Enzymatic activity and culturable bacteria diversity in rhizosphere of amaranth, as indicators of crop phenological changes

Iván Pável Moreno-Espíndola[1], María Jesús Ferrara-Guerrero[2], Fernando de León-González[3], Facundo Rivera-Becerril[2]*, Lino Mayorga-Reyes[4], and Néstor O. Pérez[5]

[1] Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico
[2] Departamento El Hombre y su Ambiente, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico
[3] Departamento Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico
[4] Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico
[5] PROBIOMED S.A. de C.V., Tenancingo, Edo. Méx., Mexico
* Corresponding author:
frivera@correo.xoc.uam.mx

Abstract

Background: Amaranth is a plant of interest in farming due to its ability to adapt into arid and semi-arid climates. Biological activity by microorganisms in rhizosphere determines plant performance and quality.
Hypothesis: The enzymatic activity is different in two types of soil, rhizosheath soil (adhered by roots) and loose soil (non-adhered by roots), in four cropping periods of amaranth.
Species study: *Amaranthus hypochondriacus* L.
Methods: Parameters of soil organic matter and several enzyme activities in the amaranth rhizosphere were assessed. Two types of soil, rhizosheath soil and loose soil, and four cropping periods were compared. Thirty-seven culturable bacterial isolates obtained from rhizosheath soil were molecularly identified.
Results: Rhizosheath soil had higher content of carbon and total nitrogen compared with loose soil; however, potential enzyme activity in both soil types was similar. Dehydrogenase and acid phosphatase activities were very sensitive to the crop phenological stages. Acid and alkaline phosphatases, cellulase and protease activities correlated to changes in soil moisture. The greatest diversity of culturable bacteria was found during the flowering stage.
Conclusions: In the rhizosphere of *A. hypochondriacus* grown in a pumiceous sandy soil, enzymatic activities in the rhizosheath and loose soils were similar, which must be considered a unique rhizosphere environment. Dehydrogenase and acid phosphatase activities were highly sensitive to changes in the crop phenology. The behavior of phosphatases and dehydrogenase activities suggests an increased dynamic soil organic matter (SOM) during the post-harvest period. In the amaranth rhizosphere, native culturable bacteria are involved in the breakdown of SOM.
Key words: *Amaranthus hypochondriacus*, dehydrogenase, phosphatase, rhizosheath, semi-arid soil, soil organic carbon.

Resumen

Antecedentes: El amaranto es una planta de interés agrícola adaptada a climas áridos y semiáridos. La actividad biológica microbiana en la rizosfera determina el comportamiento y la calidad vegetal.
Hipótesis: La actividad enzimática es diferente en dos fracciones de suelo, suelo de rizocoraza (adherido a las raíces) y suelo suelto (no adherido a las raíces), en cuatro etapas del cultivo del amaranto.
Especie en estudio: *Amaranthus hypochondriacus* L.
Métodos: Se evaluaron parámetros de materia orgánica y actividades enzimáticas en la rizosfera del amaranto. Fueron comparados dos microambientes, suelo de rizocoraza y suelo suelto, en cuatro periodos del cultivo. Treinta y siete aislados bacterianos cultivables procedentes del suelo de rizocoraza se identificaron molecularmente.
Resultados: El suelo de rizocoraza tuvo el contenido más elevado de carbono y nitrógeno total. Empero, la actividad enzimática potencial fue similar en ambos microambientes. Las actividades deshidrogenasa y fosfatasa ácida fueron muy sensibles a los estados fenológicos. Las actividades fosfatasa ácida y alcalina, celulasa y proteasa estuvieron correlacionadas con la humedad edáfica. Se observó una diversidad mayor de bacterias cultivables durante la floración.
Conclusiones: En la rizosfera de *A. hypochondriacus* las actividades enzimáticas en suelo de rizocoraza y en suelo suelto fueron similares; ambos son un mismo ambiente rizosférico. Dos actividades enzimáticas mostraron sensibilidad a las etapas fenológicas. El comportamiento de las actividades fosfatasa y deshidrogenasa sugiere un incremento en la dinámica de la materia orgánica edáfica durante la postcosecha. En la rizosfera de amaranto las bacterias cultivables nativas participan en la descomposición de la materia orgánica.
Palabras clave: *Amaranthus hypochondriacus*, carbono orgánico del suelo, deshidrogenasa, fosfatasa, rizocoraza, suelo semiárido.

