# High School Students for Agricultural Science Research

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# High School Students for Agricultural Science Research

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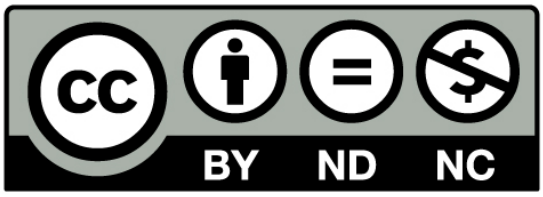

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*Editorial*

# Science Outreach from the Classroom

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Is it possible to communicate science from the classroom? This has been the challenge met by high school students in the frame of the annual Science Week at the IES Zaidín-Vergeles, and as part of the Ciencia BaSe program of the Estación Experimental del Zaidín.

Science outreach and teaching share common objectives, since in both cases the aim is to transmit scientific contents in a clear, precise and understandable way. However, while in the first case the public voluntarily approach the information, out of their own interest, in the second there is a mandatory set of contents that will also be used to evaluate the students' gained knowledge. Likely, this explains why there is an enthusiastic public eager to follow scientific advances, while science courses are among the most difficult for high school students.

A common tool used in the evaluation process at school is to demand from students that they present a report on a given topic. In most cases it consists on gathering information from literature searches, either using specific books or, most frequently, internet. Thus, these activities are limited to a compilation of data from on-line sources, and then simply changing its format to adjust it to that requested by the teacher. When one reads these reports it becomes clear that competent students, trying to do their best, make them unnecessarily complicated by adding concepts that quite often are beyond their current comprehension. In the opposite pole, there are those who simply "cut and paste" data, without any logical sequence or order. When these reports are presented in front of the class, it is easy to realize that in many cases the information included in the work is simply memorized, and not really integrated in the student's knowledge.

Science outreach implies presenting knowledge to people who do not necessarly have a scientific background, trying to correlate concepts and using metaphors and analogies, taking also advantage of different communication tools and, when possible, adding some sense of humor without losing rigour. These elements are scarce in science teaching, and might help students to approach scientific courses with a more positive attitude. With this idea in mind, a science outreach contest was set up, where students could present two types of work: radio podcasts and micro-documentaries, that could even be filmed with their own mobile phones.

The works presented included microdocumentaries with topics such as comets, earthquakes, evolution, pollen and its role in allergies (including resources from a project on pollen microscopy carried out the previous year), bacteria (combining interviews with a parallel project where bacteria were isolated from different environments), and a podcast on recent advances in genetics. All the works are compiled in the blog of the Biology Department of the IES Zaidín-Vergeles: http://biolabzv.blogspot.com.es/

Afterwards, several sessions were carried out to explain students the basics of science outreach and communication, and how to improve their work, both in terms of technical aspects of the different formats and of the way to present the information.

This pilot experience opens the way to add science communication projects to the laboratory ones carried out by high school students and teachers under the supervision of researchers in the frame of programs like Ciencia BaSe, as is the case of the three articles included in this volume. They are examples of what can be done with interested students even if most of the work is carried out in the school laboratory.

# Easy Experiments For Young Students About Simulated Acid Rain Effects On Plants[†]

Miguel Molina Morillo, Rosa Vacas Solís, Julia Shan Vida Lara, Lucas Escudero Rodríguez del Castillo, Elena Pérez Casares, Iker de la Rosa Fernández, Elena de la Torre Amat, Alberto del Castillo Esteban, Íker Fernández Muñoz, Nahuel Kessler, Ainhoa Moreno Duperón, Lupe Rosales Aguilar, Marta Roldán Martín, and Antonio Quesada Ramos[*]

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**SUMMARY**

**Air pollution causes rain to become acid, and this affects living beings. In our laboratory we have carried out some experiments with our students of the first level of ESO to assess the effect of a simulated acid rain – a dilute solution of acetic acid- on plants. Our results prove that germination is affected by acid rain and the damage cause on seeds is permanent. Plants growth was lesser in plants treated with our acetic acid solution than in plants irrigated only with water; no differences were observed in roots length. Acid rain also damaged leaves. Our students concluded that it is neccessary to take action to reduce air pollution and to avoid acid rain effects on plants.**

## INTRODUCTION

Raining is an important part of the water cycle. Rain is also very important for life; all living beings need water to live, even people, and rain brings us the water we need. But rain can be dangerous in some parts of the world because of pollution of the air. An example is what we know as acid rain.

Acid gases are produced when fossil fuels are burned. Nevertheless, nature can also produce this kind of gases in volcanoes. If they mix when water vapour in clouds this process can cause rain to become acid. Lemon juice, vinegar are acids; and sometimes rain is as acid as lemon juice.

Acids can be harmful in several ways. If there is too much acid in soil, seeds will not germinate and plants will not grow. Leaves affected by acid rain cannot make photosynthesis (Singh y Agrawal, 2008).

The effect of simulated acid rain on plants has been widely studied (Neufeld et al, 1985; Fan y Wang, 2000; Sant'anna et al, 2006; Odiyi y Eniola, 2015). Some experimental procedures can be easily adapted to secondary students. So we have carried out easy experiment in our laboratory with students of first level of ESO in order to assess the impact of acid rain on plants. To begin with, they have analysed the effect of a simulated acid rain prepared with acetic acid on the germination of lentil seeds (*Lens culinaris*). Secondly, they have studied the effect of this simulated acid rain on the growth of plants. Finally they have observed morphological alterations in leaves irrigated with a dilute solution of acetic acid.

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[†]***Note:*** *All students from 1st course of ESO have collaborated in this project. Those with a greater degree of implication are mentioned in the authors' list, but the work of all the group is acknowledged.*

Our results show that seeds treated with acid solutions do not germinate even at low acid concentrations, plants watered with acid solutions grow less than plants watered with pure water and leaves are also affected with artificial acid rain.

## MATERIAL AND METHODS

### Effect of simulated acid rain on the germination of seeds

The effect of acid rain has been studied with lentil seeds (*Lens culinaris*). Petri dishes were prepared with absorbent paper; 16 lentil sedes were disposed regularly spaced in every plate. Six of them were irrigated only with water, this was the control set. Groups of five plates were watered with acetic acid solutions (Panreac) of increasing concentration (0,1%, 1,%, 2% and 5%, v/v). In order to know if damage caused by acid is permanent, not germinated seeds were washed and transferred plates only with water. Germination was studied in the following days.

### Effect of similated acid rain on the growth of plants

Control plates from the previous experiment with germinated seeds were separated in two groups. Three plates were irrigated with water and the other three with acetic acid solution (1%). Qualitative differences in growth of small plants were observed for several days. For in vivo studies, two sets of pots were prepared with soil and ten lentil seeds were sown in every container. One of these sets were irrigated with water; the other one with an acetid acid solution (1% v/v). A spray dispenser was used to water plants. After two weeks, plants were collected and the length of stems and roots was measured by students.

### Effect of simulated acid rain on leaves.

The effect of acid rain on leaves was checked studying morphological differences between leaves treated only with water and with acetic acid solution.

## RESULTS AND DISCUSSION

### Effect of acetic acid solutions on the germination of lentil seeds

Almost all seeds germinated in control plates (95/96, 99%). No germination was observed when seeds were treated with acetic acid concentrations higher than 0,1%. In this case, only 30% (24/80) had germinated after six days of treatment (Figure 1).

In order to test if damage on seeds caused by acid is permanent, acid solutions were removed from plates without lentils germination, seeds were washed with water and transferred into plates with absorbent paper with only water. None of them germinated in the following days. In conclusion, damage in seeds caused by acid is permanent.

### Effect of simulated acid rain on the growth of plants

Students have observed the effect of simulated acid rain on the growth of plants in vitro. Control plates, all with germinated seeds, were divided into two sets. One was irrigated with acetic acid solution 0,1%; the other one with pure water. Figure 2 shows the growth of plants a week later. Plants treated with acid grew less than plants irrigated with water.

