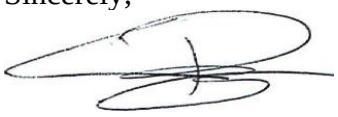
Dear ISME J Editorial team,

On behalf of all authors, please find responses to each of the requesting revisions in relation to our manuscript "*Intestinal persistence of Bifidobacterium infantis is determined by interaction of host genetics and antibiotic exposure*" below.

Each of the requested changes are presented in blue (e.g., **C1**), followed by our response in black (e.g., **R1**). Where these comments have resulted in revisions to the manuscript, we have copied this text into this response document, as appropriate. Our responses also include a line number to the revised section in the amended manuscript document. All authors have contributed to the manuscript and this revision and have seen and approved the final version.

Thank you for considering our manuscript for publication.

Sincerely,



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Editor-in-chief (Jillian Petersen)

C1: Thank you for submitting your work to our society journal. Although we intend to accept your manuscript, there are a few minor edits required - see comments below.

R1: Below, we provide a point-by-point response to all the issues raised. We confirm that the resubmission includes:

- A clean version of the revised manuscript.
- A track change PDF version.

Senior Editor (Richard Lamont)

C2: The reviewers appreciate the care taken in addressing their comments and have only a few minor suggestions for consideration.

R2: We thank the editorial team for their consideration of this work. Below, we detail our responses to each of the comments.

Specific Edits:

C4: Left justify text.

R4: Revised accordingly.

C5: Oxford commas needed consistently throughout.

R5: We have undertaken a line-by-line review to ensure consistent use.

C6: Line 89: "microorganisms" I think - viruses not included in this context.

R6: The term “microbiota” has been replaced by “microorganisms” as suggested.

Original text: "However, as fucosylated glycans are an important nutrient source for gut microbes, their absence in non-secretors has been shown to influence commensal microbiota composition (16, 17)."

Revised text (Line 91): "However, as fucosylated glycans are an important nutrient source for gut microbes, their absence in non-secretors has been shown to influence the composition of commensal microorganisms (16, 17)."

C7: Line 148: "16S" never on its own.

R7: This has been amended to "16S rRNA gene" (**Line 152-153**).

C8: Line 201: use the actual name of the kit ("Illumina MiSeq" not part of the name), line 202 needs a "S" for "MiSeq" and the instrument used for sequencing not specified explicitly in the methods. I suspect it's a MiSeq System (Illumina).

R8: We have confirmed that they name of the kit is “MiSeq Reagent Kit v3”. To avoid confusion, we have placed the kit name in a bracket (Source: https://www.illumina.com/products/by-type/sequencing-kits/cluster-gen-sequencing-reagents/miseq-reagent-kit-v3.html). In addition, we have capitalized the "S" in "MiSeq" and specified the sequencing instrument.

Revised Text (Line 205-209): "Amplicon libraries were indexed, cleaned, and sequenced according to the 16S Metagenomic Sequencing Library Preparation protocol. Paired-end sequencing was performed using MiSeq Reagent Kit v3 (600-cycle kit) (Illumina) on a MiSeq Sequencing System (Illumina), at the South Australian Genomics Centre (SAGC).”

C9: Line 251: delete "600 nm, "?
R9: Revised accordingly (**Line 259, Line 268**).

C10: Lines 277-287: R2 values to 2 decimal places only. Some of these R2 values are very low - even if significant, seems odd to highlight these as effects. Be cautious?
R10: Thank you for your feedback. We agree that R^2 values should be reported to two decimal places for consistency and clarity and have revised the text accordingly.

Regarding the R^2 values, we felt it was best to err on the side of transparency and reported them so that readers are aware that, in some cases, they are low. However, we were careful to exercise caution when interpreting these values. While significance was found in the combined (male and female) group and in the female group, we highlight that the microbiota composition difference was most evident in the male group (see Discussion section, **Line 510-512**). This conclusion is supported by the higher R^2 value in male mice (R^2=0.12) compared to female mice (R^2=0.04) and in combined group (R^2=0.03).

C11: Line 301: delete "The" and "is"
R11: Amended accordingly (**Line 323**)

C12: Line 334: delete "next"
R12: Amended accordingly (**Line 359**)

C13: Line 403: perhaps delete "Firstly," here?
R13: Amended accordingly (**Line 429**)

C14: Line 429: delete "Secondly," (mainly to ensure paragraph independence).
R14: Amended accordingly (**Line 456**)

C15: Line 461: "Although" instead of "While"
R15: Amended accordingly (**Line 499**)

C16: References need a thorough polishing edit, one by one, as per the checklist.
R16: Apologies, a further thorough review did indeed identify some errors. We believe that the reference list is now correct. We do note that there are some differences in the formatting set out in author guidelines and as formatted articles appear in ISME J (e.g., italicisation), but have adhered to the former.

