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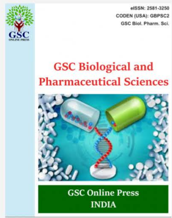

(RESEARCH ARTICLE)

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Behavior of fluorescent *Pseudomonas* under different concentrations of heavy metals (Zn and Cu)

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Abstract

The use of chemical fertilizers has resulted in the depletion of soils in mineral elements and telluric microorganisms. Thus, for an improvement in crop growth, the exploitation of telluric microorganisms such as *Pseudomonas* can be an alternative.

Laboratory work has made it possible to isolate strains of *Pseudomonas* from the rhizospheres of tomato, eggplant and onion. These strains were also tested in the presence of different concentrations of certain metals ($CuSO_4$ and $ZnSO_4$ (0, 1, 3, 4, 5 and 6 mmol/l).

The tests carried out in the laboratory in the presence of Cu and Zn revealed a very significant reduction in the number of colonies of the strains of *Pseudomonas* for the concentrations of 5 and 6 mmol/l.

The results showed better zinc tolerance for both tomato and onion rhizosphere strains. In the presence of Cu, the strain isolated from tomato rhizosphere shows more tolerance to heavy metals. These results made it possible to isolate different strains of *Pseudomonas* and to specify their tolerance thresholds for heavy metals.

Keywords: *Pseudomonas*; Heavy metals; Tomato; Onion; Eggplant

1. Introduction

Man, through strong industrialization and intensive agriculture using fertilizers and pesticides, contributes to the dissemination of metallic trace elements and their accumulation in the soil ([1]; [2]; [3]; [4]). Several of these metals such as iron, copper and zinc are essential for the proper growth of living organisms, especially plants and microorganisms [5]. But these metals can also be limiting factors for these organisms [6]. Copper and zinc are not dangerous for the organisms in themselves which is not the case for other metals such as lead or cadmium, but at an excessive level, they become toxic although the proper functioning of the metabolism of the organisms especially bacteria, is also dependent on these two metals ([7]; [8]; [9]). Although the effects of microorganisms on the availability of metal contaminants in soils are relatively well documented, the majority of these studies are based on a geochemical and not a biological approach [10]. It should be noted that in addition to fungi and lichens, bacteria play a major role in the phenomena of alteration of heavy metals. However, among the microorganisms present in the soil, there are bacteria

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of agricultural interest ([11]; [12]) referred to by the acronym PGPR (Plant Growth Promoting Growth) in this case *Pseudomonas* fluorescents ([13]; [14]). They are strict aerobic Gram-negative bacilli [15]. These bacteria are motile thanks to one or more polar flagella. They get the second part of their name from the fact that they are fluorescent ([16]; [17]). This fluorescence is due to the production of fluorescein (or pyoverdine), which is a yellow-green pigment that fluoresces under ultraviolet light [18]. They are ubiquitous bacteria, particularly abundant in soils and waters [19]. Their ability to colonize roots and maintain a high population density there is therefore remarkable [20]. They have several intrinsic characteristics that make them interesting, in particular their usefulness as biological control agents thanks to the solubilization of phosphorus, phosphate and the sequestration of iron by siderophores as well as the production of antibiotics ([14]; [21]). Although siderophores are defined as specific iron chelators, they are also able to effectively complex metal cations other than iron [22]. This great competence comes from their higher growth rate at the rhizosphere level [23]. Added to this is their ability to use a very wide range of substrates, often derived from root exudates, as a source of nitrogen or carbon [10]. They are very easy to isolate and cultivate in the laboratory and easily lend themselves to genetic manipulation [24]. It is interesting to underline that although metallic trace elements are mobilized by various organisms, they cannot be degraded into less toxic products. They persist indefinitely in the environment and pose a major problem in ecology but also for public health due to their carcinogenic and mutagenic properties [25]. The toxicity and persistence of metallic trace elements in the environment require the development of different methods to reduce contamination [3]. The objective of this work is to first study the behavior of fluorescent *Pseudomonas* isolated from the rhizosphere of tomato (To), eggplant (Au) and onion (Oi) and then to assess their tolerance to different concentrations of heavy metals Cu and Zn.

2. Material and methods

2.1. Isolation of fluorescent *Pseudomonas*

Soil samples were taken at the Center for the Development of Horticulture (CDH Cambérène station, Niayes eco-geographical zone, Dakar Region, Senegal) during the off-season (February).