Amaranth is a plant of interest in farming due to its ability to adapt into arid and semi-arid climates, where it can grow and yield seed and substantial amounts of biomass. The seed, which is gluten-free and contains relatively high levels of lysine (Clouse *et al.* 2016), is widely appreciated in Mexico as a food and its leaves are suitable for human consumption and as forage because they provide high-quality protein (Barba de la Rosa *et al.* 2009). The amaranth species studied in the current investigation, *Amaranthus hypochondriacus* L., has been cultivated in the Valley of Mexico under semi-arid conditions. Despite its agronomic and nutritional attributes, little is known about the activity and diversity of microbial communities associated with amaranth roots.

One important function of all soil microorganisms comprises the decomposition of soil organic matter (SOM). Proteases are involved in the hydrolysis of peptide bonds to release nitrogen (N); phosphatases catalyze the hydrolysis of esters and anhydrides of phosphoric acid; cellulases hydrolyze cellulose into glucose; and chitinase is capable of hydrolyzing insoluble chitin into its oligo and monomeric components (Kuddus & Ahmad 2013, Gianfreda 2015). Dehydrogenases (DH) also play a significant role in decomposition of SOM.

Microbial activity can increase in certain rhizosphere microenvironments, such as rhizosheaths, which are highly stable structures shaped by the enmeshment of soil particles (sand, silt and organic matter) by root-hairs (Othman *et al.* 2004). A high proportion of active soil microorganisms in these microenvironments benefit from the cellular debris and compounds released by roots (Lesuffleur *et al.* 2007). In the rhizosheath, greater microbial activity would be expected than in bulk soil due to the accumulation of organic compounds (Gianfreda 2015). Rhizosheaths are commonly found in the roots of grasses, but have also been found in other cultivated plants, such as amaranth (Figure 1; Moreno-Espíndola *et al.* 2007). Since plant phenology influences quantity and quality of root exudates, which determine microbial communities in roots (Bencherif *et al.* 2016, Francioli *et al.* 2018), it is expected that plant phenology influences rhizosheath traits. Microorganisms living in the rhizosphere of sandy soils promoted the for-

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Iván Pável Moreno-Espíndola designed and set up the experiment, analyzed the data and wrote the paper. María Jesús Ferrara-Guerrero designed the experiment, analyzed the data and helped to write the paper. Fernando de León-González designed the experiment, helped with interpretation of the data and critically reviewed first draft of the paper. Facundo Rivera-Becerril designed the experiment, helped with interpretation of the data and critically reviewed first draft of the paper. Lino Mayorga-Reyes was involved on molecular analysis. Néstor O. Pérez carried out analysis of DNA sequences.
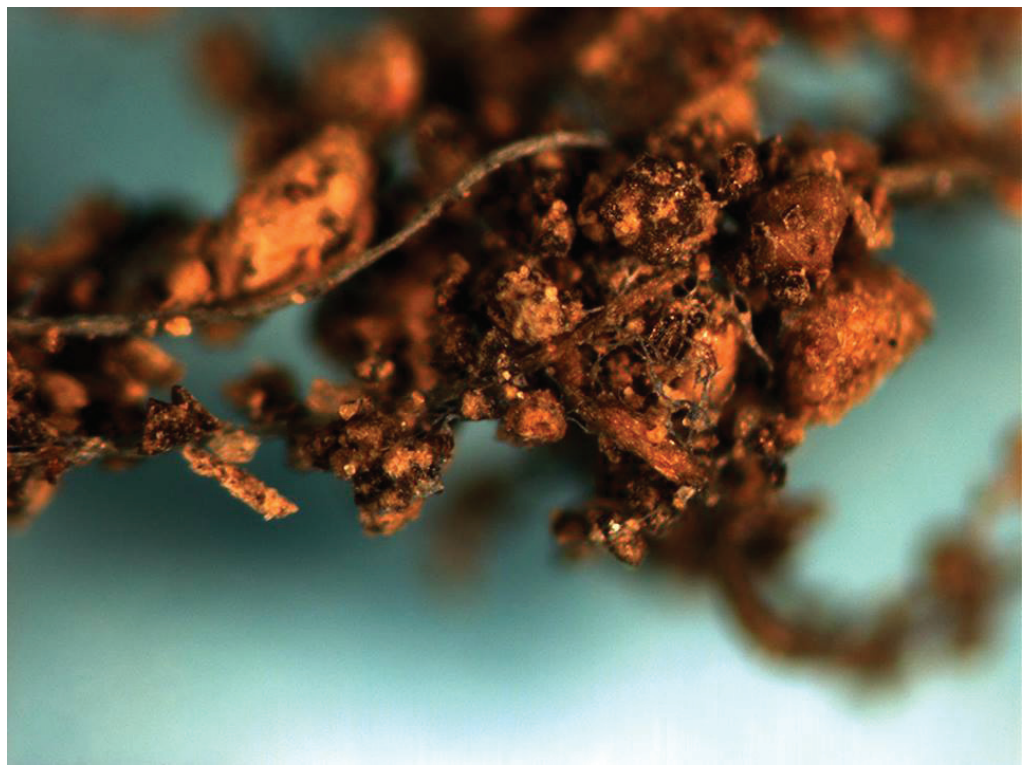
Figure 1. Rhizosheath of amaranth: Root-hairs adhering sand particles in a volcanic soil in the Valley of Mexico.