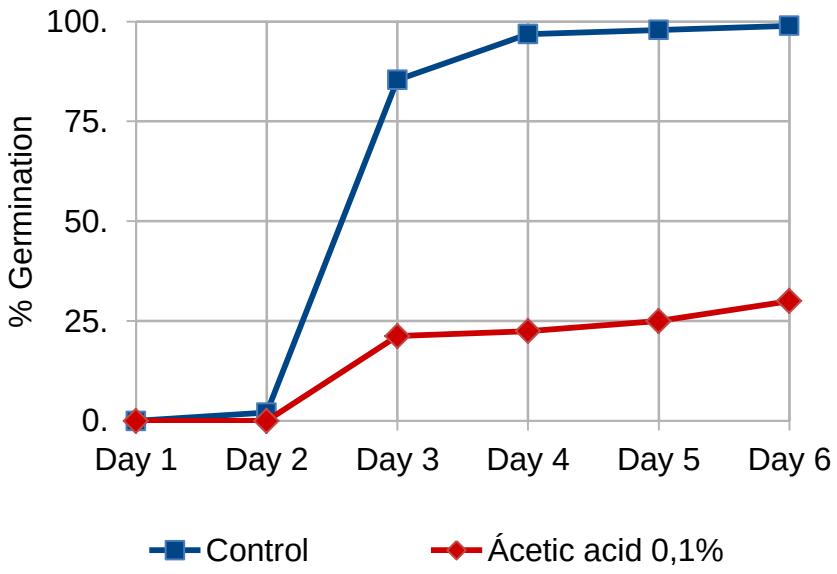


**Figure 1.** Germination of lentil seeds treated with water (blue line) and with acetic acid solution 0,1% v/v (red line).

**Figure 2.** Small plants growth irrigated with acetic acid solution 0,1% v/v (upper row) versus growth of plants irrigated with water (lower row).

**Figure 3.** Lentil plants growth. Control plants (left) and plants treated with simulated acid rain (right).

Lentils seeds were also grown in pots. Half of them were irrigated with a spray dispenser with water; the rest were watered in the same way with a simulated acid rain (acetic acid 1% v/v). Nine days later plants had grown (figure 3) and were collected. There were 65 control plants and 76 with the simulated acid rain treatment. Stem and roots length were measured by students (figure 4). Measurements of every plant are shown in table 1 and 2. Average stem length in plants treated with acid was 17,22 cm; average stem length in control plants was 20,22 cm. Average root length was 6,22 cm in treated plants versus 6,61 cm in plants irrigated with water. In conclusion, acid rain affect the growth of plants.

**Figure 4.** Students measuring stems and roots of lentil plants.

**Table 1.** Stem and roots length in plants treated with simulated acid rain.

| Plant | Stem length | Root length | Plant | Stem length | Root length | Plant | Stem length | Root length | Plant | Stem length | Root length |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 6 | 21 | 9,5 | 3 | 41 | 19 | 8,5 | 61 | 9,5 | 7,5 |
| 2 | 1,5 | 2 | 22 | 16,5 | 3 | 42 | 17 | 10 | 62 | 20 | 5 |
| 3 | 14 | 6 | 23 | 22,5 | 1 | 43 | 18 | 11,5 | 63 | 21 | 6 |
| 4 | 16,5 | 8,5 | 24 | 13,2 | 5 | 44 | 17 | 6,5 | 64 | 20 | 4,5 |
| 5 | 21 | 10 | 25 | 9,2 | 1 | 45 | 11 | 2 | 65 | 9 | 23 |
| 6 | 20,5 | 6,5 | 26 | 2,5 | 2 | 46 | 15,6 | 9 | 66 | 21 | 8 |
| 7 | 20 | 10 | 27 | 14 | 2,1 | 47 | 17 | 1,7 | 67 | 18 | 8 |
| 8 | 20,5 | 6,5 | 28 | 2,4 | 8 | 48 | 17,5 | 1 | 68 | 19 | 9 |
| 9 | 20 | 10 | 29 | 22,4 | 8,7 | 49 | 22 | 9,5 | 69 | 16,7 | 6,3 |
| 10 | 14 | 6,5 | 30 | 21,4 | 9,9 | 50 | 19,5 | 10 | 70 | 20,1 | 3,9 |
| 11 | 19,5 | 11 | 31 | 17,2 | 8 | 51 | 18 | 8,5 | 71 | 21,3 | 8,6 |
| 12 | 20 | 0,5 | 32 | 18,5 | 2,5 | 52 | 20,5 | 2,5 | 72 | 22,5 | 5,4 |
| 13 | 18,5 | 0,5 | 33 | 17 | 2,5 | 53 | 18,8 | 2 | 73 | 23 | 11,5 |
| 14 | 16 | 6,5 | 34 | 22,5 | 7,5 | 54 | 18,8 | 10 | 74 | 17,7 | 4 |
| 15 | 21 | 10 | 35 | 19 | 2,5 | 55 | 21 | 2 | 75 | 17,9 | 3 |
| 16 | 26,5 | 3 | 36 | 18 | 7,5 | 56 | 11 | 11 | 76 | 5,5 | 10 |
| 17 | 19,5 | 0,5 | 37 | 15 | 6 | 57 | 20 | 9 | | | |
| 18 | 17 | 5 | 38 | 17 | 7,5 | 58 | 23 | 10 | | | |
| 19 | 21 | 1 | 39 | 21,5 | 1 | 59 | 20 | 10,5 | | | |
| 20 | 12 | 4 | 40 | 17 | 1 | 60 | 18 | 10 | | | |

**Table 2.** Stem and roots length in plants irrigated with water.

| Plant | Stem length | Root length | Plant | Stem length | Root length | Plant | Stem length | Root length | Plant | Stem length | Root length |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 23 | 12 | 21 | 21,8 | 7,7 | 41 | 17,2 | 6 | 61 | 21 | 8 |
| 2 | 22 | 5,5 | 22 | 23,4 | 10 | 42 | 23,4 | 3,4 | 62 | 25 | 6,2 |
| 3 | 24 | 6 | 23 | 21,4 | 9,3 | 43 | 24 | 12,5 | 63 | 17 | 8,2 |
| 4 | 23 | 4,5 | 24 | 16 | 2 | 44 | 9 | 11,2 | 64 | 22 | 7,6 |
| 5 | 23 | 12 | 25 | 23 | 8,5 | 45 | 20 | 6,5 | 65 | 23,4 | 11,5 |
| 6 | 21 | 2 | 26 | 17 | 8,1 | 46 | 23,5 | 12,4 | 66 | 24 | 2 |
| 7 | 23 | 5 | 27 | 20,9 | 4,1 | 47 | 24 | 10 | | | |
| 8 | 25 | 5 | | 22,3 | 11 | 48 | 21,4 | 5 | | | |
| 9 | 19 | 5 | 29 | 22,4 | 12,5 | 49 | 17,1 | 2 | | | |
| 10 | 12 | 2 | 30 | 26,5 | 13 | 50 | 10,6 | 1,5 | | | |
| 11 | 22 | 11 | 31 | 25,5 | 12,5 | 51 | 29 | 0,1 | | | |
| 12 | 19 | 5 | 32 | 24 | 7 | 52 | 10,8 | 2,5 | | | |
| 13 | 22,5 | 3 | 33 | 25,9 | 8 | 53 | 25 | 17 | | | |
| 14 | 24 | 4 | 34 | 24,3 | 8,2 | 54 | 19 | 3 | | | |
| 15 | 11,3 | 2 | 35 | 3,9 | 1,3 | 55 | 25 | 3 | | | |
| 16 | 22,3 | 8 | 36 | 20,7 | 9 | 56 | 11,8 | 2,4 | | | |
| 17 | 24,4 | 9 | 37 | 2 | 2 | 57 | 24 | 8,5 | | | |
| 18 | 22,3 | 3,8 | 38 | 17,5 | 7,5 | 58 | 19,3 | 3 | | | |
| 19 | 16,8 | 7,1 | 39 | 12,7 | 2 | 59 | 12 | 2,3 | | | |
| 20 | 23,8 | 7,1 | 40 | 25 | 11,5 | 60 | 16,5 | 6 | | | |

**Effect of simulated acid rain on leaves**

Plants treated with simulated acid rain show damaged leaves. These leaves were smaller than healthy ones, wrinkled and they had brown spots, consistent with necrotic areas. On the contrary, plants irrigated with water had normal leaves and they didn´t show any sign of necrosis. Results are shown in figure 5.

