Jundishapur J Microbiol. 2015 September; 8(9): e27953. DOI: 10.5812/jjm.27953
Published online 2015 September 8. **Research Article**

Analysis of *Bacillus thuringiensis* Population Dynamics and Its Interaction With *Pseudomonas fluorescens* in Soil

Norma Elena Rojas-Ruiz [1]; Estibaliz Sansinenea-Royano [1]; Maria Lilia Cedillo-Ramirez [1]; Rodolfo Marsch-Moreno [2]; Patricia Sanchez-Alonso [1]; Candelario Vazquez-Cruz [1,*]

[1]Centro de Investigaciones en Ciencias Microbiológicas, de la Instituto de Ciencias, de la Benemérita Universidad Autónoma de Puebla, Puebla, México
[2]Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Ciudad de México, México

*Corresponding author: Candelario Vazquez-Cruz, Centro de Investigaciones en Ciencias Microbiológicas, de la Instituto de Ciencias, de la Benemérita Universidad Autónoma de Puebla, Puebla, México. Tel: +52-2222295500, Fax: +52-2222295650, E-mail: ecobacilos@yahoo.com

Received: March 2, 2015; **Revised:** May 17, 2015; **Accepted:** May 21, 2015

Background: *Bacillus thuringiensis* is the most successful biological control agent, however, studies so far have shown that *B. thuringiensis* is very sensitive to environmental factors such as soil moisture and pH. Ultraviolet light from the sun had been considered as the main limiting factor for its persistence in soil and it has recently been shown that the antagonism exerted by other native soil organisms, such as *Pseudomonas fluorescens*, is a determining factor in the persistence of this bacterium under *in vitro* culture conditions.
Objectives: The aim of the present investigation was to analyze the population dynamics of *B. thuringiensis* and its interaction with *P. fluorescens* using microbiological and molecular methods in soil, under different conditions, and to determinate the effect of nutrients and moisture on its interaction.
Materials and Methods: The monitoring was performed by microbiological methods, such as viable count of bacteria, and molecular methods such as Polymerase Chain Reaction (PCR) and hybridization, using the direct extraction of DNA from populations of inoculated soil.
Results: The analysis of the interaction between *B. thuringiensis* and *P. fluorescens* in soil indicated that the disappearance of *B. thuringiensis* IPS82 is not dependent on the moisture but the composition of nutrients that may be affecting the secretion of toxic compounds in the environment of *P. fluorescens*. The results showed that the recovered cells were mostly spores and not vegetative cells in all proved treatments. The molecular methods were effective for monitoring bacterial population inoculated in soil.
Conclusions: *Bacillus thuringiensis* is very sensitive to the interaction of *P. fluorescens*, however is capable to survive in soil due to its capacity of sporulate. Some of the cells in the form of spores germinated and folded slightly and remained in a constant cycle of sporulation and germination. This confirms that *B. thuringiensis* IPS82 can germinate, grow and sporulate in soil.

Keywords: Microbial Interactions; Antibiosis; Spores; *Bacillus thuringiensis*; *Pseudomonas fluorescens*

1. Background

Interactions among organisms are central to understanding any ecosystem. Soil environment is not an exception, however biotic interactions dominating soil biology differ from those in other systems because of the dominating role of some organisms and the lack of autotrophy in soil (1).

Bacillus thuringiensis are Gram-positive soil bacteria and the most successful biological control agent; however, are highly sensitive organisms to various environmental factors including germicidal effect exerted by sunlight, which has been considered as the main factor in the low persistence of vegetative cells and spores of *B. thuringiensis* in the environment.

Nevertheless, previous studies have shown that the survival of *B. thuringiensis* IPS82 is also affected by its interaction with other native soil microorganisms *in vitro*, particularly, by cohabitation with *Pseudomonas fluorescens* (2). The mechanisms by which *P. fluorescens* inhibits *B. thuringiensis* are unknown, yet the most described as the main mechanism of pathogen inhibition is by the production of secondary metabolites (3, 4) and siderophores, which may also play a role in the microbial antagonism (5, 6). This antagonism can be increased in response to several changes of conditions such as the addition of nutrients to the culture medium and the temperature change (7). Therefore, *B. thuringiensis* IPS82 has little ability to survive as a vegetative cell in interaction with other microorganisms.

2. Objectives

In this work, the population dynamics of *B. thuringiensis* and its interaction with *P. fluorescens* in soil was analyzed under different conditions. To follow the populations of both bacteria in soil, microbiological and molecular methods were used, the latter is a useful tool for the de-

tection and monitoring of bacteria and microorganisms that cannot be cultured (8).

3. Materials and Methods

3.1. Bacterial Strains

The *B. thuringiensis* and *P. fluorescens* strains used in this study were *B. thuringiensis* IPS82 (*Bacillus* Genetic Stock Center), *B. thuringiensis* recombinant strain SR08, which has a fragment of the plasmid pES8 inserted in its chromosome (9), and *Pseudomonas fluorescens* ATCC 49838 (Microbiologics Labs. St. Cloud MN USA).

