Original article

The Impact of Wheat Rhizosphere on the Distribution of *Burkholderia* spp and Soil Bacteria

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Abstract

This study was conducted on the effect of the wheat rhizosphere on the distribution of *Burkholderia* spp. and soil bacteria communities during wheat plant growth by the enumeration on plates of rhizosphere and non rhizosphere soil communities in different type of soils, which were collected from different fields, Wadi Elrabie and Janzour (East and west) of Tripoli, Libya. The results showed that the gram negative species of soil bacteria exceeded the remaining bacterial population in the rhizosphere on contrast with non-rhizosphere which dominance of gram-positive cocci, endospore and forming rods. There was no difference in the total bacterial population with plant age , but the types of colony growth were reversed between the rhizosphere and non-rhizosphere soil in 5 week (seedling stage) and 20 weeks (maturity stage).The genus of *Burkholderia* prevail in the rhizosphere of the wheat particularly in the early stages of plant growth (seedling stage). The changes in the trend of the calculated ratio of gram-negative bacteria in the rhizosphere to that of the non-rhizosphere soil cannot be generalized to all species. Results obtained with *Burkholderia* population in this experiment showed different picture.

Key words: Rhizosphere, Wheat growth stages, *Burkholderia*, Soil bacteria

Introduction

Plant roots play important roles in shaping microbial communities in soil by releasing a wide range of compounds (Garbeva *et al*., 2008). Although root-released products comprise of an important pool of organic compounds for soil micro-organisms, their composition and quality can vary according to plant species, soil type and plant development stage (Chaparro *et al*., 2013). Due to this variation in exudation, different plant species growing in the same soil type were known to select divergent bacterial communities. However, when analyzing the microbial community associated with the same plant species growing in different soil type.

The soil type might exert a great influence on microbial diversity (Wieland *et al*. 2001). In view of the fact that the plant has a large impact on microbial diversity, one might expect agricultural management to play an important role as wells (Lupwayi *et al*. 1998). Indeed, many agricultural practices, such as crop rotation, continuous cropping and tillage, induce changes in the microbial communities in soil (Alvey *et al*. 2003; Lupwayi *et al*. 1998), which may persist long after the management practice took place. Although agricultural practices induce general changes in soil microbial communities, specific microbial groups may respond differently (Clegg *et al*, 2003).

A number of studies have reported that the application of inorganic nitrogen had a significant impact on Eubacterial and Actinomycete community structures, whereas soil drainage significantly affected the community structures of *Burkholderia* spp and *Pseudomonas* spp. In addition, continuous wheat cropping affected the community structure of *Burkholderia* spp such that an increase in the population of antibiotic producing *Burkholderia* spp induced the natural suppression of Take-all disease in wheat (Raaijmakers and Weller 1998).

Similarly, the establishment of apple orchards in a field where wheat had previously been grown led to an decrease of soil suppressiveness against *Rhizoctonia solani*, which was correlated with a decrease of *Burkholderia cepacia* and *Pseudomonads putida* populations (Mazzola, 1999).

Considering the fact that agricultural management and plant species affect soil microbial communities, the main objective of this study was to get a better understanding of how land use and crop species, such as wheat affect the diversity of the genus *Burkholderia.*

As well as, this study aimed to address the effect of the wheat rhizosphere on the distribution of *Burkholderia* spp and soil bacteria which factor (land use) had a greater influence on the soil-borne populations by the enumeration on plates of rhizosphere and non rhizosphere soil communities.

Materials and methods

The experiment was conducted in a growth chamber in Soil microbiology lab at Faculty of Agriculture, University of Tripoli. Winter wheat (*Triticum aestivum* L.) was grown in glass plant tubes (25 x 150) mm were filled with 20g soil to approximately 5cm from rim. Soil were collected from two field sites from Wadi Elrabie, East of Tripoli (Loam sandy soil, pH 8.6) and Janzour, West of Tripoli (sandy soil, pH 8.1) with three replicates of each of the two soil samples, giving a total

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of 24 treatments, which explains three factors: one type of plant, two soil type (Janzour and Wadi Elrabie soil) with two sites (rhizosphere and non-rhizosphere soil) and two stages of wheat growth (seedling and maturity stage) in three replicate. All tubes were irrigated to field capacity twice a week. One inoculated seed of wheat was placed in each tube approximately 1cm below the surface of the soil.

The bacteria counting were carried out at the end of the growth cycle which was seedling stage approximately 5 weeks and maturity stage 20 weeks after sowing, by using the method of calculating the number of probabilistic (Alexander 1973). Bacterial strains were initially isolated from the rhizosphere and non-rhizosphere soil of wheat roots. Plants were uprooted from the tubes along with amount of non-rhizosphere soil.

The non-rhizosphere soil was removed by gentle shaking of the roots whereas the soil adhering strongly to the root was referred to rhizosphere soil. Ten gm of each soil samples were mixed thoroughly in 3 replicate, then transferred to 250 ml flask with 100 ml sterile distilled water and were shaken for 30 min at 150 rpm. Immediately after shaking, a series of 10-fold dilution in 0.8% (w/v) NaCl solution was prepared for each soil sample by pipetting 1 ml of aliquot into 9 ml sterile water. 0.1 ml of each dilution of the series was plated into a Petri plate with soil extract agar for rhizosphere's samples and with Dextrose ager for non-rhizosphere's samples.

Bacteria *Burkholderia* isolated from soil samples into plates with Burk's agar medium.Three replicate plates were made for each dilution plates were placed in an incubator at 30^{o} C for 8 days to estimate *Burkholderia* and soil total bacterial population, after incubation, the plates dilutions of rhizosphere and non rhizosphere microorganism were Gram-stained using standard procedures.