mation of stable macroaggregates (Moreno-Espíndola *et al.* 2007). Into this context, Moreno-Espíndola *et al.* (2013) reported the presence of a culturable bacterial community comprising 37 isolates from the amaranth rhizosheath. This community has high heterotrophic potential, which could be useful for improving the fertility of the regional farming systems. It has been demonstrated that soil bacteria (*Bacillus* and *Burkholderia*) promote growth and increase yield in grain of amaranth through higher plant P and N uptake (Pal 1998, Parra-Cota *et al.* 2014). For the development of sustainable systems, the understanding of the microbial processes in soils is required (Philippot *et al.* 2006), especially in soils in which rain-fed agriculture is practiced.

The aim of this work was to evaluate the potential activity of six enzymes (dehydrogenase, protease, acid and alkaline phosphatases, cellulase and chitinase) involved in the transformation of organic matter in rhizosheath soil and surrounding rhizosheath soil (soil not adhered by roots) of amaranth plants grown in a sandy soil. Likewise, 37 bacterial isolates obtained from amaranth rhizosheath soil were molecularly identified, as microorganisms which potentially perform these enzyme activities.

Materials and methods

Soil and experimental site. The experimental site is located in the Valley of Mexico (Tulyehualco, 19º 15'N, 99º 13"W, 2,280 m in altitude). The soil is an Entisol (Soil Survey Staff 2003), and is composed of volcanic ash and 70, 140, and 790 g kg^{-1} of clay, silt, and sand, respectively. The predominant minerals are pumice and feldspars, the climate is semi-arid, with an average annual precipitation of 537 mm, a mean temperature of 17.9 ºC (De León-González *et al.* 2006). In 2009, an experimental field was established with amaranth, which was subdivided into three plots of 12 m^2 each. Amaranth was sown under rain-fed conditions, with minimum tillage. An application of a single dose of fertilizer (80-40-00, N-P-K) at the beginning of the flowering period was carried out.

Soil sampling. Sampling of rhizosheath soil and surrounding rhizosheath soil (or loose soil), included four cropping periods of amaranth: sowing, flowering, harvest, and post-harvest (0, 45, 90, and 120 days after sowing). Three soil-root monoliths, one per plant, were obtained with a PVC cylinder (25.5 cm diameter; 37.5 cm length) used as a soil core sampler (Moreno-Espíndola *et al.* 2007) during each sampling period. Rhizosheath soil was separated from roots using a dissecting needle. The loose soil was sampled 3 cm out near the soil-root monolith. The samples were preserved in sterile plastic bags at 4 ºC.

Soil-organic carbon, microbial-biomass carbon, and total nitrogen. Soil organic carbon (SOC) was measured by the Walkley & Black (1934) method, and total nitrogen (TN), by digestion with H_2SO_4 (Bremner & Mulvaney 1982). Microbial biomass carbon (C_{mic}) was measured using the chloroform-fumigation (Vance *et al.* 1987), and an automatic carbon analyzer (Shimadzu, TOC-VCSN).

Soil pH and soil-water content. The pH of the soil samples was measured potentiometrically (HANNA HI250) in a water-soil suspension (2.5:1). At each phenological stage, soil water content (SWC) (g 100 g^{-1}) was determined *in situ* using a neutron probe (CNP® MC-1DR), by averaging six measurements obtained by introducing the probe every 5 cm into a depth of 0–30 cm.

Enzymatic activities. The enzymatic activities considered for evaluation were those of dehydrogenase (DH), acid (ACP) and alkaline (ALP) phosphatase, cellulase (CEL), chitinase (CHI), and protease (PRO). All these enzymes participate in processes of soil organic matter (SOM) decomposition. Measurement of the potential of each enzyme activity was performed following the spectrophotometric methods described by García *et al.* (2003). To determine all absorbances, a Shimadzu Dual-beam Spectrophotometer (Shimadzu UV-1700) was employed. Corresponding calibration curves were generated for each enzymatic activity and each test included a control and four replicates.

