



SDI Review Form 1.5

PART A:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	MS: 2012/BMRJ/1605
Title of the Manuscript:	Assessment of antigen preparation methods for polyclonal antibody production against <i>Streptomyces</i> spp.

(Note: Title of different sections as proposed below may differ in case of review paper)



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PART B: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part and write here 'Corrected' if not agreed, give suitable justifications)
General comment:	This manuscript describes the evaluation of a methodology to prepare <i>Streptomyces</i> spp. antigen with the purpose of producing an immune serum in rabbit.	
Specific comments:	I have minor concerns and modifications: Results and discussion line 109: from the autoclaving and sonication method, but not WITH the boiling method. line 110 (rewrite): ...higher resolution profile than either the 1 min sonication or the autoclaving methods. line 147: Rabbit anti-<i>Streptomyces</i> sp. isolate STPR78 ANTIBODY/SERUM Please use any of these words every time you refer to the specific serum.	Corrected. We have revised the manuscript in accordance with the reviewer's suggestions (Section 3.1, Page 4, line 137-139). Corrected. We added the word "antibody" after the specific serum "Rabbit anti-<i>Streptomyces</i> sp. isolate STPR78 and Rabbit anti-<i>Streptomyces</i> sp. isolate STPR84" throughout the manuscript.
Title and abstract	In my opinion, the title should be changed. The manuscript actually describes the EVALUATION of a method to prepare <i>Streptomyces</i> spp. antigen. The word "assessment" is not suitable in this case since the authors have not performed either a standardization of the methodology or statistical analysis of the results.	Corrected. We have change the word "Assessment" to "Evaluation" in the title.
Introduction (Is the problem/objective of this study original, important and well defined?)	The introduction is clear and poses the objectives of the work	
Are all the references cited relevant, adequate? Are there any other suitable current references authors need to cite?		
Materials & methods (Kindly comment on the suitability of the methods. Sufficient details of the methods should be provided to allow peers to evaluate and/or replicate the work)	The methodology is well described. When referring to the speed of centrifugation, the value should be in "g" rather than "rpm" in order to make the method reproducible by others. Instead, the specification of the centrifuge should be stated.	Corrected. We have changed the speed of centrifugation from "rpm" to "g" (lines 66, 75 and 123). We also added the specification of the centrifuge (Section 2.2, Page 2, line 65).
Results & discussion (Kindly comment on: 1. Are the data well controlled and robust? 2. Authors should provide relevant references during discussion. 3. Discussion and conclusions should be based on actual facts and figures. Biased claims should be pointed out. 4. Are statistical	In the abstract and also in the results section, the authors refers to "sensitivity detection limit" The limit of detection is defined as the lowest quantity of a substance that can be distinguished from the absence of that substance (in the	Corrected



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analyses must for this paper? If yes, have sufficient and appropriate statistical analyses been carried out?)	corresponding matrix) within a stated confidence limit The analytical sensitivity is the lowest level of a substance that can be detected by an analytic method. In fact, both definitions are similar, but one of them should be used instead of a combination of both. In addition, the limit of detection is usually expressed as a value within an interval of confidence, but not as a range. This reviewer strongly suggests the authors to calculate some parameters of precision of the method described in the manuscript. For example, it will be very useful to determine intra- and inter assay variation for the preparation of the antigen and also for the indirect ELISA to test the polyclonal serum. The calculation of the limit of the detection should be done in a certain number of independent assays so as to give statistical significance to the value obtained. In the experiment to test the antibody specificity, it is not clear which is the cut-off value of the ELISA used. It seems to be 0.08 but the reason why of choosing that value should be explained with statistical support. Finally, I think the experimental design to detect <i>Streptomyces</i> antigen in soil could be optimized. In my opinion the spike-in of the soil should be done with the whole bacteria and then that mix should be treated as described in Mat and Meth (2.3).The same procedure has to be done to calculate the limit of detection, since the matrix where the molecule to detect is, can interfere with the assay.	Corrected. We have analyzed the data for intra- and inter assay variation and revised the manuscript (Section 2.6, Page 3, line 103-108). Corrected. We performed new experiments to determine the sensitivity detection limit. These are mentioned in Section 2.7, Page 3, line 109-114. Corrected. We have revised the manuscript accordingly (Section 2.8, Page 4, line 124-125). Corrected. We agree with the reviewer's suggestion to optimize detection of <i>Streptomyces</i> in soil before application. However, the spike-in of the soil with whole bacteria prior to sonication can elicit cytoplasmic components from contaminants and environmental <i>Streptomyces</i>. For our study, antibody against the cytoplasmic antigens of <i>Streptomyces</i> was produced by immunizing with sonicated antigen. Therefore, the elicited components from contaminants or environmental <i>Streptomyces</i> may have cross-reacted or reduced the sensitivity of <i>Streptomyces</i> antibody. This is mentioned in Section 3.4, 3.5 and 4.
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Are there any ethical or competing interest issues?		
Conclusion (Is the conclusion supported by the data, discussed inside the manuscript? Conclusions should not be biased and should be based on the data, presented inside the manuscript only)	The antibodies are not suitable for soil microbiology in the conditions of the manuscript. There are not enough experiments conducted by the authors to reject the use of the immune serum. Instead, utilization of the antibodies obtained should be optimized.	Corrected. We have revised the manuscript accordingly (Section 4, Page 7, line 237-240).
References	There are no missing references. The references are adequate.	Corrected