These are soils from the rhizosphere of tomato, onion and eggplant plants in the fruiting stage and on bare soil. For each soil sample, 500 g were taken from a depth of 0 to 25 cm. From these samples, isolations of fluorescent *Pseudomonas* spp were carried out by the suspension-dilution method. A sample of 1 g of each soil was suspended in 10 ml of sterile 0.1 M of $MgSO_4,7H_2O$. After stirring for 10 minutes using a vortex, decimal dilutions (10^{-1} to 10^{-8}) were made from this suspension. 0.1 ml of each suspension was plated in Petri dishes each containing 10 ml of King B agar medium [26]. Petri dishes are incubated at 28°C in the dark.

After 48 hours of incubation, the bacterial colonies were observed under a UV lamp at 365 nm.

For each soil sample, three fluorescent pigment-producing isolates were selected and purified by streaking in three dishes containing King B's solid medium. Each isolate is designated by a code. These are the To, Oi and Au strains isolated respectively from the rhizosphere of tomato, onion and eggplant plants.

2.2. Effect of heavy metals on fluorescent *Pseudomonas* spp.

To study the effect of these heavy metals on fluorescent *Pseudomonas*, liquid KB media were prepared with different Zn and Cu concentrations: 0, 1, 3, 4, 5 and 6 mmol/l. These media were autoclaved at 120°C for 20 minutes. Then, aliquots of 100 µl of bacterial suspension of each of the *Pseudomonas* strains were spread using a micropipette in Petri dishes each containing 10 ml of the medium. The Petri dishes were incubated in an oven at a temperature of 28°C for 72 h.

3. Results

3.1. Characteristics of fluorescent colonies on U.V

After 48 h of culture, the bacterial colonies were observed on a UV table at 365 nm. For each speculation (tomato, onion and eggplant as well as bare soil), the number of fluorescent colonies varies between 3 and 4. The fluorescence was clear and sharp (Figure 1). And it was the 10^{-2} and 10^{-3} dilutions that showed the best results regarding pigmentation intensity, size, elevation as well as color and shape. No fluorescent colonies were observed with bare soil. These colonies were then subcultured on KB medium for purification (Figure 2).

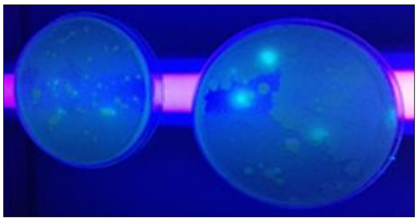

Figure 1 Colonies of fluorescent *Pseudomonas* under U.V.

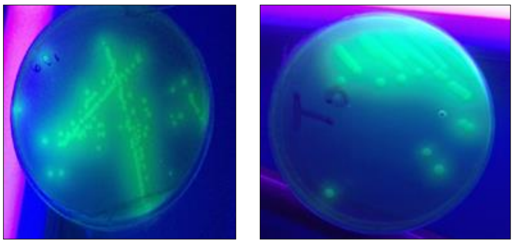

Figure 2 Colonies of fluorescent *Pseudomonas* after purification

3.2. Macroscopic aspects of fluorescent *Pseudomonas* strains

The strains showed slightly variable characteristics. (Table 1).

Table 1 Characteristics of fluorescent *Pseudomonas* strains observed under UV after 48 hours

Strains	Form	Size (mm)	Elevation	Color	Intensity of pigmentation
Au	Round	4.5	Domed	Yellow-green	+
To	Round	5	Domed	Green	+
Oi	Round	4	Flat	Green	+

3.3. Effect of Different $ZnSO_4$ Concentrations on Fluorescent *Pseudomonas*

Fluorescent *Pseudomonas* from the rhizosphere of tomato, eggplant and onion grown on KB medium for 72 hours showed variability in the number of colonies depending on $ZnSO_4$ concentration and speculation (Figure 3).

In the absence of $ZnSO_4$, the number of fluorescent *Pseudomonas* colonies is greater for the strains isolated from the eggplant rhizosphere (340 colonies). This value shows a significant difference in the number of colonies of onion rhizosphere compared to that of tomato rhizosphere (301 colonies).

For a $ZnSO_4$ concentration of 1 mmol/l, the number of colonies of strains isolated from the eggplant rhizosphere (214 colonies) decreased significantly compared to the number of colonies from the rhizospheres of tomato (324 colonies) and onion (276 settlements).

At a $ZnSO_4$ concentration of 3 mmol/l, the number of colonies of the strains isolated from the rhizosphere of eggplant and tomato are respectively 301 and 280 colonies. These values are twice as large compared to the number of colonies in the onion rhizosphere (124 colonies).