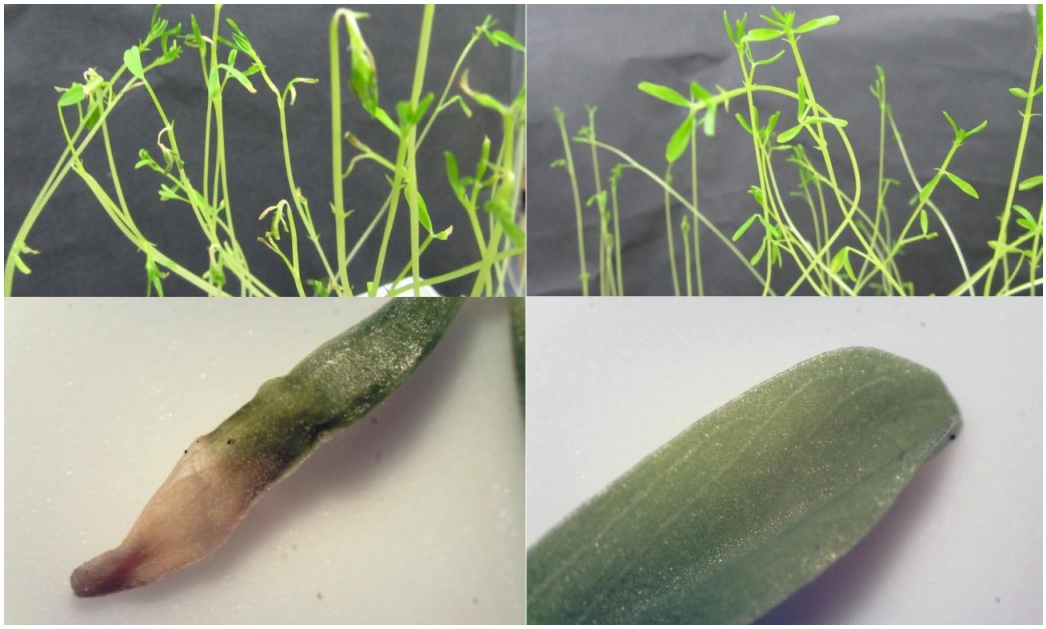

**Figure 5.** Leaves aspect in plants treated with acetic acid solution (left) and in plants irrigated with water.

**CONCLUSIONS**

- Lentils seeds germination is affected by simulated acid rain. Seeds germination was inhibited with acetil acid concentrations equal o higher than 0,5% (v/v).
- Damage caused by acid in seeds is permanent. Seed are not able to germinated if they are transferred to water.
- Only 30% of seeds treated with simulated acid rain (0,1% v/v) germinated. 99% of seeds treated with water germinated.
- Nine days after sowing, plants irrigated with simulated acid rain (acetic acid 1% v/v) grew an average of 2 cm less than plants irrigated with water. No differences in roots length were observed between plants treated with water and simulated acid rain.
- Acid rain caused damage in leaves. Leaves were smaller in plants irrigated with simulated acid rain, they became wrinkled, with brown spots due to chlorophyll loss.
- Students have carried out an easy project in the laboratory and they have been able to understand the results of the experiments. Some of them have related the results with consequences of air pollution and have proposed solutions to protect and preserve the environment.

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**MY OWN IDEAS**

Yo creo que es un experimento muy interesante, ya que podemos aprender por qué no debemos contaminar para conservar las plantas. A mi me parece muy importante que podamos aprender con experimentos como el que hemos hecho porque es mas fácil recordar lo aprendido que cuando te dicen algo. Yo pienso que se podrían hacer más cosas sobre en qué influye la contaminación como por ejemplo en los animales o incluso en nosotros. Lo que mas me llamó la atención es lo fácil que fue ver el efecto de la contaminación, en este caso con ácido, sobre la vida de las plantas. Yo no era consciente de lo mucho que pueden influir productos como el ácido acético del vinagre sobre la germinación y el crecimiento de las plantas.

*Rosa Vacas Solís*

Este proyecto me ha parecido important porque nos ayuda a aprender el desarrollo de las plantas según si las regamos con agua ácida o agua normal. Lo que más me llama la atención es que hay una gran diferencia de longitud entre el tallo y la raíz de algunas plantas. Hemos visto una planta regada con agua normal con 22 cm de tallo y 15 cm de raíz y, sin embargo, con lluvia ácida otra de 8 cm de tallo y 3 cm de raíz. Aunque las raíces, generalmente, tenían la misma longitud media.

*Lucas Escudero Rodríguez del Castillo*

At the end of the experiment, we saw that the acid affected the growth of plants. When we started we put 10 lentils in each bowl. The seeds did not germinate untile the third day, but the ones irrigateed with water lasted more. We came to the conclusion that watering the plants with acid affected the seeds even though we tried to water again with pure water. I think that this experiment is very interesting because we can observe growth.

*Julia Shan Vida Lara*

Este ha sido un experimento que organizamos los niños y niñas de 1º de ESO B que consistía en plantar semillas en unas placas e ir regando unas con agua y otras con agua mezclada con un poco de ácido. De esta manera observamos que las plantas regadas con agua normal crecieron más y más sanas.

Me parece importante porque es una manera diferente de aprender y podemos observar mejor como pasan las cosas. Además, así aprendemos a utilizar algunos objetos del laboratorio.

Creo que hubiese sido mejor que lo hubiésemos hecho con semillas de frutos o flores porque así veríamos el cambio en el fruto.

*Elena Pérez Casares*

This experiment is fantastic because is a simulation of the acid rain. We can see the effect of acid rain in the plants. With this experiment we learn that we have to contaminate less and use more bicycle and public transport. My idea is that we can use more acids with more concentration like sulfuric and nitric acids.

*Iker de la Rosa Fernández.*

El experimento trataba de hacer germinar unas plantas con ácido acético y otras con agua, y ver cómo crecían en ácido y en agua. El experimento me gustó, pero no mucho porque solo lo hicimos con dos liquídos; la vez siguiente lo podríamos hacer con Fairy, cola, etc.

*Íker Fernández Muñoz*

Siempre me ha gustado ver el crecimiento de una planta, y al saber que iban a ser regadas con lluvia ácida me motivó. Me hubiese gustado que participasen más compañeros. Podría intentarse con semillas de otras plantas, como semillas de fresa. He aprendido a cultivar una planta de manera que no se desperdicie. Me sorprendió el resultado final. En mi opinión el trabajo estuvo muy bien.

*Nahuel Kessler*

El experimento sobre lluvia ácida me ha parecido muy interesante y divertido porque era la primera vez en el laboratorio del instituto y mi primer experimento en la clase. Aunque también me pareció interesante porque no sabía que con papel podía germinar una semilla como si fuese tierra. Fue algo que me impresionó. También cuando el profesor dijo "vamos a echarle ácido" yo me sorprendí y pensé que era ácido de ese que si lo tocasse quita toda la piel y los músculos, pero cuando dijo que era parecido al vinagre me alivié. Espero que hagan más experimentos en el instituto, a ser posible cada vez más interesantes.

*Lupe Rosales Aguilar*

I believe that this experiment is very nice because you can compare the effect of the acid rain on plants and make solutions for stop it. With it, we can know in a small scale the things that we are doing since few years ago with the Earth and the environment.

Something to make the experiment better is talk aout it with people and concienciate them. In addition I think that it is a good idea and we have to share it with more schools.

*Marta Roldán Martín*

Este experimento además de divertido me ha parecido muy importante ya que nos ha servido para conocer y aprender sobre un problema medioambiental que sufren principalmente las plantas, pero que al fin y al cabo puede tener repercusiones sobre nosotros. Como ya he explicado antes nos ha ayudado a conocer este problema: sus efectos sobre las plantas, como crecen si entran en contacto con algún ácido, a qué partes de la planta les afecta el ácido… y hemos podido resolver una duda que teníamos: ¿Son capaces de crecer algunas plantas con ácido? La respuesta es sí, ya que algunas de las lentejas que

fueron regadas con un porcentaje de ácido acético (vinagre pero más concentrado) menor que el 0,5% lograron crecer. Una de las cosas que podríamos llevar a cabo es realizar este experimento con otro tipo de plantas para poder recoger más datos, o hacer este mismo experimento echando a un vaso lleno de agua procedente de alguna charca un poco de este mismo ácido y ver cómo les afecta a los microorganismos que se encuentran en ella. Lo que más me llamó la atención de este pequeño experimento es la capacidad que tienen las plantas de sobrevivir ante adversidades y condiciones tan duras.

*Miguel Molina Morillo*

# Soil Bacteria: Number, Diversity and Relevant Activities

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**SUMMARY**

**In this project, we have analyzed samples of different types of soil in Granada and surroundings, to compare the number and the diversity of bacteria in each sample. Different bacteria have been isolated to study their capacity to solubilize phosphates, the production of siderofores to capture iron, and finally if they have antimicrobial capacity. We have also made a preliminary identification of three of the strains isolated by sequencing part of the 16 rRNA gene.**

## INTRODUCTION

Soil is the surface part of the earth´s crust, biologically active, which comes from the disintegration or from the physical and chemical alteration of rocks and residues from the activities of living beings. Soil, in addition to serving as support to the plants, provides the nutrients needed for their growth. The role of soil is a fundamental part of the biogeochemical cycles and the mobility of these through the cycle of water (1, 2).

