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PART 1:

Journal Name:	British Journal of Pharmaceutical Research
Manuscript Number:	**2013_BJPR_3698**
Title of the Manuscript:	**Bacterial endophytes of the medicinal herb *Hygrophila spinosa* T. Anders and their antimicrobial activity**

General guideline for Peer Review process is available in this link:
(http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline)

- This form has total 7 parts. Kindly note that you should use all the parts of this review form.

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PART 2: Review Comments

	Reviewer's comment (# 2)	**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	From lines 80 to 84 there's a *full copy* of sentences from the Abstract of a review article available on Pub-Med (http://www.ncbi.nlm.nih.gov/pubmed/20116229); at the end of the previous paragraph, there is also another small portion of text copied ("adapted") from this same Abstract. The objectives and major content of this manuscript are well described in the title and in the '**Aims**' part of the **ABSTRACT**. The overall flow of the text is good and the reading is easy and clearly understandable. I found a few rough spots to which suggestions and corrections were provided below (as minor revision). Scientific content is sound and appropriate for the questions asked; the subject of the research and the findings are interesting. However, it must be stressed that no real novelty is presented in this paper; the new knowledge generated (endophytic bacterial isolates producing antimicrobials and other enzymes) is only of a local and/or market specific interest. Moreover, I found the Results and Discussion section merely descriptive of Results, and very poorly (if not at all!) discussed. As I mentioned at the end, this manuscript must justify a lot better its publication: it is with interesting arguments and ideas at the Discussion section that the authors can do it! I would not accept a MS so poorly discussed for publication – it would encourage authors worldwide to present only this undesirable, "too-easily-done" type of MS for publication… To me, this is not a good contribution to science and society (who ultimately pays for scientific research). I'm sorry!... Considering that the number of isolates found and characterized was not so large (only 11), I ask the authors if it would be an unsurmontable problem to sequence their 16s rDNA, so that a better taxonomic classification could be provided? This would not only improve a lot the findings of the overall functional diversity type of work that was done, but may also reveal new aspects of the isolates that can be interesting and useful in the bioprospection and biodiversity context. And, obviously, it would give a whole lot of further opportunities for discussion in the paper. See just an example of what I'm saying: the isolate HGL101 appeared as a multiple antibiotic resistant microbe – is it	

	belonging (or very close) to what kind of species? What would be the chances and, or opportunities to transfer horizontally this resistance to any other dangerous human pathogen? Would that be any kind of industrial (biotech) type of use for it, given this multiple resistance phenotype? Would that has something to do with the fact that it was the isolate with the second largest enzyme profile and one with the largest tolerance level to NaCl (Table 2) ? And so and so on… Throughout the paper, based on the nice set of data they present, the authors lead the reader to some expectation of what kind of biotechnological applications these isolates can be employed; however, this was never explored in the text. If no specific biotech application is suggested and why, a lot of the readers' interest in using and citing the information provided in this paper is lost. Finally, the results definitely do not allow the authors to conclude that there's a "high diversity" found in their work (L276)! Only 11 isolates certainly do not justify such an statement. There was only a brief mention about more than those 11 isolates in the first paragraph of Results and Discussion, but this needed to be much better explained for this "high diversity" claim to be roughly considered. Therefore, this conclusion is not valid and the statement must be rephrased. However, the conclusion that the 11 isolates showed to be very different form each other is acceptable. The other two sentences of Conclusions are questionable: first, I believe that some isolates being effective against a higher range of other bacteria (Table 6) is more relevant information that most of them being active against only two species (as it was stated); in addition, this was not a valid conclusion statement: the authors should say something like "antimicrobial activity against various bacterial species does exist in the culturable endophytes of *Hygrophila spinosa*" which is more general and suitable to one of the objectives stated in the **Abstract**. Second, without having previously explored in the MS text which are the potential biotech applications of the isolates, the last sentence, hence, lacks validity and meaning	

Minor
REVISION
comments

Abstract

L18 → "…*This study aims to determine the* ***culturable*** *endophytic bacterial diversity*…"

L21 → The word "trptic" at the end of the line is likely missing a "y"…

L22 & L30 → be consistent with the use of 'physio-biochemical': apparently this form is more common than 'physiobiochemical'.

