## INvasion

Firstly, in other bioaugmentation experiment, one of the main challenges is the survival of the inoculated strain in presence of a bacterial community [1,2,3], particularly in absence of pollutant. [([MichaelCunliffe](http://www.sciencedirect.com/science/article/pii/S0269749106000248" \l "!) ; [Michael A.Kertesz](http://www.sciencedirect.com/science/article/pii/S0269749106000248#!), Effect of *Sphingobium yanoikuyae* B1 inoculation on bacterial…)]

In our experiments, PP and PV were able to grow in both media in presence or absence of SC. This led us to think that PP and PV would be interesting candidates for bioaugemtat ion, especially as they have no problem growing with SC in mixed carbon. But as PV in toluene grew significantly less in presence of SC than alone, we think PP, which presented no difference in growth in presence of SC, would be the best candidate.

Further experiments would be required to know if this is also true in a sand medium and especially over a longer period of time. Similar experiments could also be performed on different native communities.

## Fluo (clarifier les gates gate vert et gate jaune(rouge + vert)

We noticed the second drawback when we plotted the count of SYTO-9 stained cells over time, for PP alone and PP with SC in toluene in 􀏐igure 4.

The cells count in the Syto-9 only gate (green frame in figure 2) in PP or PV alone is surprising, knowing that both PP and PV were tagged with mCherry, which emits a red fluorescence and causes an upward shift in the fluorescence graph (as explained in fig.2).

Our hypothesis is that some PP/PV cells died, but weren’t lysed. Their DNA being still nicely protected they could be stained by SYTO-9. However, their metabolic activities stopped, reducing mCherry fluorescence. Without the tag, these cells would remain in the SYTO-9 only gate and couldn’t be differentiated from the SC cells.

and., if the cells do not lyse,

The similarity between the two lines hints us that what we considered as SC cells in PP + SC represented in reality mostly dead *Pseudomonas putida* cells and not SC cells.

## SOurces

One of the main challenge in bioaugmentation is rather the survival of the exogenous strain rather then preventing it’s complete invasion [1]. In addition, boucher et al and

[1]Is bioaugmentation a feasible strategy for pollutant removal and site remediation?

[SaïdEl Fantroussi](http://www.sciencedirect.com/science/article/pii/S1369527405000512" \l "!) ; [Spiros NAgathos](http://www.sciencedirect.com/science/article/pii/S1369527405000512#!)

The rapid decline in population size of active exogenously inoculated microbial cells in soil (‘microbiostasis’ or ‘obstinacy’) is attributed to both biotic and abiotic factors. Biotic factors are often more consequential. This can be inferred by the fact that sterile soil is generally much more hospitable to external microorganisms than natural soil [[17](http://www.sciencedirect.com/science/article/pii/S1369527405000512" \l "bib17)]: factors such as predation by protists, competition with autochthonous microorganisms for nutrients or electron acceptors, and the presence of roots that release organic compounds, are biotic elements that adversely impact bioaugmentation.

Therefore, to increase the probability of the initial establishment and the long-term efficacy of an inoculum in soil, one must provide both predictable ecological selectivity (i.e. by filling a metabolic niche that is not being utilized by the indigenous microbiota) and protection from adverse circumstances (e.g. by encapsulation or structuring in a biofilm)

# Bouchez et al., ***Ecological study of a bioaugmentation failure,* ENvironemental microbiology**

Problems concerning the adaptation of the

inoculated microorganisms, the insuf®ciency of substrate,

competition between the introduced species and the indi-

genous biomass, and grazing by protozoa have been

given as possible reasons for the failure of the experi-

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Graph legend

Week 1

AUC in the different treatement. Abbreviation: PPSC\_SC, SC cells in the PPSC treatment. PPSC\_PP, PP cells in PPSC treatment.

From left to right: MixC\_SC\_w1: Sand community in mixed carbon; MixC\_PP: Pseudomonas Putida in mixed carbon; MixC\_PPSC\_SC: Sand community + Pseudomonas Putida in mixed carbon, “SYTO-9” gate; MixC\_PPSC\_PP: Sand community + Pseudomonas Putida in mixed carbon, “mChe + SYTO\_9” gate; MixC\_PPSC\_SC: Sand community + Pseudomonas Putida in mixed carbon, sum of “SYTO-9 “ gate and “mChe + SYTO\_9” gate ;

Tol\_SC\_w1: Sand community in Toluene; Tol\_PP: Pseudomonas Putida in toluene; Tol\_PPSC\_SC: Sand community + Pseudomonas Putida in toluene, “SYTO-9” gate; Tol\_PPSC\_PP: Sand community + Pseudomonas Putida in toluene, “mChe + SYTO\_9” gate; Tol\_PPSC\_SC: Sand community + Pseudomonas Putida in toluene, sum of “SYTO-9 “ gate and “mChe + SYTO\_9” gate

Week 2

From left to right: MixC\_SC\_w1: Sand community in mixed carbon; MixC\_PV: Pseudomonas Veronii in mixed carbon; MixC\_PVSC\_SC: Sand community + Pseudomonas Veronii in mixed carbon, “SYTO-9” gate; MixC\_PVSC\_PV: Sand community + Pseudomonas Veronii in mixed carbon, “mChe + SYTO\_9” gate; MixC\_PVSC\_SC: Sand community + Pseudomonas Veronii in mixed carbon, sum of “SYTO-9 “ gate and “mChe + SYTO\_9” gate ;