The biogeochemical cycles come from the cyclic movement of the elements that form the biological organisms and the geological environment. Thanks to the biological cycles, the elements are found available to be used once and again by other organisms; without these cycles, the living beings would be extinct. These are natural processes which recycle elements in different chemical forms from the environment to the organisms and then in reverse. Water, carbon, oxygen, nitrogen, phosphorus and other elements travel this cycle, connecting the living and non-living components of the earth (1, 2, 3, 4).

Bacteria and fungi are microorganisms that together with the producers, allow the existence of the cycle of matter in the biosphere. Their function is to decompose organic matter coming from vegetable remains, cadavers, excrements, and turning them into inorganic materials which are re-used by producers. The activity of the decomposers in the biosphere allows matter to be recycled. Two of the elements that follow these cycles in which the bacteria intervene are phosphorus and iron (5).

After nitrogen, phosphorus is the inorganic nutrient most required by plants and microorganisms. The plants must absorb it from soil, where it is found in soluble form in little amount. These low nutrient indices are due to the fact that soluble phosphate reacts with ions like calcium, iron and aluminum, and that causes its precipitation or fixation, lowering its availability for the vegetable (6). Therefore, the microorganisms capable of solubilizing different phosphate rocks and other sources of inorganic phosphorus are fundamental to increase the amount of nutrient available for plants.

Iron (Fe) is an essential element for practically all of the living beings, since it is necessary for important cellular functions like DNA synthesis, breathing and detoxification of free radicals. In nature, it is fundamentally found in the form $Fe^{3+}$ taking part of salts and hydroxides of very low solubility, chemical forms that make impossible their use for some organisms. To solve this problem, a lot of organisms, including bacteria, fungus and another plant, produce small molecules, non-ribosomal peptides many of them, with high affinity for iron called siderophores which act in a specific way like chelating agents to abduct iron in the presence of other metals and reducing it to $Fe^{2+}$, a much more soluble form and take advantage of it for its nutrition (7).

The limited nutrients present in soil make this environment a very competitive one. Some microorganisms are capable of producing and releasing molecules with antimicrobial activity, as a weapon to eliminate competitors.

In this work we have focused on the following objectives:

- To determine the number and diversity of bacteria in different soil types.
- To analyze if some bacteria can intervene in phosphorus or iron cycles.
- To test if there are bacteria with antimicrobial activities in these samples.
- To identify the species corresponding to some of the bacteria isolated.

## MATERIALS AND METHODS

### Samples

8 soil samples were taken from different parts of Granada and province and from different origins. In each sample, its pH was determined.

Sample 1: Collected from a pot in Cenes de la Vega.

Sample 2: Collected from a garden in Albaicín, Granada.

Sample 3: Collected in Alhendín, on and uncultivated land.

Sample 4: Collected from the orchard in the school Zaidín Vergeles.

Sample 5: Collected from a garden in Huetor Vega.

Sample 6: Collected from a pot with an ornamental plant in Granada.

Sample 7: Collected from the field in Gabias.

Sample 8: collected from a pot in Cenes de la Vega.

### Isolation of bacteria

To determine the type of bacteria in the soil, the technique of the decimal solutions and sowing in Petri plate was applied.

20 ml of saline solution were placed in a sterile tube and 10 g of soil from each sample were added. Tubes were shaken well, and allowed to settle. Afterwards, three zones can be distinguished, one on the top formed with less dense organic matter, an intermediate which

is lightest, and the last one all the way to the bottom containing more dense organic matter. The middle part was the one that used to do the cultivation.

The sample was diluted (to prevent an excessive growth of bacteria) adding 100 µl from the lighter side to 0.9 ml of M9 medium (8). Next, 100 µl from the dilution were spread on Petri dishes using glass beads. Plates were incubated at a temperature of 30°C for 2-3 days. A second culture was done in rich medium (LB). After analyzing and comparing the number of de colonies and its morphology of each soil in the two different media, several different types of colonies were picked with sterile toothpicks in new dishes to confirm the colonies´ phenotypes. The colonies were named with numbers (corresponding to the soil sample from where they came) and letters for the different morphologies.

**Siderophore production assay**

To analyze which bacteria produced siderophores, colonies were picked on King's B, a medium with low iron content:

- 10 g proteose peptone
- 1.5 g anhydrous $K_2HPO_4$
- 15 g glycerol
- 5 mL $MgSO_4$ (1 M; sterile)

After incubation at 30°C for 24 h, plates were observed under UV light to determine the appearance of a bright halo around the colony, indicative of siderophore production.

**Phosphate solubilization assay**

It was determined which bacteria carry out phosphate solubilization, growing the different colonies on NBRIP medium:

- Glucose: 10 g (source of carbon and energy)
- $(NH_4)_2SO_2$: 0.1 g (source of nitrogen and sulfur)
- $MgSO_4$: 0.25 g
- KCl: 0.2 g
- $Ca_3(PO_4)_2$: 5 g (source of insoluble phosphorus)

Plates were incubated at 30°C for several days to observe the appearance of a clear halo around the colonies.

**Antimicrobial production assay**

To analyze which bacteria produce antibiotic substances, colonies were picked on a Petri plate with LB medium in which previously a culture of *Escherichia coli* had been spread. The plates were incubated at 30°C to observe the appearance of a clear halo around the colonies, indicative of the inhibition of *E. coli* growth.

**Gram stain**

The Gram stain is a laboratory technique used in microbiological studies of bacteria to distinguish and classify different groups of bacteria based on their cell wall composition.

Selected bacteria were grown in liquid LB medium. 2 µl of each culture were placed on a microscope slide and fixed with heat. The samples were stained as follows:

- Stained with crystal violet 1', and rinsed with distilled water.
- Incubated with lugol 1', and rinsed.
- Discolored with acetone for 20" and rinsed.
- Stained with safranin 30", rinsed and allowed to dry.

Slides were then observed under the microscope. Those stained red are Gram negative and those stained purple are Gram positive.

**Polymerase Chain Reaction (PCR)**

To identify the selected microorganisms, we performed PCR amplification of part of the 16S ribosomal DNA. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Universal 16S primers containing conserved sequences were used for amplification, with colonies of the different bacteria as starting material for template. The PCR conditions were as follows:

| | | | |
|---|---|---|---|
| Initial denaturing | 95°C | 5' | |
| \| Denaturing | 95°C | 30'' | \| |
| \| Annealing | 48°C | 30'' | \| × 30 cycles |
| \| Extension | 72°C | 1' 30'' | \| |
| Final extension | 72°C | 5' | |

**Sequence analysis**

Sequencing of 16S rDNA was done at the IPBLN sequencing facility. Sequences were compared with the databases using Targeted Loci Nucleotide BLAST, available at the National Center for Biotechnology Information web (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**RESULTS AND DISCUSSION**

**Number and diversity of bacteria from different soil samples**

From the different soil samples, we analyzed the number of colonies and their morphology in minimal medium with glucose as the only carbon source. Results are shown in Table 1. Sample 4, corresponding to soil from the orchard of our own school had the largest number of bacteria (between 10 and 100 times more than any other sample, approximately). In contrast, only 6 colonies were isolated from sample 6, coming from a pot with an ornamental plant.

Based on colony morphology, we observed limited diversity of bacteria capable of growing in M9+glucose, between 2 and 3 different types in each sample:

- Sample 1: both of the colonies were similar, light color, and wet appearance.

- Sample 2: we found two types, one darker, dry appearance and the other one lighter and less dry.
- Sample 3: one colony had ochre color with mucosal appearance. The other was whiter but didn't grow when it was transferred to a new plate.
- Sample 4: both had mucosal appearance and ochre color, but one darker than the other.
- Sample 5: one had dry appearance and white color; the other ones were similar but one of them more transparent and the other more opaque.
- Sample 6: one had appearance of white-yellowish fungus (it could be *Streptomyces*). The other one had mucosal appearance and ochre color.
- Sample 7: very similar, a solid color. One might be contaminated by a fungus.
- Sample 8: one of them had mucosal and ochre appearance and the other dry and clear appearance.