Introduction

I provided some suggestions of change below, but this section has potential ethical issues to be handled (see "Part 3")

L52 → "…~~*resolving*~~ ***helping with a*** *wide variety of human health* ~~*hazards*~~ ***issues*** *[or problems]*".
L54 → "…*mentioned in ancient ayurvedic literature* ***as*** *having great economic potential*".
L74 → "…*Recent* ~~*research*~~ ***studies*** *have proven that microbial*…"
L76 → "…*source of novel natural products****, as they*** *possess*~~*ing*~~ *antimicrobial, antifungal,* ***and*** *antiviral*…"
L77 → "… *antioxidants, cytotoxic activities, etc.****, suitable*** *for exploitation*…"
L81 → "…threat***s*** of drug resistant strains of human and plant pathogen***s***…"
L83 → remove the colon (:) after the word "including".
L92 → "…*derived* ***from*** *endophytic fungi [3, 7, 10, 11].*"
L93 → "…*occurrence****,*** *as well as* ***on the*** *potential significance* …"
L96 → "…~~*Our*~~ ***In the*** *present study****,*** ***we*** *focuses*~~*s*~~***d*** ~~*attention towards*~~ ***on the*** *isolation, characterization*…"

Materials and Methods

L112 → remove the full citation "(Sun et al, 2008)" from the text.
L126 → "…*colonization frequency and isolation rate* ~~*was*~~ ***were*** *calculated."*
L147 → separate the word "Petriplate" into two: "Petri plates"…

Results and Discussion

	L161,162 → write the Arabic numbers '6', '3' and '2' (referring to the number of isolates) in words! The internationally accepted rules for this thing are that numbers < 10 (zero to nine) must be written out, except if they are followed by any measuring unit; for instance: should write "three isolates", but can write "5 mL"… L162,163 → "…***The*** *colonization frequenc~~ies~~**y** was ~~recorded~~ low**er** in leaf samples*…" L168 → the word "*more*" is missing right before "diverse types". L174-L183 → issues regarding **Table 1**. ***(i)*** "SI. No." column is not needed; please remove. ***(ii)*** The label of the last column ("Total") is confusing, as the values are *totals* only for the first three parameters (which I think would be better off called as "variables"); for the last three ones, the values are *averages* of the three plant parts rather than "totals"! ***(iii)*** I believe that consistency in labeling variables and indexes that are used worldwide is important to ease communication and understanding, and avoiding ambiguity; I suggest the use of "Shannon-Weaver" in the hyphenated form throughout the text. ***(iv)*** It would be interesting to provide some discussion in the text about what would explain a higher diversity/isolation rate in leaves than in other organs… L189-191 → same issue raised for L161, 162 above – please fix it. Besides, there is no justifiable reason to write "+ve" and "-ve" for the results of the Gram test! It is perfectly understable to write just the symbols "+" and "–", although I rather prefer them always written out fully, exactly in the same way done in L192 [Please no that it is "*three isolates were Gram negative*" (non-hyphenated), but it will be "*three Gram-negative isolates*" (hyphenated). L195-200 → issues regarding **Table 2**. ***(i)*** Use "pos" and "neg" for the entries in the "Gram nature" column, in order to avoid confusion with all other "+" and "–" in the Table. ***(ii)*** At the Table's footnote, change the first line in a way to refer to the "+" and "–" signs as *presence/absence* rather than "positive/negative response"; the same should be done for Table 3, ***but not*** for Table 4. L244 → "…*resistance genes might have **been** transferred horizontally*…" L252 → at first line of Table 5's footnote, delete "*NI=no inhibition*".	

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Optional/ General comments	My general opinion is that this is a nicely presented and well written (mostly) manuscript that brings sound scientific experimentation and interesting data and results, having a good potential to be published. To deserve publication, however, I feel their findings must be fully discussed, as a lot of interesting things are apparently happening in their experimental system; this was presented as a merely descriptive, underexplored MS that *does not contribute to science in its current form*. I also feel this paper needs to stress out more the worldwide relevance of the findings in terms of potential market of this plant species as source of pharmaceutical compounds and biological control agents through their specific content of bacterial endophytes. Besides, I suggest addition of a couple of sentences in the **Introduction** section to explain better why focusing on the culturable endophytic bacterial diversity rather than on a more throrough assessment of diversity by molecular methods, such as DGGE, T-RFLP, ARISA, 16s rDNA library making and sequencing, etc. Arguments such as that culturable isolates are required for further development of microbial-based biotechnological products and formulations, or that culturable approaches are enough for the research purposes at a lower consumables (and equipment?) costs, are valid and important in my opinion, mainly if we consider some urgent needs of developing countries…	

Note: Anonymous Reviewer