Tol\_SC\_w1: Sand community in Toluene; Tol\_PV: Pseudomonas Veronii in toluene; Tol\_PVSC\_SC: Sand community + Pseudomonas Veronii in toluene, “SYTO-9” gate; Tol\_PVSC\_PV: Sand community + Pseudomonas Veronii in toluene, “mChe + SYTO\_9” gate; Tol\_PVSC\_SC: Sand community + Pseudomonas Veronii in toluene, sum of “SYTO-9 “ gate and “mChe + SYTO\_9” gate ;

To summarise, in the first week our experiment indicates that the presence of PP affects SC differently depending on the media. In toluene, it allows a better growth but has no effect in the mix carbon media. In addition, the effect of the media on SC is important in absence of PP. SC alone grows less in toluene than in mixed carbon.

Furthermore, PP shows an important difference in AUC between the media (F =907.30, df = 1, *p* = 2*:*36 *\_*

10*􀀀*6) and no difference due to the presence of SC.

In contrast, during the second week, SC behaved differently: it was able to grow in the toluene media, even alone. No significant effect of the media nor of the addition of PV were highlighted by the anova.

# Discussion

## Staining Issue

The results are promising, however, there is a slight drawback.

Some cells were detected in the SYTO-9 only gate in PP alone and PV alone cultures in both media (fig.13). Fig.13A is a representation of PP alone in toluene, and Fig.13B of PPSC

in toluene, these graphs are almost identical, and more importantly, both

show an important count in the SYTO-9 only gate.

This is quite surprising, knowing that both

PP and PV were tagged with mCherry. This protein emits a

red fluorescence and should cause an upwards shift in the

fluorescence graph (as explained in Fig. 2a and 2b) and bring these cells in the mChe + SYTO-9 gate.

Our hypothesis is that some PP/PV cells died, but

weren’t lysed. Their DNA being still nicely protected they

could be stained by SYTO-9. However, their metabolic

activities stopped, reducing mCherry fluorescence. Without the red tag, these cells would shift downwards and remain in the SYTO-9 only gate and couldn’t be differentiated from the SC cells.

This issue makes us question whether the significant increase in AUC in the SYTO-9 only in PPSC compared to SC alone is really due to an increase of the number of SC or to the presence of theses PP cells presenting low red fluorescence values.

To circumvent this problem, another tagging method

unaffected by cell death could be used. For example, a

highly specific antibody to *Pseudomonas putida* could

be engineered. A secondary antibody, this time linked

to a fluorescent protein would bind the first one and

therefore, mark all *Pseudomonas putida* cells. This would

allow a better differentiation of our communities.

## Bacterial interaction

Resumé des résultats, en construction

To summarise, growing PP or PV with SC shows no effect on the total cell count compared to growing PP / PV alone. By looking at each population separately, in the first week our experiment indicates that the presence of PP affects SC differently depending on the media. In toluene, it allows a better growth but has no effect in the mix carbon media. In addition, the effect of the media on SC is important in absence of PP. SC alone grows less in toluene than in mixed carbon.

Furthermore, PP shows an important difference in AUC between the media (F =907.30, df = 1, *p* = 2*:*36 *\_*

10*􀀀*6) and no difference due to the presence of SC.

**In contrast, during the second week, SC behaved differently: it was able to grow in the toluene media, even alone. No significant effect of the media nor of the addition of PV were highlighted by the anova….**

## Bioremediation

In many bioaugmentation experiments, one of

the main challenges is the survival of the inoculated

strain in presence of a bacterial community [6] [7],

particularly in absence of pollutant [8].

In our experiments, PP and PV were able to grow in

both media in presence or absence of SC. This led us

to think that PP and PV would be interesting candidates

for bioaugmentation, especially as they have no problem

growing with SC in mixed carbon.

As PV showed a significantly lower growth on average

in toluene in presence of SC than PP, it would be tempting

to say that PP would be an even better candidate.

However, as said in results section B2, our data isn’t

totally reliable to compare the two strains, as the SC

extracted the two weeks behaved differently. It would

be good to test the PP and PV on the same SC.

Further experiments would be required to know if this

is also true in a sand medium (and not liquid medium)

and especially over a longer period. Similar

experiments could also be performed on different native

communities. Also, it would be interesting to vary the

concentration of toluene in these experiments.

~~Another point that could be interesting to change is~~

~~the initial concentration of PP and PV. Indeed we used~~

~~106 cells of SC and 106 cells of PP or PV. Maybe by~~

~~reducing the initial concentration of PP/PV, we could~~

~~reduce their competition with SC over resources, and~~

~~therefore observe a better growth for SC in toluene~~

~~media.~~

~~In addition, we inoculated the media with the same~~

~~number of PP or PV with SC and haven’t considered~~

~~the fact that SC community is composed of different~~

~~bacterial species. The high quantity of a single strain~~

~~may have made it easier for them to outgrow SC over~~

~~time.~~

## Conclusion

In the present state, our study shows

interesting results. However, the issue with this staining

technique might carry some artefacts that should be considered

in data interpretation. If time had allowed it, we

would have liked to perform the additional experiments

proposed above to solve this issue.