Molecular identification of bacterial isolates from amaranth rhizosheath. While enzymatic activity was determined in soils, 37 bacteria were isolated from the amaranth rhizosheath, and physiologically characterized (Moreno-Espíndola *et al*. 2013). These 37 bacterial isolates were subcultured for molecular identification. Genomic DNA was extracted using the ZRFungal/ Bacterial DNA MiniPrep™ kit according to the manufacturer's instructions. DNA quality was analyzed in a 2 % agarose gel, and quantification was achieved using a Nano-Drop spectrophotometer (Thermo). PCR amplification of the 16S rDNA fragment was performed using primers rD1 (5´AAGGAGGTGATCCAGCC3´) and fD1 (5´AGAGTTTGATCCTGGCTCAG3´) (Laguerre *et al*. 1994). The PCR mix per sample was as follows: template DNA (50 ng); primers rD1 and fD1 (0.1 μM each); 10X buffer; dNTP (20 μM); $MgCl_2$ (1.5 μM), and Fermentas *Taq* polymerase (2 U). PCR was performed in a Biometra thermocycler under the following conditions: initial denaturation at 95 ºC for 5 min; 35 cycles of denaturation at 94 ºC for 1 min; annealing at 55 ºC for 1 min, and extension at 72 ºC for 2 min; finally, an extension step at 72 ºC for 3 min. PCR products of approximately 1,500 bp were verified in a 2 % agarose gel and then purified using the QIAquick Qiagene™ kit according to the manufacturer's instructions. Last, 16 μL of the PCR products (150 ng μL^{-1}) of each isolate were sequenced using the forward and reverse primers (Biotechnology Institute, UNAM). The sequences were edited and assembled using DNASTAR SEQman II ver. 5.06 software, and low-quality sequences were eliminated. Using the basic local alignment search tool (BLAST), the sequences were analyzed and compared with those registered in the National Center for Biotechnology Information (NCBI) database to identify their similarity. Finally, the sequences were submitted to the NCBI database.

Statistical analysis. To analyze the data, a two-way analysis of variance (ANOVA) assay was performed. The test considered rhizosheath soil and soil not adhered by roots (loose soil), and the cropping period (four dates: sowing; flowering; harvest, and post-harvest) as factors. When statistically significant differences were detected ($p < 0.05$), the Tukey mean comparison test was carried out. JMP® 8.0 statistical software was employed.

Results

Properties in the rhizosphere of Amaranthus hypochondriacus. Soil organic carbon (SOC) content and total nitrogen (TN) were significantly higher in rhizosheath soil compared with samples of loose soil, but non-differences were observed concerning microbial biomass carbon (C_{mic}) even if higher levels were present in loose soil (Table 1). pH and soil water content (SWC)

Table 1. Properties and potential enzyme activities in rhizosheath of *Amaranthus hypochondriacus*, and loose soils (mean±standard error; *n* = 9). Different letters indicate different mean groups ($p < 0.05$).

	Rhizosheath	Loose soil
Soil properties		
Soil organic carbon (mg 100 g^{-1})	0.71±0.05[a]	0.41±0.03[b]
Microbial biomass carbon (mg kg^{-1})	213.28±29.45[a]	284.61±33.66[a]
Total nitrogen (mg 100 g^{-1})	0.14±0.00[a]	0.04±0.01[b]
pH	7.1±0.03[a]	7.1±0.03[a]
Soil water content (g 100 g^{-1})	10.43±2.06[a]	9.81±1.83[a]
Potential enzyme activity		
Dehydrogenase (μM INTF g^{-1} h^{-1})	5.01±0.63[a]	5.31±0.40[a]
Acid phosphatase (μM *p*-nitrophenol g^{-1} h^{-1})	0.96±0.04[a]	0.93±0.05[a]
Alkaline phosphatase (μM *p*-nitrophenol g^{-1} h^{-1})	1.16±0.06[a]	1.28±0.05[a]
Cellulase (μM equivalent glucose g^{-1} h^{-1})	0.01±0.00[a]	0.00±0.00[a]
Chitinase (μM N-acetylglucosamine g^{-1} h^{-1})	12.25±2.88[a]	7.96±2.48[a]
Protease (μM tyrosine g^{-1} h^{-1})	13.75±11.38[a]	9.89±4.24[a]

