criteria for species identification [30]. They also proposed that other properties, e.g. phenotype, should be considered in final species identification. Although different studies have identified groups of bacteria for which 16S rDNA sequences are not sufficiently discriminative and for which other gene targets have to be used.

Table 2. Utilization of carbon and nitrogen sources by the strain ANAM-39

Carbon source	Utilization	Pigmentation	Nitrogen source	Utilization	Pigmentation
D-Glucose (Positive control)	++	++	Yeast extract	++	++
No carbon (Negative control)	-	-	Peptone	+	+
L-Rhamnose	+	+	Ammonium chloride	+	+
Starch	+	+	Ammonium sulphate	+	+
D-fructose	+	+	$NaNO_3$	+	+
D-Xylose	-	-	Casein	++	++
Lactose	++	++	Beef extract	+	+
Mannitol	+	±	KNO_3	±	±
Inositol	±	±	Sodium nitrate	±	±
Sucrose	++	++	L-asparagine	±	-

The sign '++' indicates strongly positive utilization, '+' represents positive utilization, '±' denotes doubtful utilization and '-' indicates negative utilization

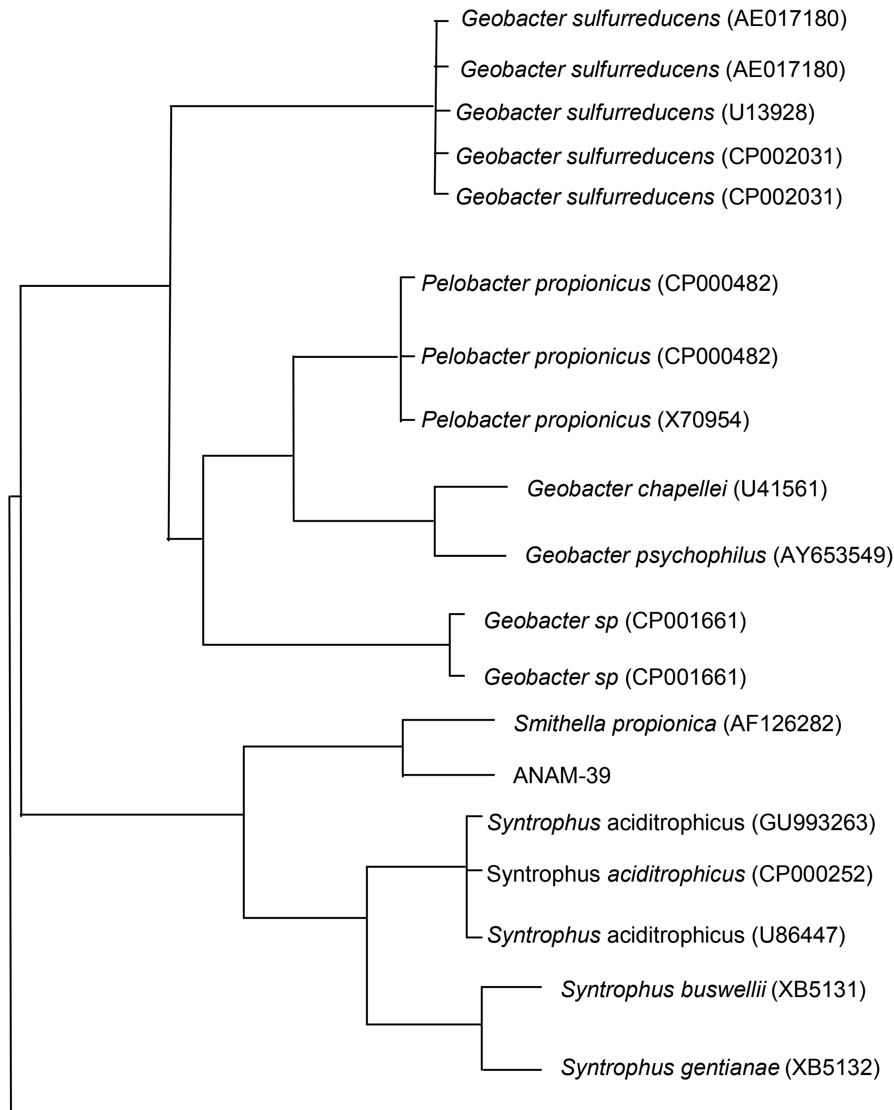


Fig. 3. Phylogenetic tree of the strain ANAM-39 based on partial 16S rDNA gene sequences (using sequence database Bacteria_TS_SSU-rDNA-16s-stringent)

Table 3. Sequences producing significant alignments with the 16S rDNA sequence of ANAM-39