3.2. Culture Media Used

In the present investigation we used the Tris G and Luria Bertani (LB) media (2) and the chemical salt employed in the media were from Sigma-Aldrich.

3.3. Evaluation of the Effect of Nutrients Addition and Moisture Adjustment on Bacterial Interaction Between Bacillus thuringiensis IPS82 and Pseudomonas fluorescens in Soil

Laboratory conditions were designed to achieve a model of bacterial interaction at at a controlled environment and to depict a series of natural conditions of survivors and antagonists between pathogen related bacteria. Primarily, 276 g of soil (with low content of organic material) held in a plastic vase, was sterilized by wet and dried heat to reduce microbial population and distilled water was added to maintain the moisture of the soil. Soil conditions were adjusted to a humidity of 80% or 40%, followed by the addition of 0.05% glucose and 0.05% yeast extract. The proportions of inoculated bacteria in the soil were: *B. thuringiensis* IPS82 and *P. fluorescens*: A (10^5: 10 colony-forming units [cfu]), B (10^3: 10 cfu), C (10^5: 10^5 cfu), D (10: 10^3 cfu) and E (10: 10^5 cfu). Control proportions for *B. thuringiensis* IPS82 were F (10^3 cfu) and G (10^5 cfu), and for *P. fluorescens* were H (10^3 cfu) and I (10^5 cfu). Nine proportions were used, with four replicates; therefore 36 vases were used for each treatment, giving a total of 144 vases. All of these vases were inoculated with the above-described proportions of microorganisms and were held at 25°C. The time period for the evaluation was 90 days. Each week 1 g of soil from the four replicated vials was blended with 3 mL of distilled water and diluted suspensions, surface-plated on LB plates with and without the chloramphenicol antibiotic (5 $\mu g mL^{-1}$). Plates were incubated at 28°C for 12 hours and colony-forming units (cfu) were counted for *B. thuringiensis* IPS82 or *P. fluorescens*.

3.4. Analysis of Bacillus thuringiensis Sporulation Percentage in Soil at Ten and Ninety Days

The percentage of sporulation of the inoculated *B. thuringiensis* proportions in soil was measured on the ninetieth day of experiment in soil. The rate of sporulation of *B. thuringiensis* IPS82 and SR08 was also measured on the tenth day. To determine the number of *B. thuringiensis* vegetative cells or spores, inoculated in soil at each of the different proportions, samples of 1 g from soil were blended in 3 mL of Tris G medium. Next, an aliquot of 100 μL was taken to determine the amount of vegetative cells in the sample, and from this aliquot serial dilutions were made (10^{-1} to 10^{-6}). All dilutions were inoculated on solid Tris G medium in Petri dishes, incubated at 28°C for eight hours, and counted as colony-forming units (cfu). Only for the selection of spores with respect to the remaining vegetative cells, the tubes with blended soil were incubated at 65°C for 45 minutes, serial dilutions were made and inoculated in solid Tris G medium. The incubation conditions were the same for vegetative cells. Finally the counting of colony forming units was performed.

3.5. DNA Direct Extraction From Soil Inoculated With Bacillus thuringiensis IPS82 and Pseudomonas fluorescens

Samples of 1.5 grams of soil were blended with 6 mL of Tris G medium and incubated in the horizontal position for six hours at 30°C. They were then centrifuged at 3000 rpm for five minutes, and 50% of the volume, free of soil, was separated and the rest with the soil was discarded. To the separated supernatant, 0.6 g of Polyvinylpolypyrrolidone (PVPP), one volume of lysis buffer (50 mM Tris, 50 mM Ethylene diamine tetra acetic acid (EDTA), 1 M NaCl pH 8) and 100 μg of lysozyme were added and the resulting solution was incubated for 30 minutes at 37°C. Subsequently, 20% Sodium Dodecyl Sulfate (SDS) was added (1 mL per 10 mL volume), samples were incubated at 65°C for one hour and shaken by inversion every 15 minutes, centrifuged at 3000 rpm for five minutes, and to 500 μL of the supernatant, 0.1 volume of 3 M sodium acetate pH 7 and 1 volume of isopropanol were added. The samples were incubated at -20°C for 10 minutes and were centrifuged at 13000 rpm for 10 minutes. Finally, the pellet of DNA was resuspended in 100 μL of TE (10 mM Tris 1 mM EDTA). The chemical compounds employed were from sigma-Aldrich (USA).