We also tried to establish if there might be a correlation between the number of bacteria and the pH of the soil sample. As shown in Table 1, the samples with a pH of 7 were the ones with higher number of bacteria, while lower and especially higher pH values seem to be disadvantageous.

**Table 1.** Analysis of pH values, number of colonies and diversity of colonies in soil samples

| Sample | pH | Colonies | morphology types |
|---|---|---|---|
| 1 | 7.5-8 | 12 | 2 (+ several fungi) |
| 2 | 6.5 | 120 | 2 |
| 3 | 7 | 144 | 2 |
| 4 | 7 | 1300 | 2 |
| 5 | 6.5 | 30 | 3 |
| 6 | 7-7.5 | 6 | 2 |
| 7 | 7 | 504 | 3 |
| 8 | 6.5 | 28 | 2 (+ several fungi) |

## Iron and phosphate solubilization

We selected 16 different colonies to test their ability to solubilize phosphate and to produce siderophores for iron capture. The results for phosphate solubilization were negative in all cases. However, it is possible that the culture conditions used were not optimal for this activity and more tests would be required to confirm the lack of activity.

Siderophores are usually complex molecules with aromatic rings, which emit fluorescence when illuminated with UV light. The halo around a colony in a medium with low iron indicates that siderophores are released to the medium, which diffuse through the agar. The results are shown in Figure 1. Colonies from samples 2, 6 and 7, and colony 4b didn't produce siderophores. In contrast, colonies 1a, 1b and 5a produced a very intense halo around the colony, indicating a significant release of siderophores. The rest of the colonies produced smaller or less intense halos, indicating some production of siderophores.

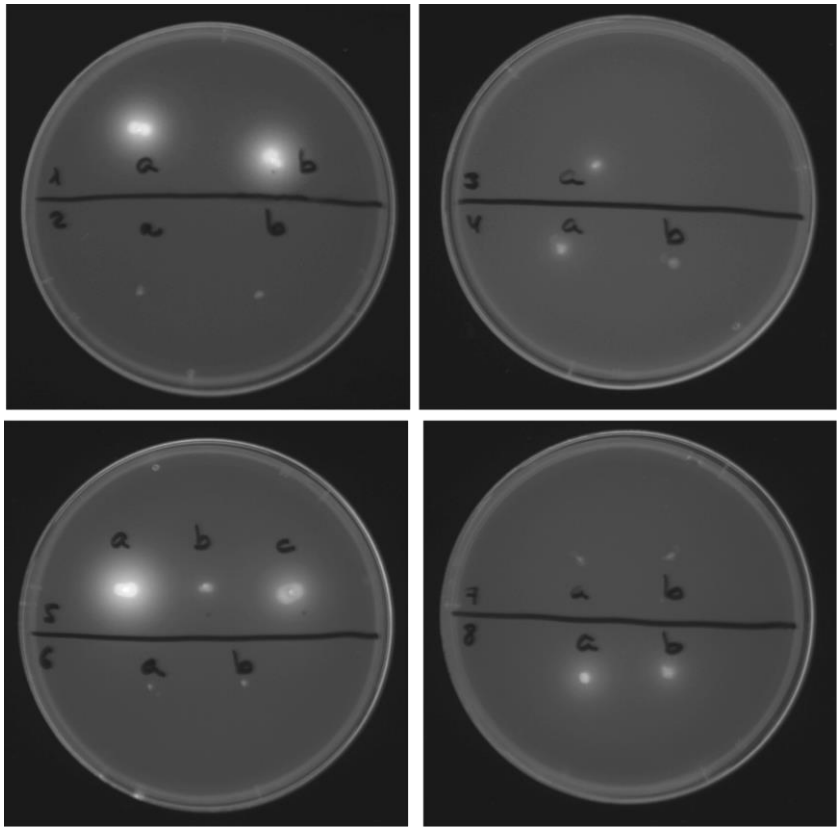


**Figure 1.**
Siderophore production by isolated bacteria

**Antimicrobial activity**

We also tested if any of the selected bacteria was capable of synthesizing antimicrobial substances that inhibit the growth of *E. coli*. These assays were done using LB plates where a lawn of E. coli was spread and each colony was picked, to observe the appearance of an inhibition halo around them. Results are shown in Figure 2. Colony 8b was the only one that produced a clear inhibitory halo. Strain 7a also appeared to produce an inhibitory halo, but it was much smaller. Finally, strain 7b appeared to produce a halo but it was not completely clear, suggesting that there might be some partial growth inhibition.

Based on the production of siderophores and possible antimicrobial compounds we selected three of the isolates to continue characterizing them: colony 5a was chosen as the one producing larger quantity of siderophore; colony 7a was chosen due to its potential production of antimicrobials; and colony 8b was chosen because it combines production of siderophores and the most evident inhibitory effect on growth of *E. coli*. Figure 3 shows an LB plate where these three bacterial strains were sown.

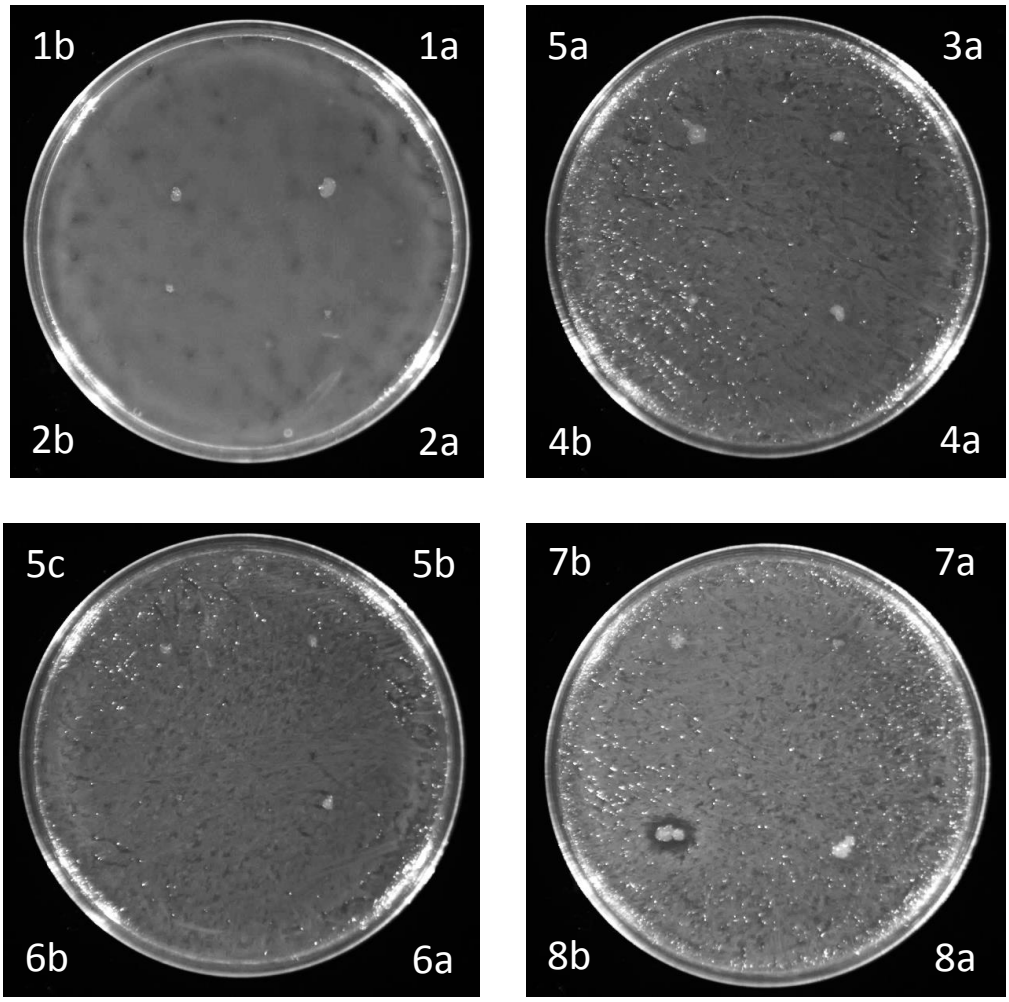


**Figure 2.** Production of antimicrobial compounds by selected bacteria

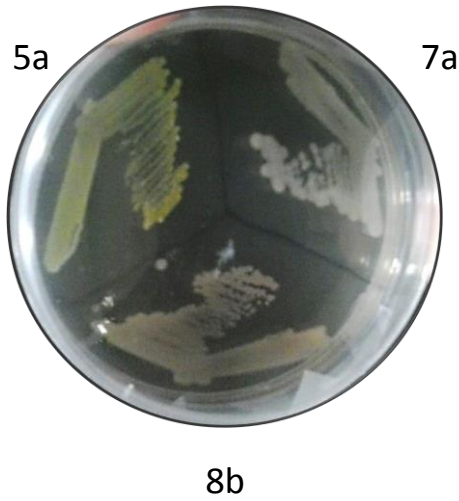


**Figure 3.** Bacteria selected for characterization

## Gram strain

The three strains were classified based on Gram stain. Results are shown in Figure 4 and indicate that strain 5a is Gram positive and the other two are Gram negative.

