



SDI FINAL EVALUATION FORM 1.1

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.

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
Thank you for correcting the paper as you have done and acknowledging the comments. It is a shame that the RTPCR data was not more insightful – I imagine that you would like to measure the toxin mRNA levels and quantify the DNA properly using standard curve, but can't due to limited funding? As it stands the RTPCR data doesn't really add anything to the paper, but that can't be helped as the analyses would need to be rerun. I recommend adding a section in the conclusion/discussion about the limitations of the method and further work that you would like to undertake to support the mechanism by which your observed effect takes place. The only other thing I recommend is that figure 5 be reworked – At the moment the sizes of the two graphs are different, the font size and type is different and the background colours don't match. Compared to the other polished figures it looks like it needs work. As I said before I've not seen any RTPCR paper that uses Ct as a result variable – I'm sure you know that Ct is not simply indirectly proportional to copy number and a small variation in Ct can represent a large difference in copies. I hope that your team will continue this research and come up with more robust data.	Thank you very much again for your valuable comments. The study was actually completed in 2011 and the reason we could not add an experiment targeting the mRNA expression was mainly because the first author, who was responsible for the lab work, is no more working in the labs, where the study was conducted. As recommended, we have added the limitations of our study and suggested further investigation. We have improved the qualities of the figures. We understand that Ct values are not the best way to report RTPCR data. However, Folkers et al. (2010) also used Ct as a result variable to compare inhibition of <i>C. difficile</i> in co-cultures containing various probiotics. Since we did not run a standard known concentration of DNA along with our samples, we could not know the copy numbers. Thus we used the Ct values as a relative way of comparing the abundance of <i>C. difficile</i> in the co-cultures with the controls. We believe that this preliminary data will help to get more funding to conduct various mechanistic studies that are needed to understand our observations indicating the inhibitory effects of LGG against <i>C. difficile</i>.