Accession No.	Species/Strain	Total score	Query coverage (%)	E value	Max identity (%)
AF126282	*Smithella propionica*	2286	100%	0.0	95.99%
CP000252	*Syntrophus aciditrophicus*	2242	100%	0.0	93.63%
X85132	*Syntrophus gentianae*	2198	100%	0.0	93.17%
GU993263	*Syntrophus aciditrophicus*	2185	100%	0.0	93.52%
X85131	*Syntrophus buswellii*	2113	100%	0.0	92.52%
U86447	*Syntrophus aciditrophicus*	1836	100%	0.0	93.93%
X70954	*Pelobacter propionicus*	1455	100%	0.0	90.59%
CP000482	*Pelobacter propionicus*	1455	100%	0.0	90.59%
AY85129	*Algidimarina propionica*	1447	100%	0.0	90.17%
AY851292	*Algorimarina butyrica*	1435	100%	0.0	90.64%

For comparison, we examined *S. propionica* and we found important differences. *Smithella propionica* is strictly anaerobic, gram-negative, syntrophic, rod shaped, 0-5 pm in diameter, contain granules of poly-P-hydroxybutyrate, weakly motile bacteria [28] but the strain ANAM-39 was aerobic, gram-positive, produce well developed substrate mycelium and aerial hyphae which are differentiated into long and extended filamentous chains with cylindrical, smooth spores occurring in chains of more than 7, colonies glabrous or chalky, folded, and with aerial and substrate mycelia and possessed an earthy odor. *Smithella propionica* grows fast with pH near neutral, could not grow at pH values of 6.3 or below and could not grow well above or below 37^0C temperature [28] whereas strain ANAM-39 showed excellent growth at 30°C and pH 6.0. *Smithella propionica* grows axenically on crotonate, producing acetate and butyrate but does not grow axenically on propionate whereas strain ANAM-39 utilizes mostly D-Glucose, Lactose, Starch and D-fructose as energy source.

Although 16S rDNA sequencing has being used increasingly for bacterial identification in clinical microbiology laboratories but there is some limitations of this technique like the ever-expanding sequence databases and taxonomic complexity, and the inaccuracies in some databases [28-29]. The use of 16S rDNA sequencing for bacterial identification depends on significant interspecies differences and small intraspecies differences in 16S rDNA sequences. Therefore, one of the major limitations is that when two different bacterial species share almost the same 16S rDNA sequence, this technique would not be useful for distinguishing between them [31]. However, there are 'blind spots' within some major genera, in which 16S rDNA sequences are not sufficiently discriminative for the identification of certain species [32-33]. 16S rRNA gene sequencing is highly useful in regards to bacterial classification; it has low phylogenetic power at the species level and poor discriminatory power for some genera [34-35].

Considering these limitations, in order to increase the accuracy of 16S rDNA sequencing for identification of bacteria, it would be necessary to interpret the results of 16S rDNA sequencing with preliminary morphological, cultural, biochemical and physiological test results. The results obtained on analyzing various characteristics and phylogenetic studies based on partial sequences of the 16S rDNA region encoding gene, demonstrate that though the strain ANAM-39 is most closely related to the organism *S. propionica* in phylogenetic development as well as sequence similarity but the various phenotypic characteristics of ANAM-39 like morphological, cultural, physiological and biochemical were completely different from *S. propionica*. So, the consignment of the strain ANAM-39 to the genus *Smithella* is not logical. Thus, further studies should be carried out in respect of the DNA relatedness studies, DNA-DNA hybridization, small subunit (SSU) sequences, cell wall composition and other characteristics to confirm the taxonomic position of the strain ANAM-39.

4. CONCLUSION

Our findings indicate that the strain ANAM-39 differs morphologically, culturally, physiologically, and biochemically from *Smithella propionica*. However, the strain was phylogenetically most similar to the genus *Smithella,* although the phylogenetic relationship was not sufficiently close to permit classification of strain ANAM-39 within this genus. So, the strain ANAM-39 may not be assigned to the genus *Smithella.* Thus to confirm the taxonomic position and to provide absolute resolution to these taxonomic problem of the strain ANAM-39, more studies should be carried out in respect to the DNA relatedness studies, DNA-DNA hybridization, small subunit (SSU) sequences, cell wall composition and other characteristics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Neidleman SL, Laskin AL. Adv AppL Micro. 2000;47:88-90.
2. Valli S, Sugasini SS, Aysha OS, Nirmala P, Kumar PV, Reena A. Antimicrobial potential of Actinomycetes species isolated from marine environment. Asi Pac J Trop Biom. 2012;45:469-474.
3. Bredholt H, Fjaervik E, Jhonsen G, Zotechev SB. Actinomycetes from sediments in the Trondhein Fjrod, Norway: Diversity and biological activity. J Mari Dr. 2008;6:12-24.
4. Marderosian AD. Marine pharmaceuticals. J Pharm Sci. 1969;58:1-30.
5. Austin B. A review novel on pharmaceuticals compounds from marine bacteria. J Appl Bact. 1989;67:461-470.

6. Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W. Culturable marine Actinomycetes diversity from tropical Pacific Ocean sediments. App Envir Micro. 2005;7:1039-1048.
7. Knowles DJC. New strategies for antibacterial drug design. Tre Micro. 1997;5(10):379-383.
8. Levy SB. The challenge of antibiotic resistance. Sci Am. 1998;278(3):32-39.
9. Imada C, Koseki N, Kamata M, Kobayashi T, Hamada-Sato N. Isolation and characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater. Actinomyce. 2007;21:27-31.
10. Bredholt H, Fjaervik E, Jhonsen G, Zotechev SB. Actinomycetes from sediments in the Trondhein Fjrod, Norway: Diversity and biological activity. J Mari Dru. 2008;6:12-24.
11. Davies FL, Williams ST. Studies on the ecology of actinomycetes in a pine forest soil. Soil Biochem. 1970;2:227-238.
12. Kampfer P. The Family *Streptomycetaceae*, Part I: Taxonomy. The prokaryotes: a handbook on the biology of bacteria (Dworkin, M et al., eds.), Berlin: Springer. 2006;538-604.
13. Thakur D, Yadav A, Gogoi A, Bora BK. Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. J Med Mycol. 2007;17:242-249.
14. Konishi M, Kawaguchi H. Dynamics new antibiotics with the 1,5 diyn-3-ene and anthaquinone subunit. Production, isolation and physic-chemical properties. J Antib. 1991;44:1300-1305.
15. Sanglier JJ, Hang H, Huck TA, Feh T. Novel bioactive compounds from actinomycetes. Resear Microbi. 1993; 144:633-642.
16. Lee HB, C. Kim J, Kim JS, Hong KS, Cho KY. A bleaching herbicidal activity of meth oxyhygromycin (MHM) produced by an actinomycete strain Streptomyces sp. 8E-12. Let App Microbi. 2003;36:387-389.
17. Berdy J. Are actinomycetes exhausted as a source of secondary metabolites? In Proceedings of the 9th Symposium. Actino. 1995;13-14.
18. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus Streptomyces? Arch Microbi. 2001;176(5):386-390.
19. Bernard B. Access excellence @ the national health museum, Isolation of Antibiotic strains from soils. (www.access excellence.org.).July 2007.
20. Alcamo E, Pomerville JC. Alcamo's Laboratory Fundamental of microbiology; 2004.
21. Shirling EB, Gottlieb D. Methods of characterization of *Streptomyces* species. Inter J Sys Bact. 1966;16(3):313-340.
22. Korzybski T, Kowszyk G, Kurilowicz W. Antibiotics, Origin, nature and properties, Translated by parayski. E. Per Pre, Oxf. 1967;47:450-725.
23. Zhao H, Parry RL, Fllis DL, Griffith GW, Goodacre R. The rapid differentiation of Streptomyces isolates using fourier transform infrared spectroscopy. Vibrat Spec. 2006;40:213-218.
24. Clarridge JE. Impact of 16S rRNA Gene Sequence Analysis for Identification of bacteria on clinical Microbiology and infectious diseases. Clin Microbi Rev. 2004;17(4):840-862.
25. Hobes G, Frazer CM, Gardner DCJ, Flett F, Oliver SG. Pigmented antibiotic production by S. coelicolor A3(2), kinetics and the influence of nutrients. J Gen Microbi. 1990;136:2291-2296.
26. Jonsbu EM, Intyre M, Nielsen J. The influence of carbon source and morphology on nystatin production by Streptomyces noursei. J. Biotec. 2002;95:133-144.
27. Mansour FA, El-Shirbiny SA, El- Metwaly NA. Demethyltetracycline biosynthesis by Streptomyces aureofaciens Sub-species viridulans as influenced by medium composition. Egyp J Microbi. 1996;31:221-235.
28. Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR. Characterization of the anaerobic propionate degrading syntrophs Smithella propionica gen. nov., sp. Nov. and Syntrophobacter wolinii. Inter J Sys Bact. 1999;49:545-556.
29. Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbi. 2000;38:3623-3630.
30. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. J Clin Microbi. 2007;45:2761-2764.

31. Woo PCY, Lau SKP, Teng JLL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbi Infect. 2008;14:908-934.
32. Kwok AY, Chow AW. Phylogenetic study of Staphylococcus and Macrococcus species based on partial hsp60 gene sequences. Int J Syst Evol Microbi. 2003;53:87-92.
33. Kwok AY, Su SC, Reynolds RP. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus Staphylococcus. Int J Syst Bact. 1999;49(3):1181-1192.
34. Gupta R, Lanter JM, Woese CR. Sequence of the 16S ribosomal RNA from Halobacterium volcanii, an Archaebacterium. Sci. 1983;221:656-659.
35. Woo PC, Chong KT, Leung K, Que T, Yuen K. Identification of Arcobacter cryaerophilus isolated from a traffic accident victim with bacteremia by 16S ribosomal RNA gene sequencing. Diag Microbi Infect Dis. 2001;40:125-127.

© 2015 Sarker et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=832&id=8&aid=8076