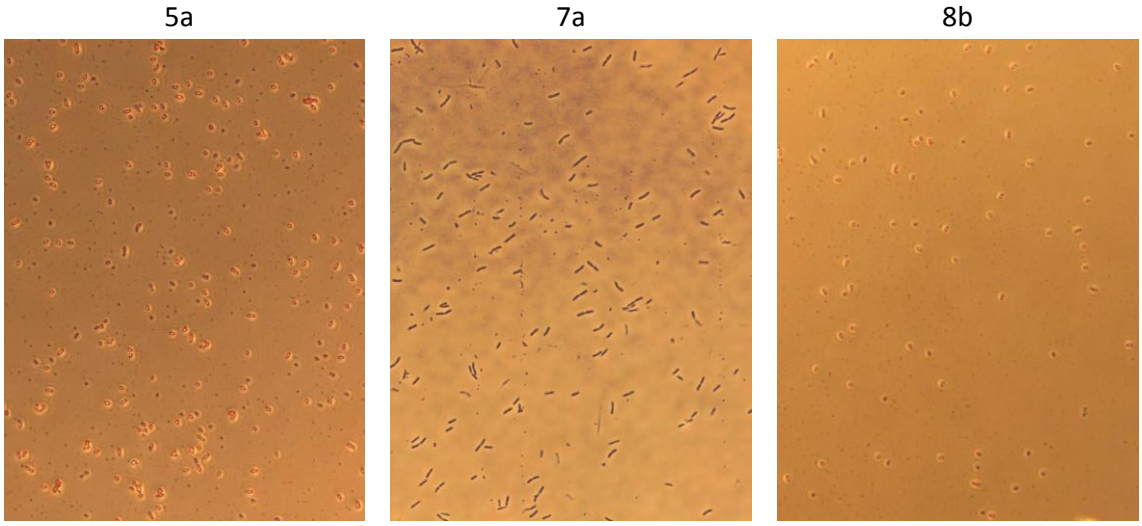


**Figure 4.** Gram stain and microscope visualization of strains

## Sequencing of a DNA region of ribosomal RNA 16S and taxonomic classification of strains 5a, 7a and 8b

In order to determine the identity of the selected bacteria, we decided to do a preliminary taxonomic classification based on partial sequencing of the 16S ribosomal DNA, which is considered the most reliable method used routinely for identification and classification of bacterial species. Using a small amount of culture of each strain as template, we did a PCR amplification with universal oligonucleotides designed to amplify a fragment of 16S rDNA, as detailed in Materials and Methods. The PCR products were sequenced using the same oligonucleotides, at the Sequencing Facility of IPBLN.

The obtained sequences (shown below in the 5'-3' orientation, after confirming the base identities in both strands) were used for comparison with rDNA database using BLAST programs [5,6]. The results of the database analysis are presented in Table 2.

Isolate 5a:

AGTCGAGCGGATGAGTGGAGCTTGCTCCATGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGT
AGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACC
TCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCCGTAAC
TGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTA
AGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCG
TGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA

Isolate 7a:

CAGTCGAGCGACCGATGGGAGCTTGCTCCCTTAGGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCT
GTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCATGGTTCAATCATAAAA
GATGGCTTTTGCTATCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAAGGGCTCACCAAGGCG
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG
TAAAGCTCTGTTGTTAGGGAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCC

ACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCG
CGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGG

Isolate 8b:
CGTCCTCCCGAAGGTTAGACTAGCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC
CCGGGAACGTATTCACCGCGACATTCTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGAC
TGCGATCCGGACTACGATCGGTTTTCTGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTCTGTACCGACCATTG
TAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG
GCAGTCTCCTTAGAGTGCCCACCATCACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACC
CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAATGTTCCCGAAGGCACCAATCCATCTC
TGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCT
TGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCAACTTAATGCGTTA
GCTGCGCCACTAAGAGCTCAAGGCTCCCAACGGCTAGTTG

**Table 2.** Identification of selected soil bacteria

| Isolate | Most similar species | % identity |
|---|---|---|
| 5a | *Pseudomonas stutzeri* | 100% |
| 7a | *Bacillus licheniformis* | 98.2% |
| 8b | *Pseudomonas veronii* | 99% |

The sequence obtained from strain 5a was 100% identical to *Pseudomonas stutzeri* and strain 8b also corresponds to a *Pseudomonas* (probably *P. veronii*). *Pseudomonas* are commonly found in soil and in association with plants, and are known to produce significant amounts of siderophores. Also, some species produce antimicrobials such as phenazines. Strain 7a corresponds to *Bacillus*, also frequently found in soils. The most similar was *B. licheniformis*, but the % of identity is not sufficiently high to ensure that this is the actual species.

## CONCLUSIONS

After finishing this project, we can extract the next conclusions:

● All of the soil from the same region, in this case Granada, share the same pH, in this case all samples oscillated around neutral pH (between 6´5 and 7´5).

● For a similar pH in different soils the number of bacteria is variable, although in different diversity.

● There is a large quantity of bacteria in the soil even though this doesn't imply a great diversity of species.

● We can observe that the number of bacteria is bigger in cultivated soils (the samples of the orchard in the high school are the ones that have more bacteria). In the soils used on gardens and pots, the number of bacteria are less.

● The percentage of the bacteria in the soil of Granada which produce siderophores is not very big, only 6 of the analyzed colonies, 4 of them in little amounts and only two in large quantity. The percentage of bacteria that solubilize the phosphorus are smaller, in our case we didn't get any positive case.

● We also find a very small percentage of bacteria with an antibiotic activity, in total only two.

● The bacteria which produce siderophores are *Pseudomonas*.

● The bacteria with most clear antimicrobial activity is classified as *Pseudomonas veronii* or a very similar secies.

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**MY OWN IDEAS**

**Jessica:** the project has appeared to me very interesting and instructive, because I´ve learned a lot about bacteria, how to difference them, when they are Gram positive or Gram negative and also on the number of bacteria that a soil has depending on where you get it and also depending on the cultivation which they are put on it will appear more or less bacteria.

**Juanma:** I´ve enjoyed doing this project since it has been my first experience of this type and it let me learn very curious data besides getting closer to the laboratory and research work. Since I want to dedicate to the field of research within the biology. This project let me prove that it results a very interesting field and reaffirm my interest for it. We have also learned working with bacteria, how to extract, cultivate, isolate them, etc., something that I have never done and it came out to be really interesting.

**Alba:** this project was for me a very interesting way of investigate and most of all recognize the characteristics and qualities that a bacterium has, also we had worked with laboratory material which it leads has on having more experience on how to use them. It is not the first time that I work on an investigation project even though I´ve learned a lot of things that I didn't know before. It has been a great experience.

**Sandra:** in my opinion, it has been a very interesting project, where we all became aware on what is on each soil and why we have to preserve it. We´ve learned the importance of the bacteria in our life, as soil mineralizers and for the production of antibiotics, and above all we learned the methodology that is carried out in identifying the types of bacteria that live on a specific soil.

**Maria:** this project made us get very interested about the bacteria that we can find on the soil, because is a topic that is not usually worked on. Another reason why I liked it is because we have joined constantly. If I had to repeat it I would do it again without even think it about it because not everyone has the privilege on doing it. From my point of view the most interesting part of it has been realizing the different types of bacteria like Gram positive or Gram negative. It is also very curious how the red or blue colors are absorbed depending on the type of bacteria wall.

# Protection of Plants Against Saline Stress by Beneficial Bacteria

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**SUMMARY**

**We have studied the influence of salinity on seed germination and plant growth, using corn and lentils as model plants. We have also analyzed if two bacterial species, *Pseudomonas putida* and *Pseudomonas stutzeri*, have plant protection effects against salt stress. Our results are preliminary but indicate that the two bacterial strains used can improve germination and plant growth in these conditions.**

## INTRODUCTION

Salinity is one of the most relevant factors that reduce the productivity of agricultural crops. It has negative effects on seed germination, plant growth and crop yield. It is a worldwide problem that affects around 25% of the world's agricultural land (nearly 50 million hectares), mainly in North and Central Asia, South America and Australia. Salinity affects plants in different ways: water and oxidative stress, ion toxicity, changes in metabolism, alteration of membranes, etc., causing reduced plant growth and survival (1).

