Review of: "Growth inhibition of Akkermansia muciniphila by a secreted pathobiont sialidase"

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Review "Growth inhibition of *Akkermansia muciniphila* by a secreted pathobiont sialidase"

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In their preprint paper "Growth inhibition of *Akkermansia muciniphila* by a secreted pathobiont sialidase" (version posted March 15, 2022), Van Muijlwijk *et al.* nicely demonstrate that the growth of *Akkermansia muciniphila* on trypticase peptone is inhibited after desialylation of this substrate by pathobiont *Allobaculum mucolyticum.* These findings derive from a delicate top-down approach starting with conditioned medium from *A. mucolyticum* and eventually pointing at *A. mucolyticum* NanH1 sialidase as the candidate enzyme most likely to exert the observed inhibitory effect, as supported by multiple lines of evidence. The fact that sialidases from other pathobionts similarly inhibit growth of *A. muciniphila* on trypticase peptone, and that the inhibitory effect can be rescued by addition of sialic acid, demonstrates the sialidase-specific and not bacterial-specific mode of action. This is interesting, given the expression of sialidases by mucin-degrading specialist *A. muciniphila* itself. Although the authors propose some interesting explanations for their findings, we have several concerns regarding the chosen experimental set-up and the lack of reporting of details that might be relevant for the interpretation of the data.

Major concerns

- A general major concern is the choice of medium, and more in particular, the selection of trypticase peptone as a proxy for glycosylated mucin. The use of Porcine Gastric Mucin or another, preferably human, mucin would more accurately represent the physiological conditions under which *A. muciniphila* resides in the human gut. Especially as the authors mention that these bacteria reside in the same mucosal niche *in vivo*, where they compete for space and nutrients (line 449-450). However, no reasoning is provided why they chose not to use more physiologically relevant mucosal nutrient sources. The work would benefit from repetition of the experimental efforts in a mucin or more mucin-replicating environment, to evaluate the relevance of the findings for the *in vivo* physiological conditions. This latter point should also be more critically taken up in the discussion.

- Alternatively, if trypticase peptone is indeed considered the best alternative, it would be informative to provide more information on the glycosylation of this substrate or, more specifically, provide evidence on sialidase content before and after desialylation.
- The evidence on the inhibitory effect of a pathobiont sialidase on growth inhibition is limited tœa single (mucin-degrading) bacterium and a single substrate. The authors do not demonstrate or discuss whether the same effect is observed for other (sialylated) substrates (see also concern above, regarding the use of mucin) or other (commensal) bacteria (is the effect observed specific for *Akkermansia* or also other glycan-degrading bacteria (such as *A. mucolyticum*?). The authors' perspective on this should at least be included in the discussion.
- Line 25-27, Line 59-62: The proposal of new therapeutic approaches is not well supported by concrete suggestions or ideas in the manuscript. This notion should therefore either be further substantiated or deleted
- Line 83 – "clinical isolate": Please state which isolate was used, especially as recent work reported on the existence of different *Akkermansia muciniphila* subspecies (Karcher *et al* 2021). It is interesting to know in which category the used clinical isolate falls, as it might have different properties from that of the well-studied *A.muciniphila* type strain Muc^T.

Minor concerns

- In some graphs, growth is reported as "% of MilliQ control", whereas in others absolute OD is reported. The latter is preferred to enable comparison between experiments and media.
- Line 390 – "Substrate specificity": We agree that substrate specificity could explain the differences in inhibitory potential observed. It would be informative for the reader, however, to include a (supplementary) figure or some lines and references on the actual preferred substrate per sialidase to support this statement.
- The exact composition of the defined minimal medium should be mentioned in the M&M
- Throughout the manuscript, the authors often refer to "Thesis chapter 3" or "Chapter 3", which should be replaced by the appropriate reference.
- Line 432-434: "these results demonstrate that *Allobaculum* inhibits *A. muciniphila* by secreting a sialidase that targets sialylated casein glycans that are critical for *A. muciniphila* growth": the authors should specify that this has only been shown in the media tested here.