



SDI Review Form 1.6

PART 1:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	MS: 2013_BMRJ_3068
Title of the Manuscript:	Lactobacillus rhamnosusGG (LGG) protects cells from Clostridium difficile toxins.

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 9 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	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)
<u>Compulsory</u> REVISION comments	1. Line 72 – Why did the authors pick this particular toxigenic strain? It would perhaps be more thorough to use a variety of clinical isolates or a supertoxigenic strain to demonstrate the generalizability of the results. 2. Line 87 – Why do the authors only dilute the C. difficile to 1×10^7 (1:100)? This doesn't offer much scope for the bacteria to grow, and means that they are transporting over a significant amount of preformed toxin in the culture supernatant. If they used a more dilute culture they may have seen more dramatic effects... 3. Line 124 – I may be missing something here...The authors attempt to measure the expression levels of TcdA and TcdB by using quantitative PCR. I did a similar study in my previous paper on the subject. However, the authors extract the DNA from sonicates of co-cultures and perform qPCR on these samples. The PaLoc of C. difficile is chromosomal, and so only exists one copy per cell. Therefore, the only thing that this PCR is showing is the number of C. difficile cells in the co-culture. Thankfully, this is consistent with their culture data! If the authors want to measure the expression levels of the TcdA and TcdB	Thank you very much for your comments. We try to address each question/suggestion accordingly. 1. Line 72-We picked this particular strain after assessing the degree of cytotoxicity induced on Vero cells. The ATCC strain caused the highest level of toxicity compared with other strains in our lab. 2. Line 87-It has been reported that C. difficile infections could result in the growth of some strains in internal organs to more than 1×10^7 in less than two days (Buckley et al., 2011). We wanted to see the effect of the probiotic at that high level. We agree that the effect would be more dramatic if the number was lower. 3. Line 124-Line 154-We would like to acknowledge the reviewer's suggestion on this point. We have reviewed the manuscript accordingly using the real time PCR data to support our results in the growth inhibition of the toxigenic C. difficile strain. 4. Line 148-We were comparing the counts in co-cultures with pure C.difficile culture counts, which grew by 2 logs after the incubation period. We have rephrased the observations according to the reviewers suggestions.



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	genes, they need to extract the mRNA from the cells and do an RTPCR followed by a qPCR on the cDNA (method in Woo et al. 2011). I recommend that this is done and the paper resubmitted. Another option would be to omit this information altogether. 4. Line 154 – Why did the authors choose to quote Ct numbers rather than absolute quantities? The beauty of qPCR is that it is quantitative. The Ct numbers are meaningless without being able to relate them to a standard curve. All we can tell from this data is that there is indeed less DNA present, but not by how much. As they should be using RNA anyway, I suggest that the authors repeat the experiment, quantify the RNA (which will undoubtedly be decreased as there is growth inhibition) and use a standard curve to present results in copy number. 5. Line 168 – You state that in co-culture the “counts were decreased by 2 logs”. This is actually untrue - the inoculum was 10^7, so in fact the <i>C. difficile</i> in the co-cultures has not changed at all (though it does grow slightly in the 1:1000 co-culture). This is perhaps a more significant result than you make it out to be! 6. Line 254 – Not so! As I said - apparently the authors are measuring DNA counts. They should measure mRNA and relate these to the growth inhibition observed – is mRNA copies/cell reduced?	5. Line 254-We have not measured mRNA, so we are using the data to support the observed growth inhibition as suggested.
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Minor REVISION comments	There are minor typos throughout the script – some probably as a result of the pdf conversion process. Please check spelling etc.	We have corrected the typos
Optional/General comments	I suspect that the cytotoxicity results that the authors obtain in this experiment are the result of growth inhibition (?nutrient outcompetition) of <i>C. difficile</i> . As the results from the co-culture show, <i>C. difficile</i> does not grow much above its starting inoculum number, and we get an increase in cell survivability from 15% to 30ish%.	Thank you very much for your comments.