Different types of bacteria have been described that can promote plant growth. Of these, a relevant group corresponds to bacteria that colonize plant roots and have a beneficial effect. These bacteria are generally called PGPR (plant-growth-promoting rhizobacteria). PGPRs improve plant growth by direct and indirect means, but the specific mechanisms involved have not all been studied in detail (2). Direct mechanisms can include synthesis of phytohormones, fixation of atmospheric nitrogen, production of siderophores that make iron available to the plant root, or solubilization of phosphorus or other minerals. Some bacteria may protect plants against stress, although this topic is not studied in detail.

Among the bacteria described as PGPR, strains of the genus *Pseudomonas* are well characterized. *Pseudomonas putida*, a rod-shaped, flagellated, gram-negative bacterium is found in soil and water. Some strains, particularly *Pseudomonas putida* KT2440, colonize plant roots and establish a mutualistic relationship with the plant. The bacteria receive root nutrients and in turn can protect the plants from pathogens. A second species, *Pseudomonas stutzeri*, is also commonly found in soil and marine environments. In soil, it is found in the rhizosphere of many plants. Some strains are denitrifiers and others are nitrogen fixers. Nitrogen fixing *P. stutzeri* could be relevant in agricultural production.

In this project we have done a preliminary evaluation of the effect of salinity on plant germination and growth and have used two bacterial strains to determine if they can protect plants from salt stress.

## MATERIALS AND METHODS

### Plants and bacterial strains

We have used commercial lentil seeds (*Lens culinaris*) and fungicide-free corn seeds (*Zea mays*, var. Girona). The bacterial strains used correspond to *Pseudomonas putida* KT2440, which was originally isolated in Japan and has been shown to have plant growth promoting activity (3, 4), and *Pseudomonas stutzeri* PS19, which has been isolated from a saline soil (MJ Lami, unpublished). Bacteria were grown in LB medium (5) before use for inoculation. For the bacterial tolerance assays, cultures were grown for 24 h in liquid LB medium before diluting them and spreading on LB plates with different salt concentrations.

### Germination and plant growth assays

Two types of assays were done to study the influence of salinity on plant germination and growth. In the first, pieces of absorbent paper were put into plastic containers, and soaked with NaCl solution prepared at two different concentrations (0.1 and 0.2 M). Between 28 and 100 seeds were spread on the paper and containers were left at room temperature for one week, replenishing the NaCl solution after 3 days.

In the second type of assay, plastic glasses filled with commercial plant growth substratum were used. Two seeds were planted in each glass, left at room temperature for one week and watered regularly using 0.1 or 0.2 M NaCl solution. Ten glasses were used for each treatment to have statistically significant data.

To test the effect of bacteria, overnight cultures grown in LB were diluted 1:100 in 10 ml of deionized $H_2O$. Seeds were incubated in this bacterial suspension for 15 minutes and then planted directly.

In all the assays, three parameters were analyzed: seed germination, root lenght and shoot lenght. The averages of these data are presented in the different graphics.

## RESULTS AND DISCUSSION

### Experiment 1: Effect of different salt concentrations on germination of corn and lentils

The effect of increasing salt concentrations on seed germination was evaluated by analyzing the number of germinated seeds after 1 week of incubation. Results showed that corn is much more resistant to salinity than lentils. All the corn seeds had germinated with 0.1M NaCl, and almost 90% with 0.2M NaCl. In the case of lentils, about half of the seeds germinated with 0.1M NaCl and none was able to germinate at the higher concentration (results shown in Table 1).

For the germinated seeds, two parameters were also measured: shoot and root length(Table 1). The average shoot length of corn was around three times less in 0.2M NaCl than in 0.1 M NaCl. In the case of roots, the length was reduced by half in the higher salt

concentration. These results indicate that salt is a factor of stress that reduces considerably plant growth. However, due to alterations in laboratory conditions, it was not possible to analyze the same parameters for control plants without salt stress, and therefore these results must be considered as preliminary. It was also interesting to observe that there was variability in the root and shoot length from one plant to another in the same conditions.

**Table 1.** Number and % of germinated seeds, and growth parameters in different salt concentrations

| Plant | NaCl concentration | % germinated seeds | average shoot length | average root length |
|---|---|---|---|---|
| Corn | 0.1 M | 100% (30 of 30) | 1.7 cm | 4.19 cm |
| | 0.2 M | 89.3% (25 of 28) | 0.47 cm | 2.17 cm |
| Lentil | 0.1 M | 48% (48 of 100) | 1.01 cm | 1.11 cm |
| | 0.2 M | 0% (0 of 100) | 0 | 0 |

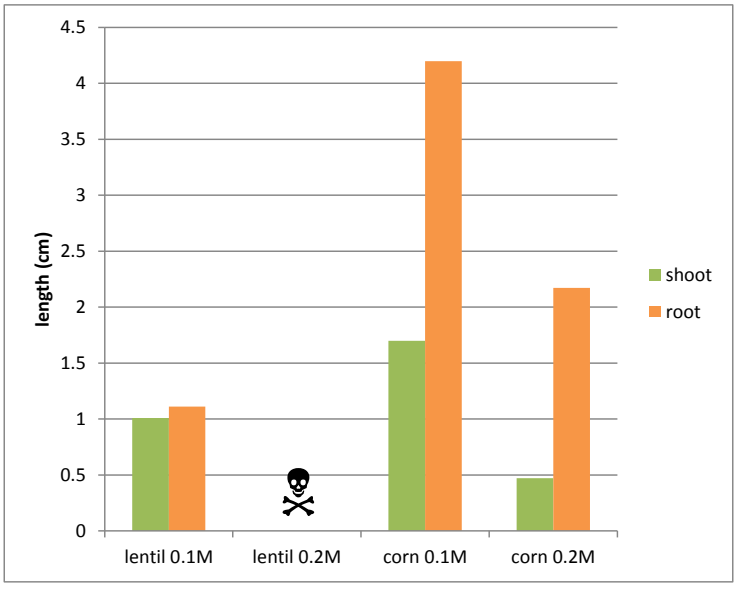


**Figure 1.** Graphical representation of average root and shoot lengths in different conditions

**Experiment 2: Effect of root-colonizing bacteria on germination and plant growth in saline conditions**

Next, we tested if certain root-colonizing bacteria have protective effect on plants under saline stress. For that purpose, seeds were inoculated with bacterial suspensions, using *Pseudomonas putida* KT2440 or *Pseudomonas stutzeri* PS19. The first is known to induce plant resistance against certain pathogens. The second has been isolated from a saline soil. After inoculation, seeds were planted and watered with NaCl solution. For corn a concentration of 0.2 M was used, since lower concentrations had no effect on germination. Both bacteria were tested with corn plants. For lentils, the experiment with *P. putida* was done with 0.1 M and the experiment with *P. stutzeri* with 0.2 M. After one week, the same parameters as in Experiment 1 were measured: % germinated seeds, shoot and root length. Results are presented in Table 2 and in figures 2, 3 and 4.

**Table 2.** Number and % of germinated seeds, and growth parameters in different salt concentrations in inoculated plants.

| Plant | NaCl | bacterial strain | % Germination | average shoot length | average root length |
|---|---|---|---|---|---|
| Corn | 0.2 M | *P. putida* | 70% (14 of 20) | 0.9 cm | 4.11 cm |
| Corn | 0.2 M | *P. stutzeri* | 65% (13 of 20) | 0.53 cm | 4.39 cm |
| Lentil | 0.2 M | *P. putida* | 50% (10 of 20) | 0.3 cm | 1.88 cm |
| Lentil | 0.1 M | *P. stutzeri* | 90% (18 of 20) | 1.98 cm | 4.3 cm |

For corn plants, inoculation with either bacterial strain caused a reduction in the percentage of germinated seeds, and therefore can be considered a negative effect. However, the average shoot length increased with respect to un-inoculated plants, the effect being more evident with *P. putida*. Root lenght also increased significanty with both bacteria, roots being twice as large as those of non-inoculated plants.

