**Reviewer's report**

**Title:** Surface-associated MUC5B mucins promote protease activity in Lactobacillus fermentum biofilms

**Version:** 1  **Date:** 21 June 2013

**Reviewer:** Mireya Felhazy

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Surface-associated MUC5B mucins promote protease activity in Lactobacillus fermentum biofilms

Overall this is a very straightforward paper with some clinical relevance in biofilm formation.

Some recommendations are provided in order to improve the content of the article as follows:

**Major Compulsory Revisions:**

**Methods:**

Lane# 100: Sample from supra-gingival dental plaque, needs clarification. I.e. How many samples were taken? From which site(s) in the oral cavity were the samples taken? Was the sample from one donor or multiple donors? What was the health status of the donor(s) and age?

Lane# 109: The authors mention a gentle centrifugation but there are not parameters indicated such as time and centrifugation force used. In the same line they mentioned about density-gradient centrifugation and there aren’t any specifications about force, time, temperature and type of centrifuge used etc.).

Lane # 110: The authors described the MUC5B-containing fractions were pooled and dialyzed. This section needs to be explained in detail. How they collect the fractions? How many fractions? Did they do an SDS-PAGE gel to verify the purity/quality of the fractions? If so it would be worth to add it to the article maybe as a supplemental figure.

Lane #136: Was there any criteria used to select randomly the areas that were imaged?

Lane #260: “Specific chaperone proteins” which ones? And give a brief explanation of what they could possibly be doing to increase and trigger this proteolytic activity.

• No WB for detection of O-sialoglycoprotein endopeptidase (lanes #178-179, lanes #244-245) was mentioned or shown neither in the results section nor in the figures.

**Discussion:**

Lane # 288: Elaborate on the conformation of the molecules is very vague and
cite references.

Lane #305-307: Where are the data corresponding to the “subsequent comparative proteomics analysis”? How was it done? There is not even a table displaying the proteomics data that is in fact necessary to add to this article or the corresponding Mass spectra figures revealing this glycoprotease high expression.

Minor Essential Revisions:

Lane # 4. The Font style is different and needs to be modified.
Lane # 26 Correct the phrase by this specie or these species.
Lane # 34: Protein core needs to be defined or explained. Is there a specific area that target? Explain briefly.
Lane # 78: Write (32-Mmubp) and remove both [, ].
Lane # 79: Remove the [ ,]and remove the words [has been].
Lane # 84: Define which membrane transporters and give references.
Lane # 87: The word [for] is repeated twice.
Lane # 111: Which method was used for calculating the MUC5B concentration?
Lane # 119: The channels were rinsed with what?
Lane # 136: Was there any criteria used to select randomly the areas that were imaged?
Lane # 138: Explained briefly how the data was analyzed using the Mann-Whitney U statistical test and add reference(s).
Lane # 144: Centrifugation parameters need to be included and it is necessary to clarify the paragraph related to the mucins surrounding the biofilm cells how they were collected (if so) before analysis by SDS and WB. This is confusing.
Lane # 159: How did they remove the bulk phase surrounding biofilm cells?
Lane # 234: Eliminate term at the origin which creates confusion. It can be replaced by “bacteria, were visualized/or identified as a single band”
Lane # 235: After weight, write the appropriate molecular weight of this band between parentheses. Also in the same line migrated is confusing. Yes there is migration or appearance of a second band of different molecular weight but I still can observe the high molecular weight one. This paragraph needs to be more elaborated.
Lane # 236: “proteolytic degradation” Could this effect be possible related to deglycosylation instead? Need references to support evidence.
Lane # 253: Explain or justify the use of Coomasie. For the authors there is silver staining compatible with Mass spectrometry analysis and Coomasie has a lower sensitivity for visualizing/detecting bands. It would be appropriate to include in supplemental data de images of the gels stained by Coomasie to see how comparable are with the ones stained with silver and shown in this article.
Lane # 293: Use term respond instead of response.
Lanes #315: The term “which are highly substituted” is incorrect. The serine and threonine are not substituted. Carbohydrates/glycans are linked to these amino acids. Authors need to mend this and cite relevant references.

Figure 4: Correct electrophoretic origin or explain the meaning of it. Also add the corresponding molecular weight markers to the corresponding bands displayed.

Figure 5: Label with arrows and names the corresponding proteins in gel #2.

Discretionary Revisions:

There should be consistency throughout the whole manuscript regarding the use of present or part tense verbs. Many sentences have a combination of both.

In addition there should be uniformity in writing the lab companies from which reagents and equipment were acquired (lanes #: 118, 125, 129, 133, 138, 147, 148, 149, 155, 156, 165, 168, 171, 174, 180, and 181). Example: use the same pattern as lane #123

Correct grammar typos.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

The reviewer declares that has no competing interests.