did not exhibit differences between rhizosheath soil and loose soil, which was also observed concerning the six enzyme activities (Table 1). At sowing date roots were not yet developed; by analyzing the other three sampling seasons (flowering, harvest, and post-harvest where the plant roots remained into the soil), TN was always higher in rhizosheath than in loose soil, as well as SOC in flowering and post-harvest seasons; C_{mic} was three-times higher in loose than in rhizosheath soil in post-harvest season (Table 2). pH and SWC were measured without discriminate between both microenvironments; pH did not show differences between phenological stages, while SWC decreased from 20 g 100 g^{-1} in sowing to 4.9 g 100 g^{-1} in post-harvest. Concerning enzyme activity, DH exhibited highest values in post-harvest season compared with the previous two seasons, with differences between the two soil types at harvest and post-harvest stages. ACP demonstrated a tendency to increase as the farming season progressed, and no differences between rhizosphere and loose soils occurred, while ALP showed differences between the two soil types only at the flowering season. CEL showed low values in all three sampling seasons. CHI had similar values in the flowering and harvest seasons without differences between soil types, with a slight decrease in post-harvest season, during which rhizosheath soil was nearly double compared with loose soil. Finally, PRO exhibited differences between soil types at harvest season, with the higher mean for rhizosheath soil (Table 2).

Correlation analysis between all variables measured in this study is shown in Table 3. ACP and ALP activities showed negative and significant correlation with soil pH and SWC. CEL activity had positive and significant correlation with pH and SWC. Finally, a positive correlation between PRO activity and SWC was observed. None of the six enzymatic activities showed significant correlation with C_{mic}, SOC, or TN (Table 3).

Molecular identification of bacterial isolates. Partial sequences of the 16S rDNA segments of each bacterial isolate were submitted to the GenBank database at NCBI (Table 4). Predominant

Table 2. Properties and potential enzyme activities in rhizosheath of *Amaranthus hypochondriacus* in every phenological stage, and loose soils (mean±standard error; *n* = 3). Different letters indicate different mean groups (Tukey; *p* < 0.05).

	Sowing	Flowering		Harvest		Post-harvest	
	Base line	Rhizosheath	Loose soil	Rhizosheath	Loose soil	Rhizosheath	Loose soil
Soil properties							
Soil organic carbon (mg 100 g^{-1})	0.48±0.00	0.89±0.00[a]	0.29±0.01[b]	0.53±0.01[a]	0.52±0.01[a]	0.69±0.00[a]	0.41±0.01[b]
Microbial biomass carbon (mg kg^{-1})	183.7±62.8	262.5±26.8[a]	179.7±24.0[a]	260.6±46.1[a]	291.1±15.7[a]	116.8±24.3[b]	383.0±49.1[a]
Total nitrogen (mg 100 g^{-1})	0.11±0.00	0.16±0.00[a]	0.10±0.00[b]	0.16±0.00[a]	0.01±0.00[b]	0.11±0.00[a]	0.00±0.00[b]
pH	7.9±0.03	7.1±0.04[a]		7.1±0.02[a]		7.2±0.04[a]	
Soil water content (g 100 g^{-1})	20.4±0.24	17.5±0.90[a]		7.9±0.06[b]		4.9±0.05[c]	
Potential enzyme activity							
Dehydrogenase (µM INTF g^{-1} h^{-1})	6.94±0.07	5.03±0.26[a]	5.45±0.82[a]	2.04±0.36[b]	3.91±0.24[a]	7.97±0.28[a]	6.56±0.37[b]
Acid phosphatase (µM *p*-nitrophenol g^{-1} h^{-1})	0.42±0.10	0.81±0.01[a]	0.72±0.06[a]	0.86±0.00[a]	0.93±0.04[a]	1.21±0.02[a]	1.12±0.02[a]
Alkaline phosphatase (µM *p*-nitrophenol g^{-1} h^{-1})	0.80±0.06	1.06±0.04[b]	1.28±0.03[a]	0.94±0.03[a]	1.10±0.07[a]	1.48±0.02[a]	1.46±0.03[a]
Cellulase (µM equivalent glucose g^{-1} h^{-1})	0.064±0.00	0.014±0.00[a]	0.006±0.00[a]	0.008±0.00[a]	0.012±0.00[a]	0.010±0.00[a]	0.005±0.00[a]
Chitinase (µM N-acetylglucosamine g^{-1} h^{-1})	13.28±3.11	13.16±6.51[a]	5.44±2.57[a]	13.80±5.33[a]	13.04±6.43[a]	9.78±4.69[a]	5.41±2.55[a]
Protease (µM tyrosine g^{-1} h^{-1})	8.51±0.99	25.62±6.02[a]	17.48±11.28[a]	12.79±1.03[a]	2.18±1.77[b]	2.85±2.28[a]	10.00±5.22[a]

Table 3. Pearson correlation coefficient of overall soil variables measured in rhizosheath and loose soils of *Amaranthus hypochondriacus* (n = 12).

