UNRAVEL THE PESTICIDE EFFECT VIA A BACTERIAL APPROACH

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ABSTRACT: A continuous increase of human population has been associated to a rapid agricultural expansion and crop production efficacy. Since around 30% of agricultural crop is lost to pests, the use of pesticides becomes essential in agriculture. Unfortunately, the adaptation of microorganisms to pesticide toxicity increased tremendously the amount and chemical diversity of toxic compounds released into environment. So, the increased crop production is counterbalanced by an often irremediable toxic damage of pesticides to the environment due to their low water solubility, environmental and food chain persistence inducing a harmful effect to organisms. Correlation between the toxic effects of pesticides to the environment and their toxic concentrations to humans is not very easy to establish, so it is imperative to find more simple biological models to follow the pesticide toxic effects and biodegradability.

In this study, we used a bacterial model as biosensors for detecting the harmful effect of pesticides as well as decontaminants of pesticide-infested environment.

"Right...a large pizza with corn, wheat, soybeans and hold the pesticides."

EXPERIMENTAL: **Bacterial growth inhibition assay**: *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were incubated at 37˚C in presence or absence of with various concentrations of chlorpyrifos-methyl. Bacterial growing rate was monitored by spectrometry at an absorbance of 600nm (A600nm).

***Determination of biochemical oxygen demand after 5 days (BOD5)**:* Briefly, 0.4g/l was incubated at 20ºC for 5 day in presence of aerobic microorganisms seeded water and BOD5 was quantified by an oxygen-electrochemical probe.

Physyco-chemical analysis of the pesticide: Detection of chlorpyrifos-methyl was performed using an Agilent 7890A series GC system (Agilent, Waldbronn, Germany) containing a split-splitless injector and Flame Photometric Detector (FPD) with phosphorus filter.

Pesticide biodegradability

In the presence of microorganisms the BOD5 increased more than 2 folds, pointing to a bacterial accelerated pesticide degradation compared to control sample.

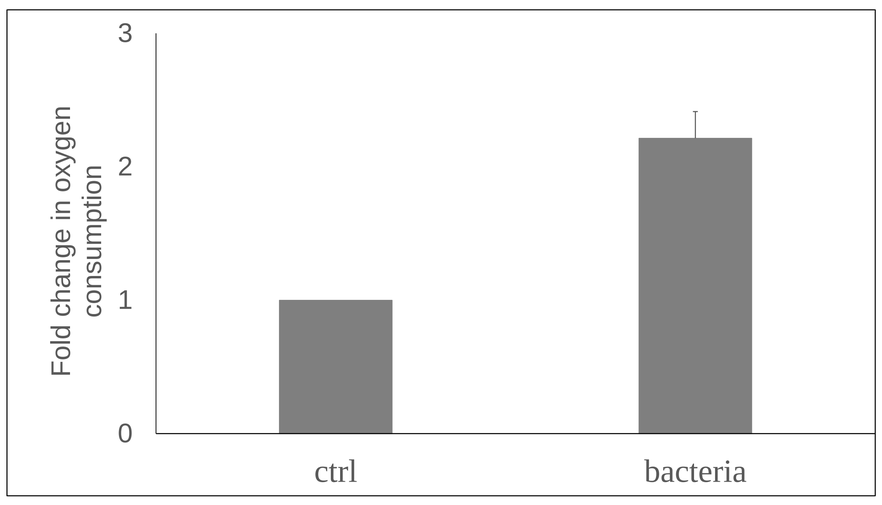

Figure legend: The chlorpyrifos-methyl biodegradability rate due to bacterial activity was quantified by BOD5 fold change between bacterial treated and untreated assay. All studies represent one of at least two independent experiments.

Bacterial growth

Both bacterial strains had shown a significant growing rate during 4 hours of monitoring by absorbance at 600nm (A600nm). *E. coli* had a higher growth rate compared to *E. faecalis* .

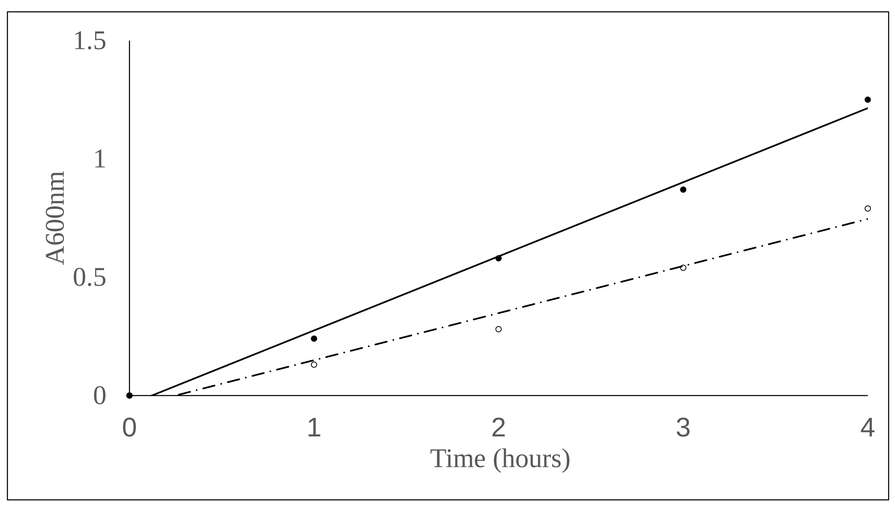

Figure legend: Bacteria, *E. coli* (-) and *E. faecalis* (_._), were incubated in their specific growth was monitored at an absorbance of 600nm (A600nm). All studies represent one of at least two independent experiments.