3.6. Detection of Populations of Bacillus thuringiensis ISP82 and Pseudomonas fluorescens Inoculated in Soil by Molecular Methods

For the detection of populations of *B. thuringiensis* ISP82 and *P. fluorescens* in soil, molecular methods such as Polymerase Chain Reaction (PCR) reaction and dot-blot Hybridization were used. To carry out the PCR reaction, 120 μg of DNA, extracted directly from soil as described above, was used. To specifically detect *B. thuringiensis* ISP82, the *cry10Aa* gene of *B. thuringiensis* was used as a probe. These primers were designed in the laboratory, based on the sequences of three conserved motifs encoding the Cry

proteins of *B. thuringiensis*, and the oligonucleotide sequences were: *Cry10Aa* (Accession number M12662) 5'TTTTGCTGCCCCTGTCTTAG3' and 3'TAGTGGGTTTAGGTGCGAGA5' (IDT Technologies, México). The amplification conditions were: denaturation at 94°C for five seconds, annealing at 50°C for 30 seconds, and extension at 65°C for one minute (30 cycles). The PCR product with expected size of 1290 bp was run on agarose (Invitrogen, USA) gel at 0.8%. To carry out dot-blot hybridization, 100 or 120 ng of DNA, extracted directly from soil, was denatured at 95°C for ten minutes and placed as drops on a nylon membrane (Merck Millipore, USA). These membranes were hybridized with a probe of total genomic DNA of *B. thuringiensis* IPS82 or *P. fluorescens* marked with ^{32}P (Merck Millipore, USA) and the autoradiography was obtained using Kodak® X-ray films, according to Sambrook et al. 1989 (10).

4. Results

4.1. Evaluation of the Effect of Nutrients Addition and Moisture Adjustment on Bacterial Interaction Between Bacillus thuringiensis IPS82 and Pseudomonas fluorescens in Soil

With the purpose of evaluating the population dynamics of *B. thuringiensis* and its interaction with *P. fluorescens,* under different conditions in soil, the effect of the nutrients addition and moisture adjustment was analyzed with the proportions of inoculated bacteria described in the materials and methods. These proportions were chosen based on the previous laboratory works, which were designed to analyze the effect of the interaction between *B. thuringiensis* and *P. fluorescens in vitro*. The results determined that there was an antagonistic effect when these bacteria interacted, thus it was interesting in the present work to analyze whether such event was also present in soil.

The results of the soil analysis showed that when a high proportion of the population of *B. thuringiensis* IPS82 with respect to *P. fluorescens* in A proportion (10^5: 10 cfu), only in the treatment with the addition of yeast extract, was inoculated, there was a disappearance of the population of *B. thuringiensis* on the sixtieth day, while in the rest of the treatments, *B. thuringiensis* IPS82 was recovered even on the ninetieth day. For *P. fluorescens* in the same proportion, two days after being inoculated, the bacterial population increased four or five orders of magnitude in different treatments (Figure 1). Thus, moisture adjustment had no effect on both bacterial populations while the addition of yeast extract had an effect on the *B. thuringiensis* population because it disappeared by the sixtieth day. In the B proportion (10^3: 10 cfu) similar results were obtained (data not shown).

At the C proportion (10^5: 10^5 cfu) similar to the A proportion, moisture adjustment had no effect on both bacterial populations yet the addition of yeast extract had an effect on *B. thuringiensis* population because it disappeared in thirty-two days (Figure 2).

At the E proportion, when the proportion of *B. thuringiensis* is lower than *P. fluorescens* (10: 10^5 cfu), in all treatments, *B. thuringiensis* population were eliminated during a period of ten to thirty-two days, while populations of *P. fluorescens* remained present on the ninetieth day (data not shown). In the case of the control proportions, wherein only one of the two populations was inoculated, *B. thuringiensis* IPS82 survived in order of 1×10^4 cfu until the ninetieth day of the trial while the population of *P. fluorescens* at the end of the analysis was 1×10^5 cfu (data not shown). These results indicated that the disappearance of *B. thuringiensis* IPS82 was not dependent on the moisture but the composition of nutrients that could have affected the secretion of toxic compounds into the environment of *P. fluorescens* or the sensitivity of vegetative cells to the toxic compounds of *P. fluorescens* secreted in soil.

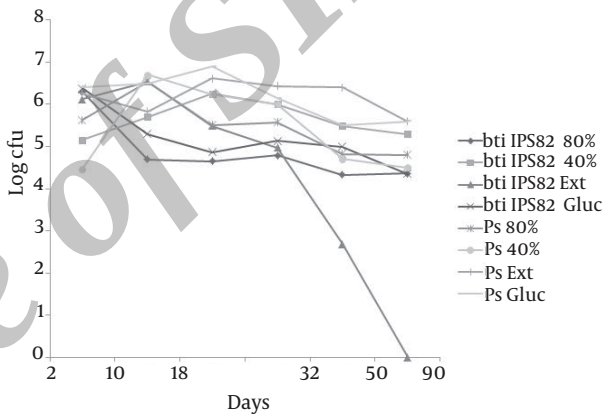


Figure 1. Evaluation of the Effect of Nutrients Addition and Moisture Adjustment on Bacterial Interaction Between *Bacillus thuringiensis* IPS82 and *Pseudomonas fluorescens* in Soil With the A Proportion (10^5: 10 cfu)