	pH	SWC	SOC	TN	DH	ACP	ALP	CEL	CHI	PRO	C_{mic}
pH	1.00										
SWC	0.53*	1.00									
SOC	-0.13	0.07	1.00								
TN	0.08	0.43	0.48*	1.00							
DH	0.43	0.06	0.01	-0.19	1.00						
ACP	-0.66**	-0.84**	0.18	-0.30	0.15	1.00					
ALP	-0.46*	-0.54*	-0.11	-0.41	0.39	0.77**	1.00				
CEL	0.91**	0.59*	-0.02	0.14	0.30	-0.70**	-0.63*	1.00			
CHI	0.14	0.10	0.26	0.21	-0.11	-0.06	-0.30	0.28	1.00		
PRO	-0.12	0.46*	0.22	0.37	-0.14	-0.16	-0.09	-0.07	0.11	1.00	
C_{mic}	-0.25	-0.22	-0.12	-0.42	-0.22	0.24	0.08	-0.22	-0.06	0.12	1.00

SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen; DH, dehydrogenase; ACP, acid phosphatase; ALP, alkaline phosphatase; CEL, cellulase; CHI, chitinase; PRO, protease; C_{mic}, microbial biomass carbon; *, **, significant at $p < 0.05$ and $p < 0.01$, respectively.

genera found during the four sampling seasons included *Bacillus* (29 isolates), followed by *Enterobacter* (3 isolates), *Streptomyces* (2 isolates), *Stenotrophomonas* (1 isolate), *Pseudomonas* (1 isolate), and *Arthrobacter* (1 isolate). *Streptomyces* was detected only during the sowing season. In summary, the flowering season showed highest bacterial diversity, whereas the harvest season had lowest bacterial diversity.

Discussion

The similarity in the general potential enzyme activity in rhizosheath and loose soils indicate biochemical likeness in both microenvironments in the amaranth rhizosphere. This could be explained by the dominant mineral in soil, pumice, which favor equality in water-retention capacity in both soil types (Segura-Castruita *et al*. 2005), and, by the presence of abundant hyphae in association with the amaranth roots in the same soil (Moreno-Espíndola *et al*. 2007), which increases the influence zone of the roots. Othman *et al*. (2004) reported that in the rhizosheaths of two grasses (*Panicum turgidum* and *Stipagrostis scoparia*), SOC and TN were two-fold higher than in loose soil. This result is consistent with the fact that grasses are not exposed to mechanical tillage, which allows the accumulation of exudates and organic matter. In our study, the amaranth crop was established with minimum tillage, which favor microbial growth and potential enzymatic activity in the loose soil located in the vicinity of the main root zone.

When comparing potential enzymatic activity between rhizosheath and loose soils for each cropping period, significant statistical differences were found. These results confirm the role of DH activity as a sensitive agronomic indicator (Fuentes-Ponce *et al*. 2016); in the current study, DH changed with phenological stage and soil microenvironment. Likewise, higher ALP activity during the flowering period in loose soil in comparison with rhizosheath soil coincides with the larger area of influence attributed to this enzyme, which is produced only by microorganisms and not by roots (Spohn & Kuzyakov 2013). Activities of DH and both phosphatases were highest during the post-harvest stage. One factor that may intervene in higher enzyme activity during post-harvest period is the soil–atmosphere gas exchange. It is assumed that, as the soil dries the water-free pores are filled with air and further shrinkage of root and post-harvest root decay increase the number of pores that can be filled with air. Such conditions favor aerobic respiration and increase DH activity during post-harvest. The effects of these changes on rhizosphere conditions over time were also observed in ACP and ALP, which increased as each period elapsed, while CEL behavior was contrariwise. In the current study, the slow degradation rate of cellulose-rich residues is due to a decrease in soil moisture during the dry season

Table 4. Molecular identification based on the 16S rDNA segment of 37 bacterial strains from rhizosheath soil of *Amaranthus hypochondriacus* and their potential roles.

