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JoVE Editorial Staff,

This letter is in reference to our original manuscript submission **JoVE59219 - [EMID:006dbda986a0ecac]** titled *"IDBac: An Open-Source MALDI-MS Platform for Analysis of Microbial Protein and Specialized Metabolite Data."* Note: we revised the title to read: "*Using the Open-Source MALDI TOF-MS IDBac Pipeline for Analysis of Microbial Protein and Specialized Metabolite Data*." Herein we submit a revised manuscript.

Below is a detailed response to each reviewer critique. We only included major issues as comments below; all grammatical or minor structural changes were made per request of the reviewer. Reviewer comments are indented and in blue font, while our responses are left aligned. If you require any further material or wish to discuss this over the phone, we would be happy to speak with you. Thank you for your time and consideration,

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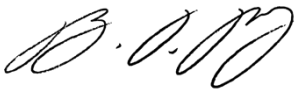

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Response to reviewer comments:

> The authors briefly mention in the discussion, "Performing the full analysis on isolated colonies will provide the best results, as mixed colonies/biofilms may provide spurious peaks that interfere with analyses." While that is the case, some bacteria do not produce all of their specialized metabolites when grown in isolation but may produce them in coculture - couldn't this pipeline then could also be used to analyze the impacts of mixed colonies on the specialized metabolites? I therefore wonder whether these 'spurious peaks' are potentially biologically interesting results, or whether the authors have reason to believe that they are in fact some sort of artifact?

We agree and removed the sentence as we thought it may confuse readers. However, to answer the question- yes, IDBac could be/is being used to study the effect of culture conditions on metabolite production.

> In the procedure, under number 5: Cleaning the target plate the protocol for cleaning the target plates should be included, and the reference just left to the Sauer paper: the article referenced for the cleaning is not an open-access article so not everyone will be able to view it. It would therefore be best to simply include a brief overview of the preferred/recommended ways to clean the plates for the novice user.

We have included a general method for cleaning the plates. We left the reference to the article as we adapted their method.

> Table 1 (which was to include the general parameters for data acquisition) was not included.

We corrected this.

> In addition, it is not clear how challenging it will be to use data collected on instruments other than the Bruker Autoflex - when I tried to input some Microflex files into IDBac, they were not in the correct format for preprocessing (i.e. "The Raw data file will be one folder that contains individual folders for each MALDI plate…" etc.) It is possible I was just using older data that doesn't conform to this format, and newly collected data would (perhaps especially if collected according to the missing Table 1?) but will this be a broader issue for data collected from other MALDI instruments/software? Is there a workaround that involves creating these folders? If so, what are the required parameters to facilitate this?

It can be difficult to maintain compatibility across vendor software versions. To help ameliorate this we have added the ability to use mzXML, mzML, txt, and csv files as input and will incorporate more input-types when users request them.