



SDI Review Form 1.6

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	2013_IJTDH_5808
Title of the Manuscript:	Rv2485c, a putative lipase of M. tuberculosis: expression, purification and biochemical characterization
Type of the Article	Research Paper

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:

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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	1. The English is poor. The authors have to have their manuscript edited by a native English speakers or an English-editing company. 2. Some descriptions of the results and experiments are ambiguous, and need to be modified or corrected. The authors should double check their manuscript throughout. 3. L199-200, L407, and else: The data under the single condition (5mM H₂O₂, pH 4.5, 1 x phosphate buffer saline) are not enough to make a conclusion for overall acidic, oxidative, nutrient stress conditions.	(Reviewers kindly note that due to addition of some data and lines into the manuscript, the line numbers have been changed, so they are requested to check for the corrections accordingly and look for the highlighted parts in the manuscript) 1. Manuscript has been edited for English language and modified. 2. Corrections added 3. The stress conditions that are used in the research are adapted from previous established research articles. References number 22,23,24 are newly added in the main manuscript.
Minor REVISION comments	1. In the Abstract and Results sections: the expression assays of the Rv2485c gene in M. tuberculosis cultures should be placed before the studies of cloning and expression of the gene in E. coli, thus providing the in vivo meaning of the gene.	1. Correction is made as per suggestion.



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	2. In the Abstract: what is your conclusion? 3. L88: Please list the GenBank number of Rv2485c. 4. L229: No data present for ML1243. 5. Please use consistent phrases or words for kDa/daltons, pNP palmitate/pNP-palmitate, etc. throughout text. 6. L296: PMSF and DEPC inhibited the enzyme activity to nearly 60% and 80%, instead of 50% and 60%. (figure 4). 7. L326: Which kinds of amino acid changed from the Ser-249, Asp-344 and His-377 for site-directed mutagenesis of LipQ? 8. L340: Please make it clear for the no expression of Rv2485c: the expression in the control was used as a reference or it couldn't be expressed in the control.	2. Conclusion in the abstract has been added as per suggestion 3. GenBank number of Rv2485c: 887876 4. There was no % similarity of ML1243 and <i>M. abscessus</i> gene product with Rv2485c protein, therefore both the species name has been removed from the table. 5. The required corrections are made. 6. PMSF and DEPC inhibited the enzyme activity to nearly 60% to 80% according to the figure 4. Line Corrected. 7. For site directed mutagenesis, the amino acids were changed as followed: Ser249 - Ala249 Asp289 - Ala289 Asp344 - Ala344 His377 - Pro377 8. The gene Rv2485c is not expressed at all in conventional RT PCR. And in real time PCR, the expression was almost equal to 1 i.e. negligible in the control. The histogram is added in the main manuscript showing the expression
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	9. L392: The optimum temperature of lipQ was at 40°C, but PMSF and DEPC were pre-incubated at 50°C. please explain it.	analysis of Rv2485c, which further explains the table showing the values for gene expression. 9. Although the optimum temperature of the protein is 40°C but it was preincubated at 50°C with PMSF and DEPC to make the active site accessible to inhibitors because in many cases the proteins active site may be buried inside a hydrophobic core . The protein itself did not loose much activity after incubation at 50°C for 5 min (more than 95% activity retained, data not shown).
Optional/General comments	The studies in this paper described for the cloning, expression, purification and biochemical characterization of Rv2485c are simple and routine. The RT-PCR results of the Rv2485c gene in <i>M. tuberculosis</i> H37Ra under different stress conditions make some sense.	