C17: Figures - Any axis label that should end with (%) should do so and, when doing so, all % values shown for tick labels should be removed. Tighten up spacing accordingly. Note that Fig. 3D missing a y axis label altogether.
R17: Axis labels have been adjusted to end with (%) where appropriate, % values for tick labels have been removed, and spacing has been tightened. The y axis label for Fig. 3D has been added.

C18: Panel letters should be same font as rest of the figure font (Arial or Helvetica) and should not be followed by periods.
R18: Panel letters have been adjusted to match the rest of the figure font (Helvetica) and periods have been removed (**See revised Figure 1-5**)
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C19: Figure 4 and Figure 5 have a key at the top that is surrounded by a long box with a grey fill. Try removing these boxes/fills and see if this helps declutter?
R19: Amended accordingly (**See revised Figure 4 and Figure 5**)

REVIEWER #1:
C20: The authors have appropriately addressed all comments raised by the referee. One final suggestion is to modify the sentence on line 297 to "Lactobacillus, a genus with GH29 and GH151 that comprise a-1,2-fucosidases," as GH29 also includes a-1,3-, 1,4-, or 1,6-specific fucosidases.
R20: We have made the requested modifications to the sentence (**Line 317**).

REVIEWER #2:
C3: In addition to the points below, Reviewer #2 would like to see responses R13, R16, R17, R18 from the previous critique incorporated in the manuscript if possible. Regarding Reviewer #3's comment, please just confirm all sequencing data were deposited in NCBI under PRJNA1011386.
R3: We have incorporated responses R13, R16, R17, R18 from the previous critique into the manuscript as requested. Additionally, we confirm that all sequencing data have been deposited in NCBI under the accession code PRJNA1011386 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1011386/).

Line 519-529 (R13 in previous response document)
We acknowledge the importance of considering blood antigens/ABO phenotypes in interpreting the influence of *FUT2* gene on the gut microbiome, as indicated by recent studies [61, 62]. Indeed, in humans, *FUT2* is responsible for the generation of the H antigen, which can be further modified to give the OLewis^{b}, ALewis^{b}, or BLewis^{b} antigens [63]. Each of these glycans can modulate the competitive advantage of particular microbes capable of cleaving the oligosaccharide constituents. In the absence of *FUT2*, these Lewis^{b} antigens are not displayed, leading to a Lewis^{a} antigen. While our study did not address these blood type variations, it should be noted that even in humans, a secretor O blood group and a non-secretor O blood group are not the same. The impact of this on the gut microbiome is evidenced by studies reporting an association between H antigen concentrations and gut microbiome characteristics [58].

Line 468-476 (R16 in previous response document)
It should be noted that our antibiotic mix contained a cocktail of ampicillin and neomycin, designed to deplete a wide range of bacteria. While most *Bifidobacterium* strains are resistant to neomycin, the tested strains are sensitive to ampicillin [47]. We designed the experiment so that gavage with *Bifidobacterium* was immediately after ceasing antibiotic depletion to maximize colonisation without competition from other bacteria. It is possible that residual antibiotics in the intestine deplete *Bifidobacterium* over the first days of gavage. For this reason, we performed gavage for 5 days, a time period that extends beyond the activity spectrum of the administered antibiotics. Such an antibiotic combination is common for mouse models [48, 49], as well as empiric for suspected sepsis in humans [50].

Line 240-243 (R17 in previous response document)
We investigated the extent to which 16S amplicon sequencing could discriminate between different *Bifidobacterium* species. As expected, level 7 resolution (species-level output) was

unable to differentiate bifidobacterial strains, reflecting a well-recognised limitation of this approach. Given that, the quantification of … was performed

Line 145-147 (R18 in previous response document)
Given the heterogeneous nature of the gut microbiome, each mouse was considered as a biological replicate rather than a technical replicate, even within cohoused littermates.

REVIEWER #3:
Q21: The authors have soundly addressed all my comments. No further comments from my side. Prior to publication, the authors should deposit their sequence data in a public database.
R21: We thank Reviewer 3 for their constructive feedback. We confirm here that all sequencing data have been deposited in NCBI under the accession code PRJNA1011386.