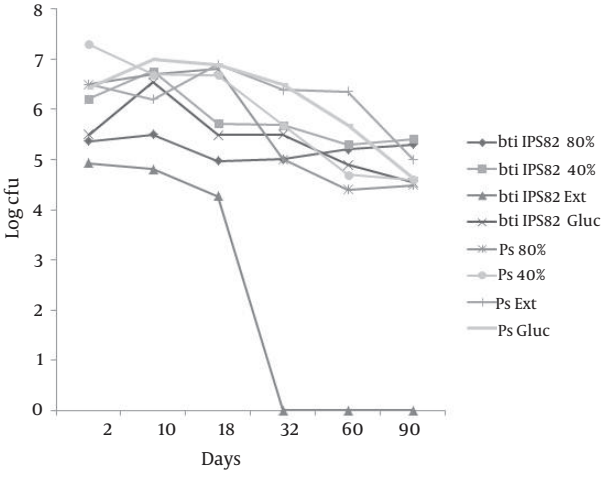


Figure 2. Evaluation of the Effect of Nutrients Addition and Moisture Adjustment on Bacterial Interaction Between *Bacillus thuringiensis* IPS82 and *Pseudomonas fluorescens* in Soil With the C Proportion (10^5: 10^5 cfu)

4.2. Analysis of Sporulation of Bacillus thuringiensis IPS82 in Soil

Bacillus thuringiensis IPS82 is able to transform from a vegetative cell to spore in soil, and vice versa; this transformation could help the survival of the bacteria resisting the antagonism by *P. fluorescens*. *Bacillus thuringiensis* IPS82 sporulation percentage in soil was determined on the ninetieth day of the experiment for different inoculated proportions in soil. The results indicated that the recovered cells were mostly spores and not vegetative cells in all proved treatments (Table 1).

In A and B proportions, 100% of recovered cells, were spores. In D and E proportions, when low amounts of *B. thuringiensis* were inoculated with respect to *P. fluorescens*, neither vegetative nor spore cells were recovered.

Information on the reproductive biology and the sporulation process of *B. thuringiensis* IPS82 in soil is scarce; however, it is possible to speculate that *B. thuringiensis* IPS82 survives in soil for a brief period of time, as a vegetative cell and it must sporulate for its maintenance in the environment. The sporulation percentage of *B. thuringiensis* was determined every 24 hours during ten days. The results indicated that recombinant and wild type strains of *B. thuringiensis* have similar behavior under the same conditions. However, the most interesting result was obtained from the analysis of the onset of sporulation. It was expected for all vegetative cells to sporulate on the first day and remain so, for over ten days (due to low nutrient content in the soil). The results shown in Figure 3, indicate that on the first day, the amount of *B. thuringiensis* IPS82 obtained from the total population (vegetative cells + spores) and from only the spores, was similar (10^6 cfu) therefore, all cells were spores, as expected (Figure 3A). However, from the second day there was a decrease in the number of spores and a slight increase in the number of total cells (Figures 3A and B), indicating that some of the cells in the form of spores germinated and multiplied slightly, maintained a constant cycle of sporulation and germination. This confirms that *B. thuringiensis* IPS82 similar to *Bacillus cereus* can germinate, grow and sporulate in soil. The phenomenon of the increment of cells is probably due to the presence of other microorganisms in soil. These microorganisms could segregate some components into the soil, which serve as nutrients for *B. thuringiensis* development.

Table 1. *Bacillus thuringiensis* IPS82 Sporulation Percentage at Different Proportions Inoculated in Soil on the Ninetieth Day

Inoculated Proportions of *B. thuringiensis* IPS82/*P. fluorescens* in Soil (cfu)	*B. thuringiensis* IPS82 Viability on the Ninetieth Day (cfu)	*B. thuringiensis* IPS82 Sporulation Percentage
A) 10^5: 10	1.5×10^4	100
B) 10^3: 10	3.25×10^4	100
C) 10^5: 10^5	1.22×10^5	80
D) 10: 10^3	0	0
E) 10: 10^5	0	0
F) 10^3	1.87×10^4	90