When the $ZnSO_4$ concentration is 4 mmol/l, the numbers of fluorescent *Pseudomonas* colonies in the onion and tomato rhizosphere are five times greater than the number of colonies isolated from the eggplant rhizosphere.

For a $ZnSO_4$ concentration of 5 mmol/l, it can be seen that the number of colonies of fluorescent *Pseudomonas* isolated from the eggplant rhizosphere (65 colonies) has significantly decreased compared to that of tomato (137 colonies).

For all the strains, the lowest values are obtained with a concentration of 6 mmol/l. The number of colonies of the different speculations did not vary significantly at this concentration (Figure 3).

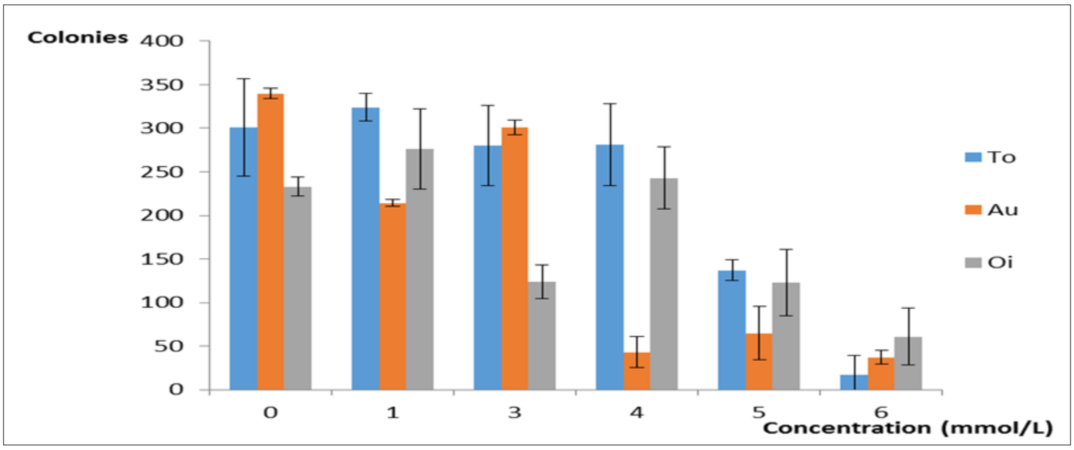

Figure 3 Effect of different $ZnSO_4$ concentrations on fluorescent *Pseudomonas* colonies

3.4. Effect of Different $CuSO_4$ Concentrations on Fluorescent *Pseudomonas*

Fluorescent *Pseudomonas* from the rhizosphere of tomato, eggplant and onion, cultured on KB medium for 72 hours showed a variability in the number of colonies depending on the concentration of $CuSO_4$ and speculation (Figure 4).

In the absence of $CuSO_4$ (control), there is no significant difference between the number of colonies from the tomato rhizosphere (301 colonies) and that of the eggplant rhizosphere (313 colonies). However, these values show a significant difference compared to the number of colonies obtained at the level of the rhizosphere of the onion.

For the $CuSO_4$ concentration of 1 mmo/l, the colonies of fluorescent *Pseudomonas* from eggplant (23) and onion (14) are very weak or even negligible compared to those from the tomato rhizosphere (335).

At 3 mmol/l of $CuSO_4$, the number of colonies of fluorescent *Pseudomonas* from the tomato rhizosphere (124) is significantly higher compared to those from the eggplant (68) and onion (49) rhizospheres.

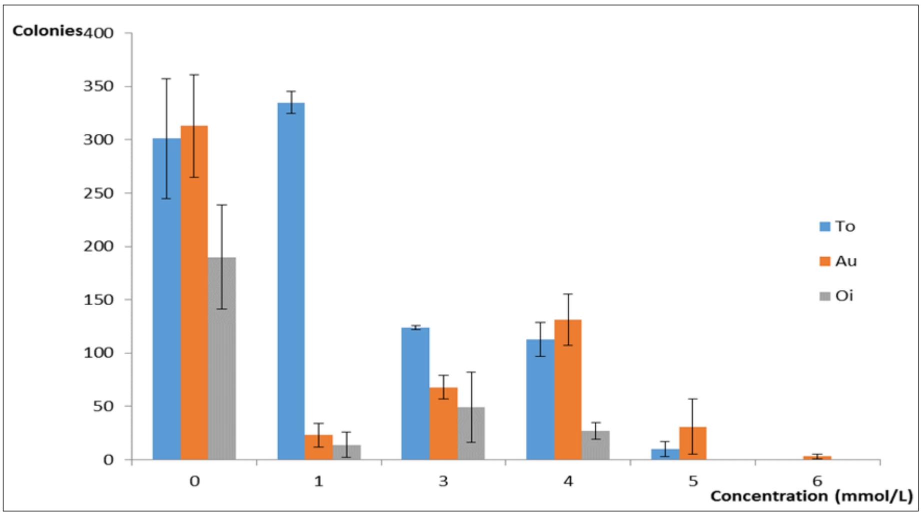

Figure 4 Effect of different concentrations of $CuSO_4$ on colonies of fluorescent *Pseudomonas*

