Metabolic activity of *pseudomonas* spp isolated from agricultural soils

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INTRODUCTION

Due to their characteristics, microorganisms actively participate in various functions and offer ecological services in soils, highlighting the maintenance of primary production and soil recovery. Among the microorganisms that contribute significantly to ecological soil services are bacteria, which have the ability to adapt to a wide variety of biotic conditions (Marcano, 2008). Bacterial metabolism confers the ability to use a large number of compounds as a source of carbon and energy, facilitating their survival in unfavorable environments and facilitating the transfer of compounds that are not available to plants (Bakhshandeh *et al.*, 2015). The objective of the present work was to evaluate the metabolic processes in bacterial isolates in conventional and agroecological agricultural soils. Samples were carried out in plots with conventional and agroecological management of the state of Puebla and Tlaxcala, Mexico.

METHODOLOGY

Conventional and agro-ecological soil sampling was performed in the areas of Puebla and Tlaxcala, Mexico (Fig.1). Each sampling was 2 kg of soil and was carried out according to NOM-021-SEMARNAT-2000 in which it is indicated that the agricultural land take must be 0-15 cm deep. One gram of each soil sample was weighed and resuspended in 10 mL of nutrient broth, incubating at room temperature and stirring at 40 revolutions per minute/24 hours. 10 µL were reseeded in nutrient agar and incubated at 30°C/24 hours, the colonies being selected with greater viability. The identification of each isolation was carried out with the "Nissui" EC™ Compact Dry chromogenic medium.

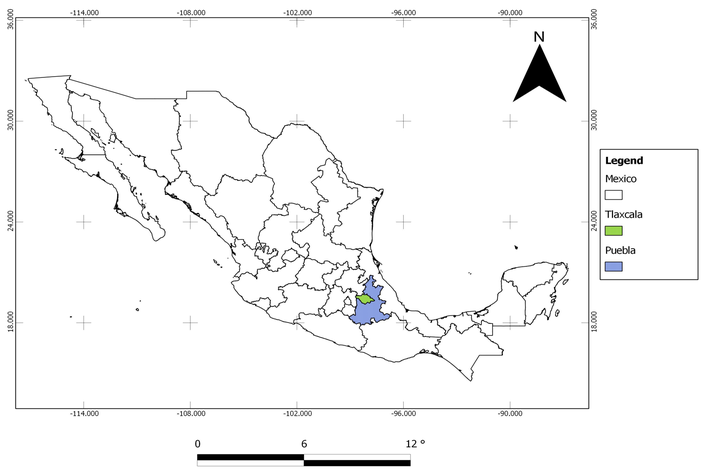

Fig. 1: Areas where soil sampling was performed, indicating the states of Tlaxcala with agroecological system and Puebla with conventional system.

For the quantification of biofilm, the nutrient broth growth of each of the isolates was carried out by adjusting the inoculum to $1X10^6$ CFU/mL. The different samples were placed in 96-well microplates, stained with violet crystal and following the technique described by Stepanovic *et al.* (2004) to obtain the cut-off point in the biofilm formation. To determine the degradation of pesticides (esteron, malation and carbofuran) by bacterial isolates, a silty soil (control) was used, of which 300 grams were weighed and divided into three 100 gram fractions, to each fraction was added a pesticide in a solution of 100 mL and a concentration of 100 ppm. Each sample was then interacted with 1 mL of the bacterial inoculum, allowing them to interact for evaluation days. The UV-Vis spectra were determined in a spectrophotometer (Lamba 20), with the absorbance observed in the maximum of each spectrum the concentrations were calculated using the calibration curve of each pesticide to compare its degradation in the absence and presence of each bacterial isolation.

RESULTS

Based on the isolates obtained from both types of soils (conventional and agroecological), the identification was carried out in the Compac Dry "Nissui" EC™ chromogenic medium, identifying *Pseudomonas* spp. For the evaluation of metabolic activity the isolates of the agroecological system showed positive test in 20% and 40% in dextrose and sucrose, respectively, and in the conventional system the isolates were positive to sucrose and dextrose in 44% and 50%, respectively. DNase activity only in the conventional system a 20% positive test is reported. The data on the determination of biofilm formation of the isolates are presented in table 1.

