The Effect of Bacterial Interactions on Fitness

Summary: In this paper, the authors set out to test the effects of adding two candidate strains for bioaugmentation to soil in the presence of a pollutant, toluene. They use flow cytometry to quantify population sizes over time and compare different growth media and the presence or absence of the bioaugmentation strains. They find that one of the strains increases the growth of the resident community on the pollutant.

General comments:

The text is well written throughout the paper, despite some typos and unclear parts. The abstract is clear, except for the last sentence. The introduction is also very well written: the motivation for the experiments and the hypotheses are very clear. I am not sure, however, about your definition of bioremediation. Also, you talk about fluorescent markers without mentioning which strains carry these markers.

The methods section is quite complete and well-explained.

I don't like the figures very much. If you are calculating the area under the curve anyway, it would be nicer to include one plot with all the AUC's together in the main text (rather than the appendix).

The authors seem "certain" that the difference in growth of P. putida in mixed carbon sources and toluene is due to concentration of carbon, but the values are not provided. How much carbon is in the mixed carbon sources?

One issue is unclear in the discussion and Figure 4: "the count of SYTO-9 stained cells", do you mean SYTO-9 and not mCherry? Otherwise, it's normal that Pseudomonas cells also get stained with SYTO-9.

In the discussion section, it is very good to discuss future work and ideas on what went wrong and how to improve the system. However, there are also interesting findings in this paper (that sand cells can grow better in toluene in the presence of Pseudomonas putida) that need to be summarized again, highlighted and put in context of the literature and what is known about interactions of an "invading" species into a sand or soil community, or whether you think the strains tested would be good candidates for bioaugmentation. This should be expanded.

Overall, the paper is well-written, and the results clear. However, the figures could be improved and the discussion could be expanded to situate this study in the bigger picture of the field.

Detailed comments:

- The title of the article needs to be more specific.

The effect of the addition of toluene degrading bacterial strain on a natural community in the case of heavy toluene contamination.

- Methods: What is the concentration of substrates in your mixed carbon?

- When you say "plated on a 96-well plate" you mean "diluted"?

- What are the samples used to generate Fig. 2? It should say this in the caption.

- The lines in the figures are not very visible and the text should be much larger.

Evan:

Articles potentiellement intéressants :

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2698150/>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC124669/>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5094676/>

Literature:

*Bioaugmentation has been proven tricky to apply. The new strain is often less competitive or has predators and thus doesn’t last long. Usually, a diminution of the pollutant’s concentration causes the added strain to disappear.*

Bioaugmentation has proved to be tricky to apply, because the new strain usually doesn’t last long, being less competitive, or having predators. Usually, as soon as the pollutant is reduced, the new strain disappears.

Our experiment:

*In our case, the new strain shows a good adaptation to both the toluene polluted media and the mixed carbon media. Pseudomonas putida F1 seems to be an interesting candidate for bioaugmentation.*

In our case, the new strain has adapted well, in a polluted media and, more interesting, in a mixed carbon media, with no pollutants. This seems to indicate that our Pseudomonas putida would be an interesting specimen for bioaugmentation.

However, our experiment was limited in time and original communities. This means that we do not know if the *Pseudomons putida* would last over time, and we may have had no competitors out of sheer luck.

“We noticed a slight drawback when we plotted the count of SYTO-9 stained cells over time, for PP alone and PP with SC in toluene in figure 5. Indeed, the fact that we get a green-only fluorescence in PP and PV alone is surprising, knowing that both PP and PV were tagged with mCherry, which emits a red fluorescence and causes an upwards shift in the fluorescence graph (as explained in fig. 2). Moreover, the number of green-fluorescent cells in PP or PV alone looks a lot like the number in PP(or PV) + SC.

It is even more visible in fig.6 [trucmuche]; {fig.6 a) is a representation of PP alone in toluene, and fig.6 b) of PP+SC in toluene}. These graphs are almost identical, and both show a green-only fluorescence.”