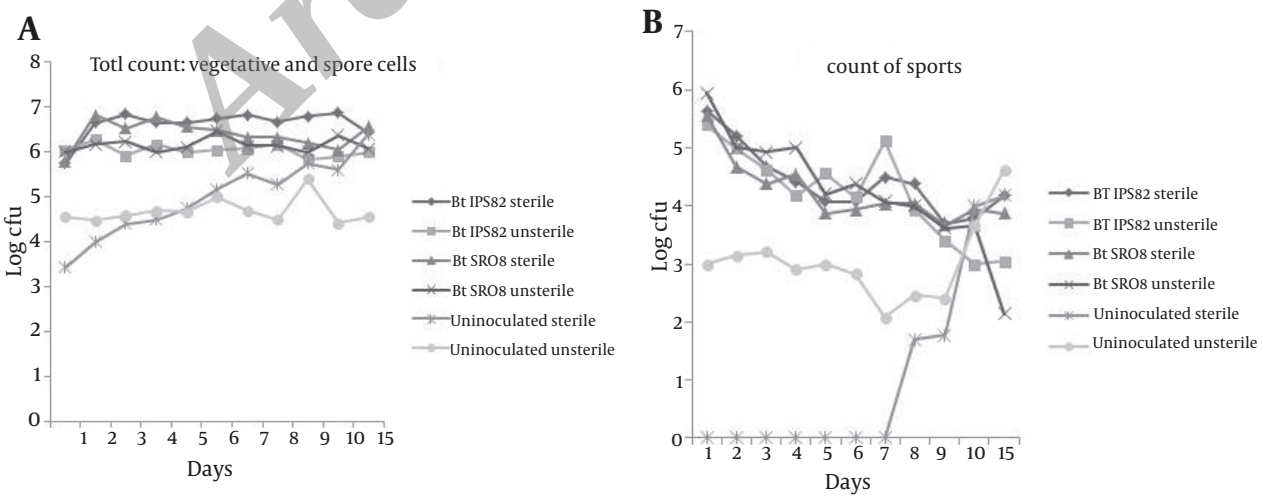


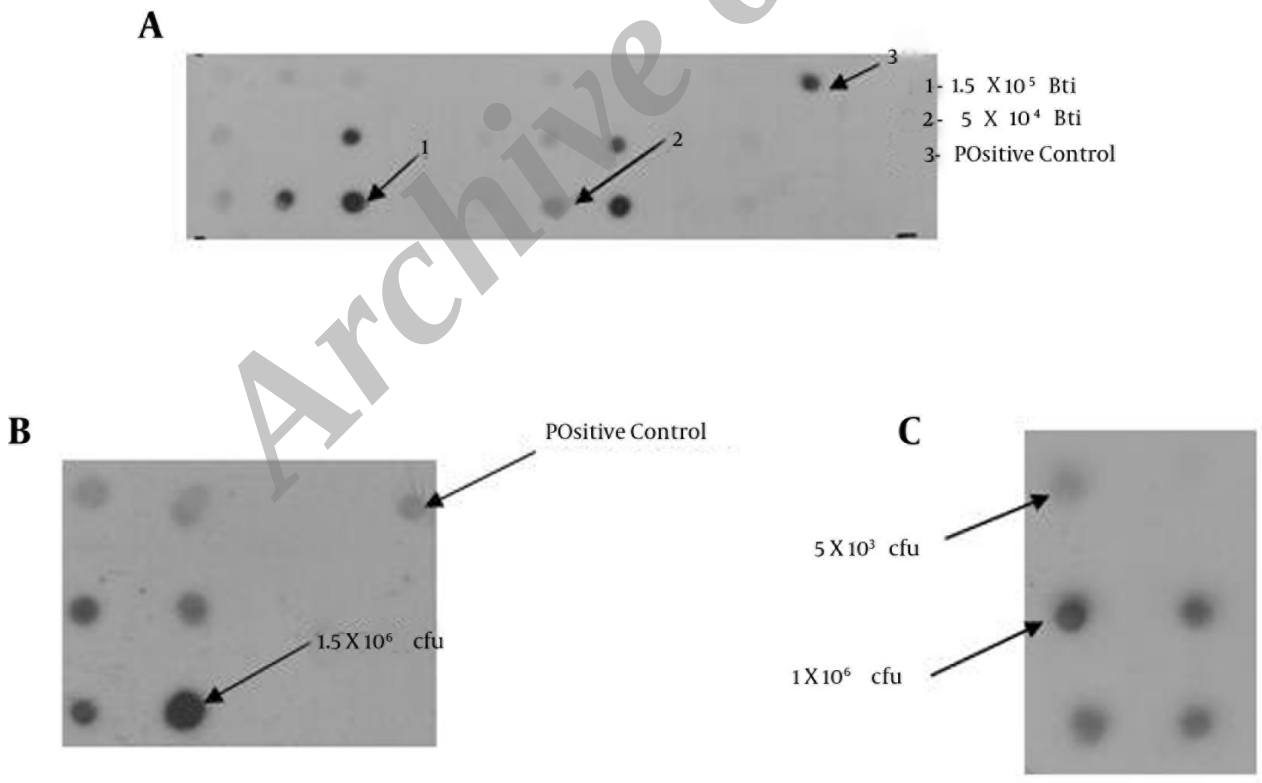
Figure 3. Total Count of Vegetative Cells and Spores of *Bacillus thuringiensis* IPS82 and Recombinant Strain SR08