Strain	Accesion number in the GenBank	Highest homology (accesion number in the GenBank)	Potential role in the soil (references)
Sowing			
AN1E1	KC336020	*Streptomyces zaomyceticus* (EU593685)	Degradation of recalcitrant organic matter, cellulolytic activity (a, b, e)
AN3E1a	KC336021	*Bacillus* sp. (AB773240)	P solubilization (a, b, e)
AN4E1a	KC336022	*Bacillus* sp. (JN132107)	P solubilization (a, b, e)
AN5E1b	KC336024	*Bacillus* sp. (HQ600985)	P solubilization (a, b, e)
AS1E1	KC336025	*Bacillus cereus* (EU661712)	P solubilization, PGPR (a, b, e)
AS2E1b	KC336027	*Streptomyces* sp. (JQ812096)	Degradation of recalcitrant organic matter, cellulolytic activity (a, b, e)
AS4E1b	KC336029	*Bacillus* sp. (JN872500)	P solubilization (a, b, e)
AS5E1a	KC336030	*Bacillus cereus* (JX855262)	P solubilization, PGPR (a, b, e)
Flowering			
AN1E2	KC336032	*Bacillus* sp. (JX402435)	P solubilization, PGPR (a, b, e)
AN2E2	KC336033	*Bacillus* sp. (JQ396537)	P solubilization, PGPR (a, b, e)
AN3E2	KC336034	*Enterobacter* sp. (EU331414)	PGPR (a, c)
AN4E2	KC336035	*Stenotrophomonas* sp. (AY689048)	Chitinolytic activity, degradation of xenobiotics (a, b)
AN5E2	KC336036	*Bacillus cereus* (EF488087)	P solubilization, PGPR (a, b)
AS1E2	KC336037	*Pseudomonas* sp. (HQ224640)	PGPR (a, b)
AS2E2b	KC336039	*Enterobacter* sp. (HQ677832)	PGPR (a, c)
AS3E2a	KC336040	*Enterobacter* sp. (GU272374)	PGPR (a, c)
AS4E2a	KC336042	*Bacillus* sp. (JN210907)	P solubilization, PGPR (a, b, e)
AS5E2a	KC336044	*Bacillus* sp. (KC121044)	P solubilization, PGPR (a, b)
Harvest			
AN1E3	KC336046	*Bacillus subtilis* (HM744709)	P solubilization, biological control, amylase and cellulase production (a, b, e)
AN2E3	KC336047	*Bacillus pumilus* (JX847116)	P solubilization, PGPR (a, b, e)
AN3E3a	KC336048	*Bacillus pumilus* (JX625990)	P solubilization, PGPR (a, b, e)
AN4E3	KC336050	*Bacillus pumilus* (JN082266)	P solubilization, PGPR (a, b, e)
AN5E3	KC336051	*Bacillus pumilus* (HQ218989)	P solubilization, PGPR (a, b, e)
AS1E3	KC336052	*Bacillus megaterium* (AB738793)	P solubilization, PGPR (a, b, e)
AS2E3	KC336053	*Bacillus megaterium* (AB738793)	P solubilization, PGPR (a, b, e)
AS3E3	KC336054	*Bacillus aryabhattai* (JX460818)	P solubilization, PGPR (a, b, e)
AS4E3	KC336055	*Bacillus megaterium* (JX312585)	P solubilization, PGPR (a, b, e)
Post-harvest			
AN1E4b	KC336057	*Bacillus* sp. (JX897963)	P solubilization, PGPR (a, b)
AN2E4b	KC336059	*Bacillus* sp. (JX566587)	P solubilization, PGPR (a, b)
AN3E4b	KC336061	*Bacillus cereus* (JX273683)	P solubilization, PGPR (a, b, e)
AN4Eb	KC336062	*Arthrobacter* sp. (JF768708)	Degradation of xenobiotics (d)
AN5E4b	KC336064	*Bacillus* sp. (JX266343)	P solubilization, PGPR (a, b)
AS1E4	KC336065	*Bacillus* sp. (HM104462)	P solubilization, PGPR (a, b)
AS2E4	KC336066	*Bacillus megaterium* (AB738793)	P solubilization, PGPR (a, b, e)

Table 4. Continuation.

Strain	Accesion number in the GenBank	Highest homology (accesion number in the GenBank)	Potential role in the soil (references)
AS3E4	KC336067	*Bacillus* sp. (JF309237)	P solubilization, PGPR (a, b)
AS4E4a	KC336068	*Bacillus megaterium* (HM357355)	P solubilization, PGPR (a, b, e)
AS5E4	KC336070	*Bacillus aryabhattai* (JX460818)	P solubilization, PGPR (a, b, e)

Strain names were reported by Moreno-Espíndola *et al*. (2013); PGPR, plant growth-promoting rhizobacteria. References: a) Andrade (2004), b) Yang *et al*. (2012), c) Schütz *et al*. (2003), d) Vaishampayan *et al*. (2007), e) Yasir *et al*. (2009).

and low temperatures during winter. These climatic conditions can explain the low levels of CEL activity, which are mainly driven by anaerobic bacteria. Moreno-Espíndola *et al*. (2007) reported high soil porosity in this same soil, which could increase O_2 availability, resulting in possible inhibition of cellulolytic microbial groups, hence, of their activity. Moreover, Yaroslavtsev *et al*. (2009) reported that a decrease in soil moisture promoted CHI activity in sandy soils. In contrast, we found a decrease in CHI activity when SWC decreased. SOC and TN results showed that rhizosheaths possessed more favorable conditions for soil organic matter (SOM) accumulation.

