Reviewer’s report

Title: Cariogenicity features of Streptococcus mutans in presence of rubusoside

Version: 1 Date: 23 Nov 2015

Reviewer: Cristiane Koga-Ito

Reviewer's report:

The authors revised the manuscript however some points still should be addressed:

1- Although most of the references on caries etiology had been revised, the main concept still has to be reviewed. The authors stated that "Dental caries is a multifactorial and chronic bacterial disease that involves the destruction of tooth hard tissue structure. It is directly caused by the acid produced by oral bacteria fermentation of dietary carbohydrates in dental plaque". The cited reference is from Dr. Zero (1999). However, in the last decades a lot of information has been produced on this issue and should be included in the manuscript. Please, see the manuscripts on the extended caries ecological hypothesis and others.

2- Please add reference to the statement "Rubusoside widely used as a natural sweetener for seasoning and additive in food industry".

3- In response to the question "The authors reported "the original cell suspension (1 mL) prepared was added to 15 ml of the various test solutions". What were these various test solutions? Please describe." , the authors answer that "S. mutans was cultured in a culture medium containing no sugar, and the pH values were the same in the 24 hours and 48 hours". Please, clarify this point. How S. mutans grew in a medium containing no sugar?

4- The authors stated that "In order to make S. mutans the best possible metabolizing all the sugar/substitute available in the culture medium, 48 hours was choosed.". However, it seems
that for determining the best incubation period, the establishment of a growth curve should be necessary. Please, clarify this point.

5- Regarding the answer for the question on the item 2.5., the authors stated that "This study is mainly to study the planktonic bacteria, without considering the single strain bacterial biofilm. The methodology used were based on some references. This is the deficiency of this study. The future study will to study the rubusoside impact on the biofilm."

- The cultures were incubated for 48 hours in the presence of carbohydrates. In our opinion, there is a single species biofilm formation. Do the authors disagree? Why do they believe that they are working with planktonic bacteria at this point?

6- The statement "Rubusoside may not be used as an energy source by MS so that no significant production of acids by these organisms will be followed. It may not act as the substrate for the synthesis of glucan by GTases of MS, however it may inhibit glucan synthesis from sucrose.", should be better clarified in the text.

7- As suggested before, the possible mechanisms of rubusoside should be included in the Discussion section.

Please, clarify the answer for the question

Respond to Reviewer: These various test solutions were described in Item 2.3. pH readings only after 48 hours is based on the references and our pre-experiment. (1. Drucker DB. Comparative effects of five chlorosucrose analogues on acidogenicity and adherence of the oral bacterium Streptococcus mutans in vitro. Arch Oral Biol. 1983;28(9):833-7. 2. Liu TJ, Gui HM, Xiao XR. Effects of Steviosin on acidogenicity and adherence of Streptococcus mutans in vitro. West China Journal of Stomatology 1986; 4:165-168.) S. mutans was cultured in a culture medium containing no sugar, and the pH values were the same in the 24 hours and 48 hours. In order to make S. mutans the best possible metabolizing all the sugar/substitute available in the culture medium, 48 hours was choosed.

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.
No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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