4.3. Detection of Bacillus thuringiensis IPS82 and Pseudomonas fluorescens Populations Inoculated in Soil by Molecular Methods

Considering the efficiency of molecular methods, in this study, for the detection of the persistence of these populations, the DNA of *B. thuringiensis* IPS82 and *P. fluorescens* populations inoculated in soil was extracted directly from the soil and PCR reaction and dot-blot hybridization were carried out. In the PCR reaction, the *Cry 10Aa* gene of *B. thuringiensis* was detected. The design of oligonucleotides for the detection of *Cry 10Aa* gene was done based on the sequences of three conserved motifs encoding the Cry proteins of *B. thuringiensis*. The amplification of this fragment was useful and used as a specific probe for monitoring inoculated populations of *B. thuringiensis* in soil. The PCR reaction showed that the DNA, directly extracted from soil, was suitable to obtain a successful amplification for the *Cry 10Aa* gene, only when the extracted DNA was diluted (data not shown). This is probably due to some soil components, which could inhibit the reaction, as has been reported in the literature.

In the analysis to detect the inoculated populations of *B. thuringiensis* IPS82 and *P. fluorescens* in soil by dot-blot hybridization, the directly extracted DNA from soil was hybridized with a total genomic DNA probe of the analyzed bacteria, *B. thuringiensis* IPS82 or *P. fluorescens*. Figure 4 A visualizes the detection of *B. thuringiensis* IPS82 for hybridization with the *B. thuringiensis* IPS82 probe of total genomic DNA. The minimal level of detection of this microorganism was 5×10^4 cfu per gram of soil at 10 days, whereas the maximum was 1.5×10^5 cfu per gram of soil, which was found in combination with *P. fluorescens*. The *P. fluorescens* detection for hybridization, with the probe of total genomic DNA, from the tenth and the ninetieth day (Figure 4B and C) shows that the minimum level of detection for *P. fluorescens* was 5×10^3 cfu (on the ninetieth day of the experiment). These results confirm that through genomic hybridization it was possible to monitor the efficiently of the microbial populations introduced in soil, for extended time periods.

Figure 4. Detection of Bacterial Populations of *Bacillus thuringiensis* IPS82 and *Pseudomonas fluorescens* Through Dot-Blot Hybridization in Samples Extracted From Inoculated Soil



A, *Bacillus thuringiensis* IPS82 detection in samples after ten days of inoculation. B, *Pseudomonas fluorescens* detection in samples after ten days. Pseudomonas *fluorescens* detection in samples after 90 days.

5. Discussion

Due to the differences and changes of various environmental conditions at different sites, field studies tend to show large fluctuations in microbial populations (11). Although *B. thuringiensis* can be isolated from various environments, its role in the ecosystem is not clear. One way to elucidate its role in the ecosystem is studying what happens when these bacteria are introduced into the soil as an ending environment. Earlier, studies showed that the survival of *B. thuringiensis* IPS82 is affected by interaction with other native soil microorganisms *in vitro*, particularly, by cohabitation with *P. fluorescens* (2). Therefore, it is possible to consider *B. thuringiensis* as a casual soil microorganism, due to its susceptibility to other microorganism antagonistic present in soil (2). In this study the method of counting in plates to follow the inoculated proportions, was an effective tool to estimate the population dynamics and distinguish the change between vegetative cells and spores.

Previous studies have shown that the dormant spores of *Bacillus* species are much more resistant than their vegetative cell counterparts to a variety of treatments, including heat, pressure, radiation and various chemicals (12). In this study, it was evident that vegetative cells were more sensitive to interaction with other soil microorganisms, in comparison with spores. This study showed that *B. thuringiensis*, similar to *Bacillus cereus*, is able to germinate, grow and sporulate in soil (13, 14). A study found a considerable persistence of spores in the soil, yet their number decreased over time; after 135 days from the last application of spores, the number of spores that could be detected was approximately 6.8 log spores g^{-1} soil (15). This phenomenon is in agreement with the current investigation, wherein was possible to carry out counting of spores in soil with the treatments proved at 90 days of the inoculation.

Other studies showed the persistence of spores of *Bacillus* sp. in soil for months or years; this data clearly indicates that soil is a favorable reservoir for viable spores, whether the soil is in a rural or urban environment (16, 17). It is also possible that these bacteria use some soil natural components, such as nutrients; according to literature reports, *B. thuringiensis* BT27a grew in artificial soil with humic acid as the sole carbon and energy source (18). The divergence of the availability of nutrients in different types of soils, explains the difference of isolation of spores in various environments. The exact moment at which the sporulation process was initiated is unknown. In the present investigation two types of populations were inoculated in the vegetative form; *B. thuringiensis* IPS82 strain and one *B. thuringiensis* SR08 recombinant strain obtained in the laboratory (9). The *B. thuringiensis* population sporulation-germination behavior, depended on the availability of nutrients, which could indicate that *B. thuringiensis* populations fluctuate such as zymogen soil microorganisms do.