For a $CuSO_4$ concentration equal to 4 mmol/l, colonies of fluorescent *Pseudomonas* from the rhizosphere of tomato (113) and eggplant (131) did not show any significant difference. However, we find a significant decrease in the number of colonies of fluorescent *Pseudomonas* originating from the onion rhizosphere (27).

At 5 mmol/l of $CuSO_4$, the numbers of colonies of fluorescent *Pseudomonas* originating from the rhizospheres of tomato (10) and eggplant (31) fell sharply and did not show any significant variation, on the other hand a total absence of colonies originating from the onion rhizosphere.

At 6 mmol/l of $CuSO_4$, only the strains isolated from the eggplant rhizosphere were recorded with a very low value (3 colonies) (Figure 4).

4. Discussion

Cultures in the presence of heavy metals may require the presence of microorganisms to ensure their growth. Thus, soil microorganisms provide a physical and chemical link between soil and plant roots. They participate in the recycling of plant nutrients and in maintaining soil stability as well as in the detoxification of toxic chemicals ([27]; [28]). On a larger scale, in the environment, essential or non-essential metals cause considerable modifications within the microbial community and their activities. Furthermore, *Pseudomonas* have developed mechanisms to deal with toxic metals [29]. With the strains of *Pseudomonas* isolated in the rhizosphere of tomato, eggplant and onion, we observe a very significant decrease in the number of colonies for concentrations of 5 and 6 mmol/l. These results are in agreement with those of Chen et *al.*, [29] who showed that from concentrations of 3 and 5 mmol/l, there is a significant decrease in the number of colonies of *Pseudomonas* respectively in the presence of Cu , and Zn. However, these microorganisms were related to *Pseudomonas* Putida based on 16S ribosomal DNA sequencing. For these levels of Cu and Zn, the numbers of colonies observed were higher for tomato and onion if it is Zn. In the presence of Cu, the strain of *Pseudomonas* from tomato rhizosphere was found to be more tolerant. The effect of heavy metals on the growth of colonies of *Pseudomonas* shows that the intensity of the effect of metallic trace elements varies according to the level and the nature of the metal and could also vary according to the cultivated speculation ([30]; [10]).

The results show better zinc tolerance for strains isolated from tomato and onion rhizosphere (Figure 3). In the presence of Cu, the strain isolated from the tomato rhizosphere shows better tolerance (Figure 4). Additionally, we found that the toxicity of copper was greater than that of zinc.

The effect of heavy metals on ecosystems and the mechanisms of their transfer from soil to living organisms are still poorly understood [31].

5. Conclusion

Tomato, onion and eggplant are three crops widely used by the populations of the Sahel where they play an important economic and socio-economic role. CDH soils showed a significant potential for rhizospheric microorganisms. However, this study showed that the different strains of *Pseudomonas* did not have the same level of tolerance to heavy metals. Our work has made it possible to define, under in vitro conditions, the tolerance thresholds for heavy metals (Zn and Cu) for the strains of fluorescent *Pseudomonas* isolated in the rhizosphere of the different speculations used.

Indeed, the results obtained showed that the colonies of *Pseudomonas* from the rhizosphere of tomato and onion were more tolerant to $ZnSO_4$ (5 mmol/l) compared to the strains isolated from the rhizosphere of eggplant which are more sensitive to $ZnSO_4$. The effect of $ZnSO_4$ from a concentration of 4 mmol/l. For Cu, colonies of *Pseudomonas* in the tomato rhizosphere are more tolerant to $CuSO_4$ (4 mmol/l) followed by those of eggplant and finally those of onion. Overall, it appears from this study that strains of *Pseudomonas* from the tomato rhizosphere are more tolerant to different abiotic constraints, namely the presence of heavy metals (Zn and Cu).

Compliance with ethical standards

Acknowledgments

The authors the Mushroom Biotechnology Laboratory.

Disclosure of conflict of interest

Authors declare that no conflict of interest exist.

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