The organophosphorus pesticide modulates bacterial growth

Both bacterial strains had shown a significant growing rate during 4 hours of monitoring by absorbance at 600nm (A600nm). *E. coli* had a higher growth rate compared to *E. faecalis* .

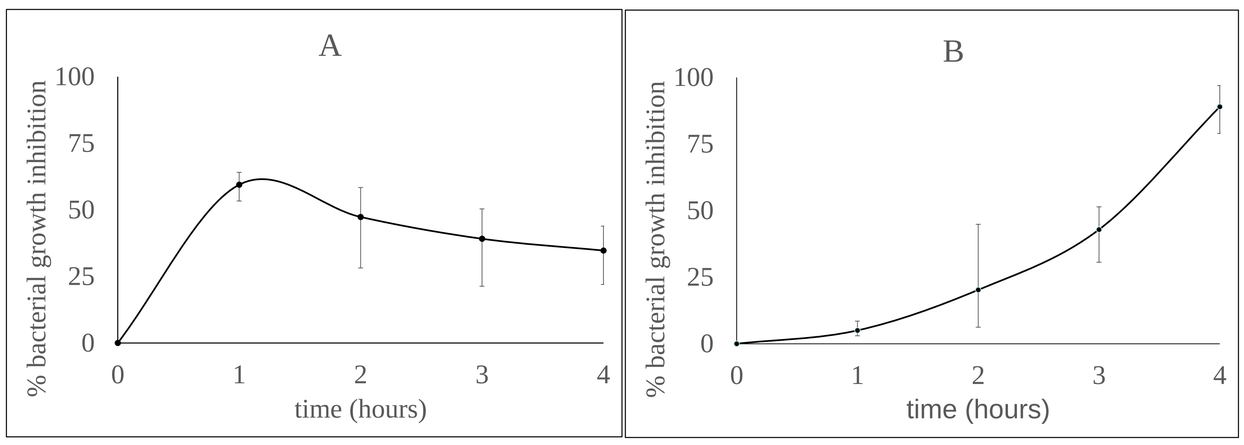

Figure legend: *E. coli* (A) and *E. faecalis* (B) were incubated 4h at 37ºC in presence of 0.4 g/l chlorpyrifos-methyl and their inhibition growth rate was determined in function of the bacteria growth without pesticide treatment. All studies represent one of at least two independent experiments.

Chlorpyrifos-methyl monitoring during bacterial growth

Addition of bacterial strains induced changes in pesticide final concentrations, especially in the presence of *E. faecalis* where after 4h of incubation the final concentration of the pesticide decreased to 50% of the starting point (Fig. 5B). Moreover, concentration of chlorpyrifos-methyl constantly decreased, during 4h incubation in *E. coli* growing medium, up to 70-75 % of the initial concentration (Fig. 5A).

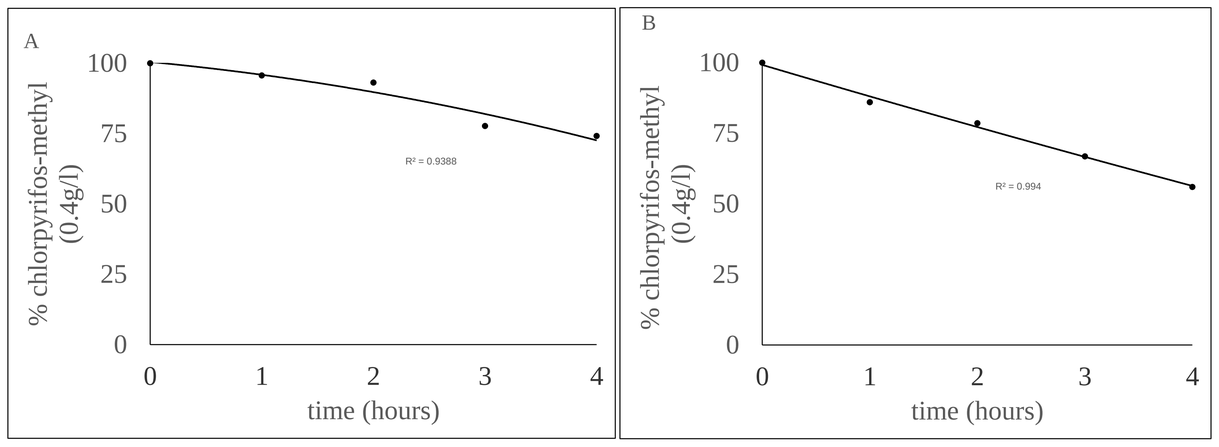

Figure legend: The influence of bacteria on pesticide concentration. *E. coli* (A) and *E. faecalis* (B) were incubated 4h at 37ºC in presence of chlorpyrifos-methyl (0.4 g/l) and the pesticide concentration was monitored by GC system. All studies represent one of at least two independent experiments.

CONCLUSIONS:

- Bacteria enhanced pesticide (chlorpyrifos-methyl) biodegradability;
- Chlorpyrifos-methyl modulated the bacterial growth rate (*Escherichia coli* and *Enterococcus faecalis*);
- *Escherichia coli* and *Enterococcus faecalis* defense mechanisms against chlorpyrifos-methyl toxic effect seemed to be based on the efflux pumps;
- Bacteria is a simple and efficient model for (1) detection and characterization of the pesticide toxicity as well as (2) enhancing the pesticide biodegradability.

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