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February 13<sup>th</sup>, 2020

Dear Editors,

We thank the reviewers for their comments on the manuscript and we have edited the manuscript to address their concerns.

We hope that the manuscript is now suitable for publication in PeerJ.



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Nor Azlan Nor Muhammad

On behalf of all authors.

## Reviewer 1 (Karla Yotoko)

### Basic reporting

*1) I have concerns about the concept of a cluster: "The definition of a cluster in this work is the cluster formed by majority of the terminal nodes of the tree (> 50%) that belong to the same class under Bacteroidetes" (lines 285 and 286). They used this particular concept instead of monophyletic clades containing the Bacteroidetes classes.*

In the previous manuscript, monophyletic clades concept is used in addition to that definition. In order to make it clearer, the manuscript has been revised; the definition is removed to avoid confusion and the monophyletic clade is added to replace it (Lines 290-294, 303-305, 367-368, 425-426, 475-476).

*2) I think the authors could reproduce the 16S tree coloring the OTUs according to the cited classes.*

The BI tree of 16S rRNA has been reproduced. The method (Lines 216-251), result (Lines 324-344), and discussion (Lines 400-424) sections of the manuscript have been updated to reflect that. Figure 7 is added to illustrate the constructed 16S rRNA tree.

*3) They compared the PorS and PorR trees and argued that they have the same "clusters" of Cytophagia (blue) and Bacterioidia (green). The problem with this state is that the "conserved clades" of PorS tree contain only one genus of Bacterioidia (Porphyromonas) and only one genus of Cytophagia (Hymenobacter).*

Due to limited number bacterial species were found to acquire PorS protein homologs in this study, we have decided to construct the BI tree for UgdA instead. Both *porR* and *ugdA* genes are within the intervening DNA segment that is flanked by insertion sequences. Both genes are involved in the Wbp pathway, which is one of the pathways in the A-LPS biosynthesis. The method (Lines 266-280), result (Lines 361-375), and discussion (Lines 470-487) sections of the manuscript have been updated to reflect that. Figure 9 is added to illustrate the comparison between PorR and UgdA BI trees.

## Experimental design

*1) The authors treated as "well supported", clusters with posterior probability (PP) values > 0.8 (line 288), which is unusual since we consider as well supported clusters with PP > 0.95 when using BI.*

In the current revision, only branches with PP > 0.95 are considered having a strong support. Those branches are denoted as black coloured branches in **Figs. 2-7** and **Fig 9**. In those figures, the identified monophyletic clades that formed by terminal nodes that belong to the same class under Bacteroidetes are denoted by solid curves. The monophyletic clades or its subclades with a strong support are denoted by dashed curves. The reason behind those annotations is because we would like to cater for the reader that is interested to see only the clades with a high support and also for the reader that is interested to see the clades with/without a high support as there is still a chance for those clades to appear even though the probability is low. The result (**Lines 290-294, 330-333**) section of the manuscript has been updated to reflect that.

*2) In Abstract and Discussion, the authors refer to clusters in terms of their position at the printed figure, which is unusual in phylogenetic studies. At lines 48-49 they stated that: "General topology of PorR BI tree is different from the other 19 BI trees that are multifurcating at its centre into three big clusters that are approximately 60o apart from each other except for PorN BI tree that has two big clusters that are almost 180o apart. However, PorR BI tree has one big cluster at bottom half of the tree and branches sequentially diverging out from its centre at top half of the tree". At lines 397-399, they repeated the same idea: "This is because the 19 BI trees are multifurcating at its centre into three big clusters that are approximately 60o apart from each other (or two big clusters that are almost 180o apart from each other for BI tree of PorN) (Figs. 2-6). However, the BI tree of PorR has one big cluster at bottom half of the tree and branches sequentially diverging out from its centre at top half of the tree (Fig. 3)".*

Agreed. The instances of such statements in **abstract** and **discussion** sections are removed in the revised manuscript.

## **Validity of the findings**

*1) I could not understand the point of the horizontal transfer of genes in this work. It would be useful to inform (or infer) the consequences of the transfers for the organisms that received the genes. I think that authors had a great job in working with the Bayesian Inference. Still, I feel that the work would gain with the aid of a phylogenist to help them to describe the trees and interpret their results.*

We combine our response below.

## **Comments for the authors**

*1) However, I still have concerns about the interpretation of the results. My main suggestion is that the authors invite a phylogenist to help them interpret their findings. Also, it will be necessary to investigate the possible consequences of horizontal transfers of some of the T9SS family genes among Bacteroidetes.*

The aim of the work is to report about the phylogeny of T9SS protein components that hasn't been covered yet by the literature as most works on this bacterial secretion system have been focusing on functional characterisation of its protein components. We observed that the BI tree of PorR is rather different from the other 19 BI trees of T9SS protein components. Thus, this work also aims to establish the differences between the BI of PorR tree and other 19 BI trees. The point of the horizontal gene transfer in this work is just a mean to explain the differences that we observed between the BI tree of PorR and other 19 BI trees. It is interesting to infer the possible consequences of such transfer but we are afraid that we don't know any close expert that we could invite to interpret the result from that perspective.

## Reviewer 2

### Comments for the authors

*1) I recommend the authors to change one thing to improve. In Figure 1, A-LPS attachment property should be RgpB, not Kgp. This must be a mistake.*

Figure 1 has been edited to reflect that (Figure 1).