



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	2014_BMRJ_15907
Title of the Manuscript:	Characterization and Phylogenetic Studies of Marine Bacteria Isolated from Sundarbans, Bangladesh
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Abstract Authors should describe for what the techniques presented in Study design are used? MATERIAL AND METHODS DNA extraction, PCR and sequencing should be referenced.	We have described what the purpose or necessity of using the techniques in abstract section. All the corrections were making as per reviewer comments. Please see the corrected manuscript.
Minor REVISION comments	Abstract There is one strain which was isolated, so the aim In abstract should be changed to 'a new marine bacterium strain'. Methodology: change "from the different location" by "from different locations" Change "ANAM-39 were selected" by "ANAM-39 was selected" Change 'as it was exhibited highest" by "since it exhibited the highest" Not all the strains were assessed in phenotypical characteristics, thus the word "strains" in the followed sentence "its closely associated strains" should be changed by "<i>Smithella propionica</i>" Change "further studies is needed » by « further studies are needed" Introduction From line 23 to line 32, there are some repeated ideas, thus the whole paragraph should be shortened. I suggest to change the followed idea "bacteria have the potential to mutate known resistance mechanisms to combat these" (line 37) by "bacteria have the potential to develop new resistance mechanisms to combat these molecules" In line 37, remove one of these two sentences because they present the same idea. "The need for new, safe and more effective antibiotic is a major challenge	All the corrections were making as per reviewer comments. Please see the corrected manuscript. All the corrections were making as per reviewer comments. Please see the corrected manuscript.



SDI Review Form 1.6

	to the pharmaceutical industry today, especially with the increase in antibiotic resistance. Or "For this constant increasing of bacterial resistance, it is now desperate need to search for new sources of antibiotics". In 45, to avoid repetition, remove this followed idea "Marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites" because it has already been described. In line 49, the followed idea should be reformulated "Marine derived antibiotics are more efficient at fighting microbial infections than the terrestrial bacteria have not developed any resistant against them"!!!. Add 2 comma , after the word actinomycetes and Streptomyces in line 51 and 52, respectively. Change the followed sentence "only small fraction of the repertoire of bioactive compounds produced " by "only a small fraction of the repertoire of bioactive compounds was produced " (line 65). Remove "that" in line 70. MATERIAL AND METHODS What are the bacteria used in antibacterial activity? Remove the letter s from the word" isolates" in line 86. Remove "means of" in line 95 To avoid repetition in the paragraph "biochemical and physiological characterization", remove the followed idea "such as production of melanin, optimization of pH and temperature, utilization of carbon and nitrogen sources etc." (lines 116, 117). Change "was" to "were" in line 123. Authors should determine the composition of the basal medium used in optimization of nitrogen sources. Every microbiologist knows the importance of phylogenetic studies and phenotypic characteristics in identification, So I think that the idea about these techniques in lines 130-133 should be removed. This is a suggestion, authors can very simply say "Phylogenetic studies were also performed. Thus, for the preparation of genomic DNA, the isolated colony from the agar plate was dispersed ..." Add "s" in primer. (line 141). What are the names of the forward (5'-AGAGTTTGATCCTGGCTCAG -3') and the	All the corrections were making as per reviewer comments. Please see the corrected manuscript. The bacteria that were used for testing antibacterial activity are <i>Streptococcus agalactiae</i>, <i>Bacillus cereus</i>, <i>Escherichia coli</i>, <i>Shigella dysenteriae</i>, <i>Shigella sonnei</i> and <i>Pseudomonas aeruginosa</i>. All the corrections were making as per reviewer comments. Please see the corrected manuscript.
--	--	--



SDI Review Form 1.6

	reverse (5'-GGTTACCTTGTTACGACTT -3') primers used in PCR? Primers used in sequencing are the same used in PCR or they are different? Authors should add that detail. Change "constsructed" to" constructed" in line 149 Change "was" by "were" in line 149 Result Change « a new marine bacteria strain" into "a new marine bacterium strain" (line 154). Change "the isolates ANAM-39" by "the isolate ANAM-39"(line155). Add photos in results to highlight the antibacterial activity of the chosen isolate. Change the title of the figure 1; white colonies appear after 24h as was reported by authors in results and not 21 days. Thus, the title of the figure should be divided into 2 part according of each picture (while colonies and brown colonies, respectively). To say that "the strain ANAM-39 shows morphology and properties of typical <i>Streptomyces</i>" authors should add in table 1 characteristics of a referenced <i>Streptomyces</i> to make comparison between the isolate and <i>Streptomyces</i> (lines 178- 179). Remove the "±' sign utilization doubtful" from the title of table1. Change was by were in line 210. Change metabolite to metabolites in line 214. Add a comma after For comparison line 250 Add "we" before the verb found in line 250 I suggest to change "is being used" by "has being used" Change limitations by limitation in line 314. Reference Re write references as it required by the journal guidelines.	Both primer that were used in our study are universal primer and Primers used in sequencing are the same used in PCR. The photos of ANAM-39 presenting the antibacterial activty are added. Thanks for your suggession. As we all know that the standard morphology and characters of <i>Streptomyces</i> and it is well understood with the help of Bergeys Mannual of Bacteriology, so we think this comparison can be avoided. Yes, we have re write references as it required by the journal guidelines.
--	---	--



SDI Review Form 1.6

<u>Optional/General</u> comments	MATERIAL AND METHODS Did authors use electrophoresis in 1% agarose gel after PCR to verify whether DNA was amplified or not? Results After sequencing, what is the number of nucleotides contained in each sequence? If this number is less than 600, then, the identification could not be well done. In this case, extraction of DNA, PCR and sequencing should be repeated. More you have a big number of nucleotides (above 600), more the homology can reach 97% and more. As was reported by authors, according to Drancourt, 97% of homology can determine the genus and 99% of homology can determine the species. Thus, homologies of 95% and less could not help authors in identification. I suggest the best thing before performing phenotypical tests or hybridation to repeat sequencing. Another thing why authors should repeat sequencing; that because the strain was closely related to <i>Smithella propionica</i> as was showed by figure 2 but after performing phenotypical tests between the two bacteria, some differences were revealed. If the number of nucleotides of the obtained sequence by authors is larger than 600, thus sequencing should be repeated by other primers which targeted the whole RNA 16 gene to increase your changes to have a big homology. As a perspective of the work, authors can optimize the production of metabolite by using a medium containing glucose (lactose or sucrose), yeast extract, casein at 30°C and pH 6.	Yes, we did it. After sequencing, The length of the sequence was 1523 bp. Now, it is somewhat impossible for us to carry out sequencing repeatedly withy some other primers and immediately as we are citigen of developing country and we can't serve the cost of repeated sequencing now. But, in future, we must try it. Thanks for your suggession. Yes, you are absolutely right. We already optimize the production of metabolite by using a medium containing glucose and yeast extract, We also want to extract and purified the active antibacterial constituent of the isolate ANAM-39.
--	--	---