**Reviewer’s report**

**Title:** Genetic polymorphism of scrA gene of Streptococcus mutans isolates is not associated with biofilm formation in severe early childhood caries

**Version:** 0  **Date:** 15 Mar 2017

**Reviewer:** Celine Levesque

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The aim of this study was to investigate if there was a correlation between genetic polymorphisms of scrA gene and biofilm formation using clinical strains of S. mutans isolated from caries-free (CF) children and children with severe-early childhood (S-ECC) caries. The authors tested 60 clinical strains of S. mutans (CF: 30; S-ECC: 30) for their ability to form biofilm in the presence of sucrose and found that strains isolated from S-ECC patients produced higher biofilm biomass compared to the strains isolated from the CF subjects. They also sequenced the scrA gene encoding the sucrose-specific IIABC component. They identified 17 different missense mutations but no significant difference between the two clinical groups was observed. The design of experimental setup seems appropriate. I have listed below some suggestions to help improve the manuscript.

1. P4, L16. Please provide more details regarding the biochemical testing performed for the correct identification of S. mutans species in the different plaque samples, especially the CF population. Different biochemical tests exist on the market. Although some of these kits are acceptable from a clinical point of view, the same kits cannot be used to differentiate all streptococcal species. PCR amplification of htrA gene (S. mutans-specific; Chen et al., 2007. FEMS Microbiol. Lett. 272:154-162) will confirm the presence of S. mutans and will help discriminate between S. mutans and S. sobrinus species.

2. Provide a schematic representation of scrA gene on the chromosome of UA159, the genome sequence reference strain, with the location of the primer pairs used for PCR amplification. Do the PCR amplification products overlap?
3. Delete Fig. 2.

4. Suggestion: Provide a typical DNA agarose gel showing the PCR bands obtained with the five different primer pairs used to amplify the scrA gene in S-ECC and CF vs. UA159 as positive control.

5. ScrA is a multiprotein system. In order to provide a more detail analysis of the results presented at Table 3, a figure showing the nucleotide and aa sequences of scrA locus of UA159 chromosome should be presented. On the figure, the following information must be provided: i) active site; ii) phosphorylation site; iii) Hpr interaction site; iv) different domains (EIIA, EIIB, EIIC). Several missense mutations were identified at codon 10, 1487, 1822, and 1863. Where are these codons located? Any specific domain? Are they representing mutation hotspots?

6. Proofread the manuscript for typos and italicize all bacterial names (text and references).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

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