Morphology characterisation was determined using a compound microscope in oil immersion (1000 X) about 100 colonies were chosen at random at all the colonies from the rhizosphere whatever their size, shape and colour were transferred onto other plate to check for purity. All the colonies grown on the plates were about 1mm diameter and white with flat margins initially glossy and gummy but turned into glistening colonies with clear slime upon further growth. Some of the species were defined by direct use of microscopic morphological characteristics and compared to some of the known and available cultures and then were characterised using the criteria of Bergey and Holt (1994).

Results and discussion

This experiment showed the impact of the wheat rhizosphere on the distribution of *Burkholderia* spp and soil bacteria by the enumeration on plates of rhizosphere and non-rhizosphere soil as shown in table (1). A difference between the rhizosphere and non rhizosphere concerned the colonies growth on the plates. All morphological characteristics conformed to descriptions of those known from the most of the rhizosphere colonies of two stages of wheat growth (seedling and maturity stages) from two soils (Wadi Elrabie and Janzour). There had a very homogeneous morphology bacteria rod, ovoid cells treated like species of *Burkholderia, Psedomonas, Azotobacter, Agrobacterium* and endospore as *Bacillus sp, Streptomyces.* All colonies had grown after 4 days of incubation, on the contrast the non rhizosphere soil had a vast of colonies rod and ovoid, rod forming and endosperm cells as *Streptomyce,s Cellulomonas, Azotobacter, Cytophaga* and *Pseudomonas* grown after 4 days and some of them grow after 8 days.

Table 1: The common genuses of soil bacteria and their growth on plates listed downward in Wadi Elrabie and Janzour soils.

	Seedling stage (5 weeks)	**Maturity stage (20 weeks)**
Rhizosphere	1- Gram negative bacteria, rod, and ovoid cells and treated like species of *Burkholderia, Psedomonas, Azotobacter and Agrobacterium.* 2- *Bacteria gr*am positive, rod and endospore, *Bacillus sp*	1- Gram negative bacteria, rod and ovoid cells. *Cytophaqa, Coccobacil, Azotobater, Burkholderia.* 2- Gram positive bacteria, endosperm forming rod. *Bacillus Streptomyces.*
Non-rhizosphere	1- Gram positive bacteria, Cocci, endospore *Staphylococcus*, forming rod *Streptomyces*. 2- Gram negative bacteria, rod *Pseudomonas, Burlholderia* and ovoid cells like species of *Azotobacter.*	1- Gram positive bacteria. *Streptomyces* forming rod *Cellulomonas.* 2- Gram negative bacteria, rod and ovoid cells. *Azotobacter* and *Cytophaga.*

It can confirm the fact in a number of previous researches revealed that gram-negative bacteria population prevails in rhizosphere, while the gram-positive bacteria population is numerically superior in non rhizosphere and comes second in rhizosphere (Macura 1967 and Briwb 1975). It also notes that the plant age and stages of growth does not affect the distribution of microbes in the appearance and disappearance of some specie as a result of the nature of the root outputs by releasing a wide range of organic compounds that change from one stage to the other stages of wheat growth. It had indicated the ratio

between the numbers of gram negative bacteria in the rhizosphere to their numbers in the non-rhizosphere become even greater with plant age in between stage seedling and maturity stage (Salles *et al*.(2006); Ben Mahmoud 2008).

This study indicates the diversity of the *Burkholderia* population (which was a negative-gram bacteria) associated with rhizosphere was consistently higher in the rhizosphere than in the non-rhizosphere There was a greater number of *Burkholderia* population at seedling stage (5 weeks) in rhizosphere than in non-rhizosphere soil, while thereafter the numbers of *Burkholderia* declined to the lowest number at the maturity stage (20 weeks) were recorded from 7 x 10^5 to 4 x 10^4 cfu /gram soil at Wadi Elrabie and from 5 x 10^4 to 2 x 10^3 cfu /gram soil at Janzour soil. However, the other bacterial communities' population of the non rhizosphere in both soils were increased from seedling to maturity stages of wheat growth, as shown in table (2 and 3).

Table 2: Total *Burkholderia* and soil bacteria in wheat rhizosphere and non rhizosphere in Wadi Elrabie soil

	Seedling stage (5weeks) cfu /gram soil		Maturity stage (20 weeks) cfu /gram soil	
	Burkholderia	Soil bacteria	*Burkholderia*	Soil bacteria
Rhizosphere	7 x 10^5	4.5 x 10^2	4 x 10^4	8 x 10^3
Non- rhizosphere	5 x 10^2	6.5 x 10^4	2 x 10^2	7 x 10^5

Table 3: Total *Burkholderia* and soil bacteria in wheat rhizosphere and non rhizosphere in Janzour soil

	Seedling stage (5weeks) cfu /gram soil		Maturity stage (20 weeks) cfu /gram soil	
	Burkholderia	Soil bacteria	*Burkholderia*	Soil bacteria
Rhizosphere	5 x 10^4	3 x 10^2	2 x 10^3	7.3 x 10^4
Non- rhizosphere	2 x 10^2	4.5 x 10^3	1.2 x 10^2	3 x 10^5

To sum up, the *Burkholderia* population present in the wheat rhizosphere higher than in non rhizosphere (Pallud *et al*.2001) might be due to the high substrate availability observed around the roots, indeed the bacterial communities population of the wheat rhizosphere seem to have lower level of culturability than non rhizosphere as proven by Van Elsas *et al*. (2002); Salles *et al*. (2006).

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