Table 1. Determination of biofilm production in the isolates of *Pseudomonas* spp.

Samples	Conventional system	Agroecological system
1	Not producing	Moderate producer
2	Weak producer	Not producing
3	Weak producer	Not producing
4	Not producing	Weak producer
5	Weak producer	Not producing

The quantification of pesticide degradation by UV-Vis spectrum showed the following data: when comparing the degradation of the ester with and without inoculation of the isolates of *Pseudomonas* spp., a greater degradation was observed in the first seven days, on day three the sample with bacterial inoculation showed a 26.28% greater degradation difference compared to the test where the bacterial inoculation was not applied. Malation with bacterial inoculation showed the greatest degradation in the first nine days and for carbofuran it was observed that on days seven and fifteen without bacterial inoculation there was greater degradation (Fig.2).

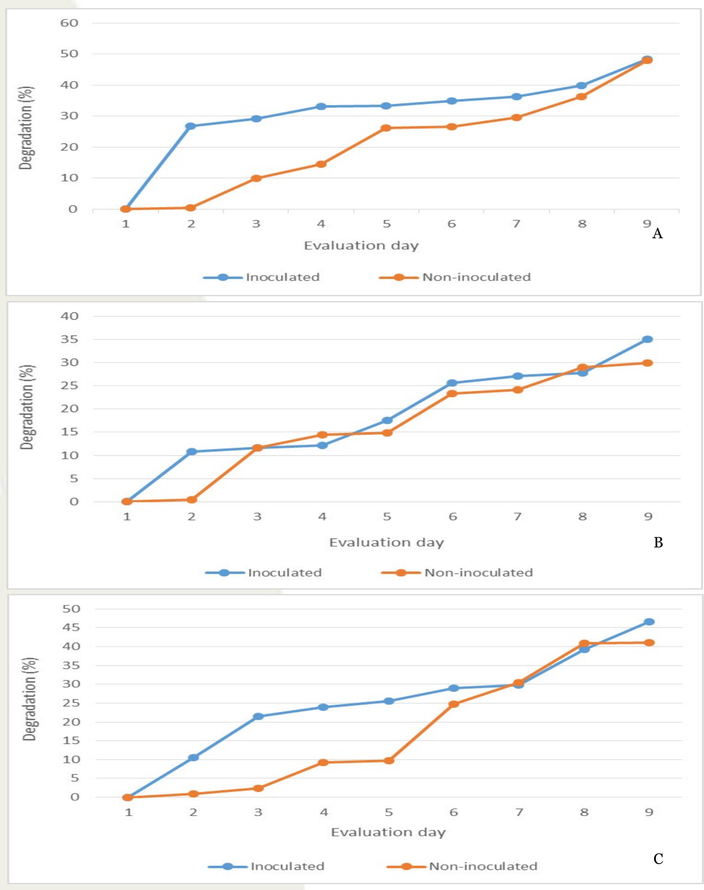

Fig.2: Degradation of esteron (A), carbofuran (B) and malation (C) in the presence and absence of bacterial inoculation, presenting the best degradation activity against esteron (A).

Isolates of the conventional system presented different activity due to the use of pesticides, which modifies their metabolic activity (Griffiths *et al.*, 2000; Gravina *et al.*, 2017). Pesticides can have an inhibitory effect on bacteria, causing alterations in biomass formation and metabolic activity, and as a result of these interactions bacteria develop the ability to form biofilms, favoring their ability to survive and adaptability (Dong *et al* ., 2011; Bjarnsholt *et al.*, 2013).
The isolates showed the highest degradation against the ester, followed by malation and carbofuran, the efficient degradation is related to the ease that bacteria have to metabolize chlorinated organic compounds (Koranda *et al.*, 2014).
In conclusion, the isolates can be considered together with other agroecological practices to degrade or decrease the concentration of pesticides in some soils affected by these compounds, taking advantage of the biodiversity of soil microorganisms.