Reviewer’s report

Title: Cariogenicity features of Streptococcus mutans in presence of rubusoside

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Reviewer: Cristiane Koga-Ito

Reviewer's report:

The study aimed to evaluate the effects of rubusoside (a sugar substitute) on Streptococcus mutans cariogenicity features. Growth, adherence, and acidogenicity in the presence of rubusoside, xylitol and dietary sugars were evaluated.

It is an interesting subject, since rubusoside has been described as a potential sugar substitute. Previous studies on the effects on Streptococcus mutans cariogenicity were not detected. However, two questions have to be considered: Is rubusoside used as sugar substitute by the population? Does ruboside remain stable in the oral milieu to be absorbed and metabolized by S. mutans? The responses to these questions are not described in the paper.

Deep revision in Introduction and rationale description is needed. Evaluation of biofilms and their composition in the presence of rubusoside and other sugar/sugar substitutes would be very important and should be included in the paper. Discussion section should be revised, as follows.

Title:

The title should be revised. Suggestion: "Cariogenicity features of Streptococcus mutans in presence of rubusoside"
Introduction

Most of the references cited in the first paragraph regarding caries etiology and control are very old (mostly from '80). Much literature has been produced in the last years on the multifactorial etiology of dental caries. Please revise.

The literature on rubusoside is also very old (references 7, 8 and 9; from 1998, 1998 and 1982, respectively). Please revise. Some information is not cited in the revision, but is essential to justify this study: Rubosside is currently used as sugar substitute by humans? I there any study on rubosside toxicity?

Recently, Prakash et al. (Nat Prod Commun, 2015), published an article on the degradation of rubusoside under acidic conditions. To what extent, the authors believe that the rubusoside would be degraded by the acid produced by S. mutans in the oral milieu. Would this possible degradation interfere in the absorption of rubusoside by S. mutans?

Material & Methods

The authors reported that "Sucrose, glucose, maltose, fructose and xylitol were reagent-grade." - Please be more specific, include the manufacturer's reference.

Item 2.6 - 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.

Did the authors perform pH readings only after 48 hours? It is important to consider how many hours S. mutans took to metabolize all the sugar/substitute available in the culture medium.

Item 2.5 - The authors reported that S. mutans suspension in presence of sugar/substitutes were incubated for 48 hours at 37°C and the number of cells adhered to glass specimens was evaluated. Do the authors believe that at this point (after 48 hours of incubation) the influence on S. mutans adherence capacity can be still assessed? Is there a biofilm formation?

After this period, the specimens were submitted to agitation in a mixer and the number of cells was obtained. After 48 hours, the biofilm formed in the glass surface is probably firmly
adhered due to the presence of dense extracellular matrix. The better way to recover the cells should be sonication. To what extent the authors believe that this could be a limitation of the study?

Results

Figure 4 shows that S. mutans produces the same amount of acid when grown in presence of rubusoside and rubusoside +sucrose. What is the proposed mechanism that could explain this phenomenon?

Discussion

In the last years, the literature showed that caries is not a S. mutans dependent disease. Nowadays, the multifactorial etiology associated to ecological theory (Takahashi et al., JDR, 2011) is widely accepted. What are the limitations of studying the effect just on S. mutans cariogenicity features? This should appear in the Discussion Section.

For caries etiology, see also: Nyvad et al. (Caries Res, 2013); Belda Ferre et al. (Caries Res, 2015); Simón-Moro (J Oral Microbiol, 2014).

The authors stated that "Sucrose substitutes that are not metabolized by plaque-forming bacteria, particularly Streptococcus mutans, have been proposed as a promising approach to caries prevention." A reference should be cited. If the authors consider this statement as true ("rubososide is not metabolized by S. mutans"), how rubusoside interferes in S. mutans cariogenicity features? What is the possible mechanism?

Are the methods appropriate and well described?

If not, please specify what is required in your comments to the authors.

No

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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I am able to assess the statistics

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