In the case of lentils, however, germination increased notably with inoculation, from 48 to 90% at 0.1 M NaCl, and from 0 to 50% at 0.2 M NaCl. At 0.1 M NaCl, shoots of inoculated plants were twice as large as those of non-inoculated plants, and roots were four times longer. Therefore, both bacterial strains had a clearly positive effect on lentils under salt stress. However, these data must be considered partial, since it was not possible to test the two bacterial strains in the two conditions (0.1 and 0.2 M).

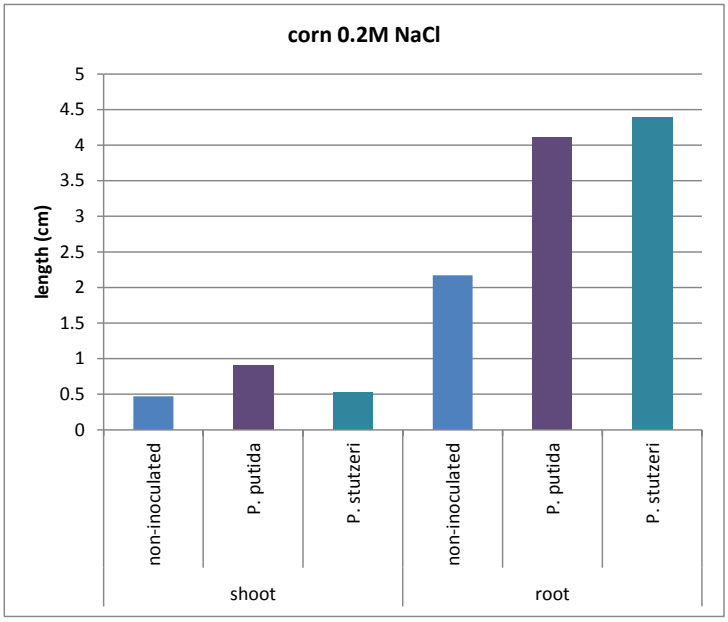


**Figure 2.** Influence of inoculating corn plants with *P. putida* KT2440 or *P. stutzeri* PS19 on shoot and root length after 7 days in the presence of 0.2 M NaCl.

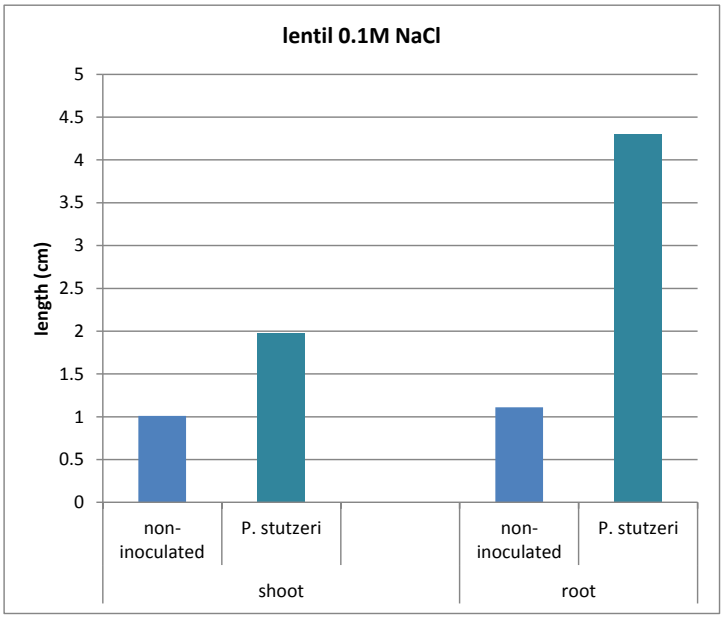


**Figure 3.** Influence of inoculating lentil plants with *P. stutzeri* PS19 on shoot and root length after 7 days in the presence of 0.1M NaCl.

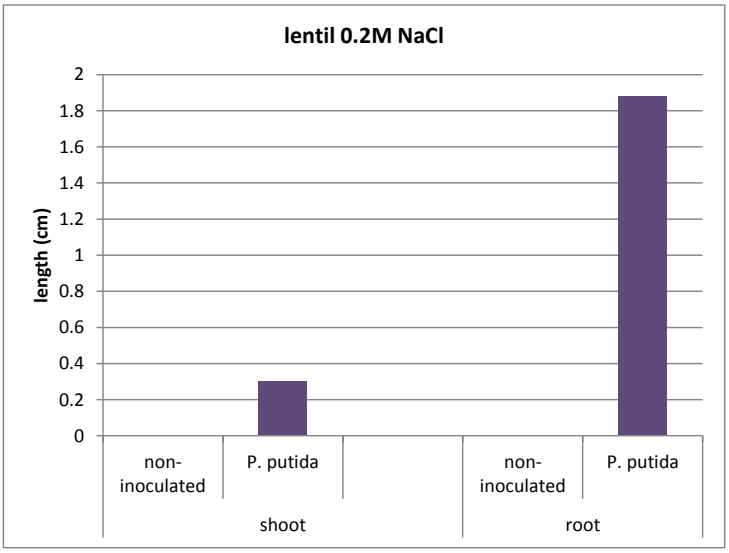


**Figure 4.** Influence of inoculating lentil plants with *P. putida* KT2440 on shoot and root length after 7 days in the presence of 0.2M NaCl.

**Bacterial tolerance to salt stress**

Since *P. putida* was capable of promoting germination and growth of lentils at 0.2 M NaCl, which inhibits germination of non-inoculated seeds, we decided to test the tolerance of this bacterium to increasing salt concentrations. For that, a suspension of bacteria was spread on Petri plates containing LB with 0, 0.5, 0.75 and 1 M NaCl. The number of colonies able to grow in these conditions was counted after 24 h of growth at 30°C. Results are detailed below and indicate that even at high salt concentrations *P. putida* is able to grow, its viability being only reduced by half when the concentration was very high (1 M).

- Control (no NaCl added): 370 colonies
- 0.5 M NaCl:~300 colonies
- 0.75 M NaCl: >200 colonies
- 1 M NaCl:~150 colonies

**CONCLUSIONS**

- Salt is a relevant stress that limits seed germination and plant growth. However, the sensitivity of different plant species to salt can be very different. Corn is more resistant than lentils to salt stress.
- Some bacteria can protect plants from the negative effects of salt, increasing the % of germination and the root and shoot lenght of plants.
- The preliminary results obtained in this project will allow to design new experiments for a more precise analysis of salt stress and plant protection by bacteria.

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**MY OWN IDEAS**

Me ha parecido un proyecto interesante por la cantidad de usos que se le podría dar si da buenos resultados. Pero también creo que los resultados deberían ser demasiado buenos como para que merezca la pena usar la bacteria *Pseudomonas putida*.

Creo que con este trabajo se ha demostrado el daño que hace la salinidad al suelo y que hay determinadas bacterias que pueden ayudar a apaciguar los afectos negativos de la salinidad.

*Fran Santander López*

Ha sido un proyecto interesante, con un propósito muy útil en futuras investigaciones e incluso técnicas de cultivo. Sin embargo falló algo en el procedimiento, y es que durante algunas tardes se bajaban las persianas modificando las condiciones a las que eran sometidas las semillas y probablemente impidiendo conocer el potencial real de las bacterias empleadas en el experimento.

Teniendo un poco más de cuidado con estos detalles e introduciendo una salinidad más propia de un campo de cultivo se podría continuar investigando y sacar buenos resultados. A su vez, como conclusión, es que en medios salinos, estas bacterias ayudan bastante a la germinación, con lo que podría mejorarse mucho la productividad a largo plazo en zonas costeras y con altas concentraciones de sales.

*Marcos Molina Fernández*

Para mi el proyecto ha sido muy interesante y se aprenden cosas nuevas. También ha sido importante puesto que no siempre hay oportunidades de poder estudiar algo que interesa y que en un futuro te puede servir para tus estudios y puede que incluso para tu carrera. En este proyecto he aprendido mucho y he observado lo que hemos hecho y me ha gustado mucho.

Estudiar como afectaban las bacterias a las semillas ha sido muy interesante.

Lo que más me ha gustado del proyecto ha sido observar las colonias al colocar las semillas dentro de las placas de Petri.

*Almudena Martín Martínez*

Ha sido un proyecto diferente, ya que gracias al proyecto no ha sido todo horas de teoría, es decir, así aprendemos también cosas de laboratorio.Me ha gustado mucho ver como crecían las bacterias y cómo se reproducían. Lo veo una forma increíble de aprender ciencia y dar las horas de Biología y de Física y Química. Espero poder participar en otro trabajo así o semejante.

*Lidia López López*


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