At present, culture independent methods based on extraction and analysis of DNA from environmental samples are becoming more popular for assessing the population structure of indigenous or introduced bacterial communities (19-21). In this work molecular methods such as PCR and hybridization using DNA, which was extracted directly from bacterial populations inoculated in soil, were effective to detect and monitor population dynamics, yet PCR was more sensitive to the inhibitor effect of soil components. The literature reports that humic acid, which was present in the soil, could have been interfering with the reaction, inhibiting the activity of enzymes, such as restriction endonucleases and DNA polymerase (8, 22-24). Due to the little information that is available about the *B. thuringiensis* IPS82 behavior in natural conditions and its interaction with *P. fluorescens* in soil, this work evaluated the interaction of these populations, using microbiological methods such as traditional culture and molecular methods such as PCR and dot-blot hybridization.

With the purpose of evaluating the population dynamics of *B. thuringiensis* and its interaction with *P. fluorescens* under different conditions in soil, the effects of nutrients addition and moisture adjustment were analyzed with the proportions of inoculated bacteria. The results indicated that the disappearance of *B. thuringiensis* IPS82 was not dependent on the moisture content but the composition of nutrients that could be affecting the secretion of toxic compounds in the environment of *P. fluorescens* or the sensitivity of vegetative cells to the secreted toxic compounds of *P. fluorescens* in the soil.

Bacillus thuringiensis IPS82 sporulation percentage in soil was determined on the ninetieth day of the experiment in the inoculated proportions in soil. The results indicated that the recovered cells were mostly spores and not vegetative cells, in all proved treatments. The sporulation percentage of *B. thuringiensis* IPS82 was determined every 24 hours during ten days. The results indicated that some of the cells in the form of spores germinated and and were increased and maintained in a constant cycle of sporulation and germination. This confirms that *B. thuringiensis* IPS82 can germinate, grow and sporulate in soil. On the other hand, in this study, for the detection of the persistence of these populations, the DNA of *B. thuringiensis* IPS82 and *P. fluorescens* populations inoculated in soil was extracted directly from the soil and the PCR reaction and dot-blot hybridization were carried out. The results confirmed that these methods were efficient for monitoring and detecting the populations inoculated in soil without traditional culture methods.

The data of this work suggests that *B. thuringiensis* IPS82 can form part of the soil zymogen microorganisms. *B. thuringiensis* IPS82 can be found in soil in low amounts; its presence in this environment perhaps can be due to soil contamination with this bacterium and the persis-

tence of its spores, so it would be more appropriate to consider this bacterium such as an entomopathogenic microorganism and not as a soil germ due to its ability to kill insects.

Acknowledgements

We acknowledge the CONACYT-Consejo Nacional de Ciencia y Tecnologia de Mexico and BUAP-Benemerita Universidad Autonoma de Puebla for their financial support and fellowships provided for Norma Elena Rojas Ruiz and Estibaliz Sansinenea Royano. We also acknowledge Estela Anastacio Marcelino for her technical assistance.

Authors' Contributions

Norma Elena Rojas-Ruiz performed the main research as part of her PhD project. Estibaliz Sansinenea-Royano obtained the recombinant *Bacillus thuringiensis*. Maria Lilia Cedillo-Ramirez was the environmental supervisor. Rodolfo Marsch-Moreno directed the DNA and *Pseudomonas fluorescens* manipulation. Patricia Sanchez-Alonso was the molecular supervisor and performed the data analysis. Candelario Vazquez-Cruz was the main director of the research and provided the grants.

Funding/Support

Funding was provided by the CONACYT, under Grant number 143058.

References

1. Karlovsky P. Secondary Metabolites in Soil Ecology. In: Karovsky P, editor. *Secondary metabolites in soil ecology.*. Berlin Heidelberg Germany: Springer-Verlag Berlin Heidelberg; 2008. pp. 1–17.
2. Rojas-Ruiz NE, Vazquez-Cruz C, Sanchez-Alonso P, Sansinenea-Royano E. Population analysis of Bacillus thuringiensis interaction in vitro with soil bacteria. *Agrociencia.* 2010;**44**(8):941–53.
3. Haas D, Keel C. Regulation of antibiotic production in root-colonizing Peudomonas spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol.* 2003;**41**:117–53.
4. Brodhagen M, Henkels MD, Loper JE. Positive autoregulation and signaling properties of pyoluteorin, an antibiotic produced by the biological control organism Pseudomonas fluorescens Pf-5. *Appl Environ Microbiol.* 2004;**70**(3):1758–66.
5. Mossialos D, Meyer JM, Budzikiewicz H, Wolff U, Koedam N, Baysse C, et al. Quinolobactin, a new siderophore of Pseudomonas fluorescens ATCC 17400, the production of which is repressed by the cognate pyoverdine. *Appl Environ Microbiol.* 2000;**66**(2):487–92.
6. Godert AM, Jin M, McLafferty FW, Begley TP. Biosynthesis of the thioquinolobactin siderophore: an interesting variation on sulfur transfer. *J Bacteriol.* 2007;**189**(7):2941–4.
7. Budde IP, Ullrich MS. Interactions of Pseudomonas syringae pv. glycinea with host and nonhost plants in relation to temperature and phytotoxin synthesis. *Mol Plant Microbe Interact.* 2000;**13**(9):951–61.
8. Sagova-Mareckova M, Cermak L, Novotna J, Plhackova K, Forstova J, Kopecky J. Innovative methods for soil DNA purification tested in soils with widely differing characteristics. *Appl Environ Microbiol.* 2008;**74**(9):2902–7.
9. Sansinenea-Royano E, Sanchez-Alonso P, Anastacio Marcelino E, Ibarra-Rendon J, Olmedo-Alvarez G, Vazquez-Cruz C. Homologous recombination to Bacillus thuringiensis chromosome in one step. *Agrociencia.* 2010;**44**(4):437–47.
10. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: A laboratory manual+ Cold Spring Harbor.* New York: Cold Spring Harbor Laboratory Press; 1989.
11. Song HG, Kim OS, Yoo JJ, Jeon SO, Hong SH, Lee DH, et al. Monitoring of soil bacterial community and some inoculated bacteria after prescribed fire in microcosm. *J Microbiol.* 2004;**42**(4):285–91.
12. Setlow B, Loshon CA, Genest PC, Cowan AE, Setlow C, Setlow P. Mechanisms of killing spores of Bacillus subtilis by acid, alkali and ethanol. *J Appl Microbiol.* 2002;**92**(2):362–75.
13. Vilain S, Luo Y, Hildreth MB, Brozel VS. Analysis of the life cycle of the soil saprophyte Bacillus cereus in liquid soil extract and in soil. *Appl Environ Microbiol.* 2006;**72**(7):4970–7.
14. Melo-Santos MAV, Araújo AP, Rios EM, Regis L. Long lasting persistence of Bacillus thuringiensis serovar. israelensis larvicidal activity in Aedes aegypti (Diptera: Culicidae) breeding places is associated to bacteria recycling. *Biol Control.* 2009;**49**(2):186–91.
15. Guidi V, Patocchi N, Luthy P, Tonolla M. Distribution of Bacillus thuringiensis subsp. israelensis in Soil of a Swiss Wetland reserve after 22 years of mosquito control. *Appl Environ Microbiol.* 2011;**77**(11):3663–8.
16. Van Cuyk S, Deshpande A, Hollander A, Duval N, Ticknor L, Layshock J, et al. Persistence of Bacillus thuringiensis subsp. kurstaki in Urban Environments following Spraying. *Appl Environ Microbiol.* 2011;**77**(22):7954–61.
17. Haddad M, Polanczyk RA, Alves SB, Garcia M. Field persistence of Bacillus thuringiensis on maize leaves (Zea mays L.). *Brazilian Journal of Microbiology.* 2005;**36**(4): 309-314.
18. Ellis RJ. Artificial soil microcosms: a tool for studying microbial autecology under controlled conditions. *J Microbiol Methods.* 2004;**56**(2):287–90.
19. Kuske CR, Banton KL, Adorada DL, Stark PC, Hill KK, Jackson PJ. Small-Scale DNA Sample Preparation Method for Field PCR Detection of Microbial Cells and Spores in Soil. *Appl Environ Microbiol.* 1998;**64**(7):2463–72.
20. Hurt RA, Qiu X, Wu L, Roh Y, Palumbo AV, Tiedje JM, et al. Simultaneous recovery of RNA and DNA from soils and sediments. *Appl Environ Microbiol.* 2001;**67**(10):4495–503.
21. Ticknor LO, Kolsto AB, Hill KK, Keim P, Laker MT, Tonks M, et al. Fluorescent Amplified Fragment Length Polymorphism Analysis of Norwegian Bacillus cereus and Bacillus thuringiensis Soil Isolates. *Appl Environ Microbiol.* 2001;**67**(10):4863–73.
22. Burgmann H, Pesaro M, Widmer F, Zeyer J. A strategy for optimizing quality and quantity of DNA extracted from soil. *J Microbiol Methods.* 2001;**45**(1):7–20.
23. Dong D, Yan A, Liu H, Zhang X, Xu Y. Removal of humic substances from soil DNA using aluminium sulfate. *J Microbiol Methods.* 2006;**66**(2):217–22.
24. Fitzpatrick KA, Kersh GJ, Massung RF. Practical method for extraction of PCR-quality DNA from environmental soil samples. *Appl Environ Microbiol.* 2010;**76**(13):4571–3.