In a semi-arid region, plant species increased the organic matter content in their rhizospheres, where enzymes such as phosphatase and urease increased their activity in contrast to a soil without vegetation; rhizosphere induces the synthesis of such enzymes (Garcia *et al*. 2005). Other semi-arid degraded soil, amended with compost, improved soil water holding capacity, which in turn increased activity of β-glucosidase, phosphatase and DH (Hernández *et al*. 2015). These results reinforce the need to study the rhizosphere footprint of plant species and the microbial ecology process (York *et al*. 2016).

In the microbiome identified, the *Bacillus* genus was the most abundant. During the crop-sowing stage CEL activity was highest, which was consistent with the presence of *Streptomyces* isolates that play a significant role in aerobic cellulose degradation (Andrade 2004). High cellulose accumulation in soil derives from root tissues of amaranth from the last post-harvest period. During the post-harvest season when phosphatase activities (ALP and ACP) were highest, the bacterial species *Arthrobacter* spp., *Bacillus cereus*, *B. megaterium*, and *B. aryabhattai* were found. These species have been reported as phosphate-solubilizing bacteria (Yang *et al*. 2012).

In the present study, highest bacterial diversity was observed during the flowering period which can be related, in part, with the unique fertilization dose applied to the soil, while, during crop harvest, *Bacillus* was the sole genus present. *Stenotrophomonas* has been reported as rhizosphere inhabitant cohabiting with *Bacillus* and *Pseudomonas*, and their presence is influenced by plant type and by carbon sources (Kapoor & Mukerji 2006). These genera have also been reported as plant growth-promoting rhizobacteria (PGPR) because they produce and release growth hormones (Schütz *et al*. 2003). The soil bacterial strains identified in the current study have a potential in biotechnological uses (i.e., native microbial consortia useful for biological soil fertility) and for cropping amaranth and other plants under the concept of sustainability (Scotti *et al*. 2015) in semi-arid conditions.

In conclusion, in the rhizosphere of *Amaranthus hypochondriacus* grown in a pumiceous sandy soil, potential enzymatic activities in the rhizosheath and loose soils were similar, which must be considered a unique rhizosphere environment. Dehydrogenase and acid phosphatase activities are highly sensitive to changes in the crop phenology. Acid and alkaline phosphatase, cellulose and protease activities correlated to changes in soil moisture. The behavior of phosphatases and dehydrogenase activities suggests a process of active degradation of organic materials and increased dynamic SOM during the post-harvest period. In the amaranth rhizosphere, native bacteria are involved in the breakdown of SOM and they possess potential for biotechnological applications for agriculture as inoculants for the design of novel biological inputs. Other molecular biology methods should be employed for the study of total microbial diversity.

Literature cited

Andrade G. 2004. Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. *In*: Varma A, Abbott L, Werner D, Hampp R. Eds. *Plant Surface Microbiology*. Berlin Heidelberg: Springer-Verlag, 51-69. ISBN: 978-3-540-00923-8

Barba de la Rosa AP, Fomsgaard IS, Laursen B, Mortensen AG, Olvera-Martínez L, Silva-Sánchez C, Mendoza-Herrera A, González-Castañeda J, De León-Rodríguez A. 2009. Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. *Journal of Cereal Science* **49**: 117-121. DOI: https://doi.org/10.1016/j.jcs.2008.07.012

Bencherif K, Boutekrabt A, Dalpé Y, Lounès-Hadj-Sahraoui K. 2016. Soil and season affect arbuscular mycorrhizal fungi associated with *Tamarix* rhizosphere in arid and semi-arid steppes. *Applied Soil Ecology* **107**: 182-190. DOI: https://doi.org/10.1016/j.apsoil.2016.06.003

Bremner JM, Mulvaney CS. 1982. Nitrogen-total. *In*: Page AL, Miller RH, Keeney DR. Eds. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Madison: American Society of Agronomy, 595-622. ISBN: 0-89118-072-9

Clouse JW, Adhikary D, Page JT, Ramaraj T, Deyholos MK, Udall JA, Fairbanks DJ, Jellen EN, Maughan PJ. 2016. The amaranth genome: genome, transcriptome, and physical map assembly. *The Plant Genome* **9**: 1-14. DOI: https://doi.org/10.3835/plantgenome2015.07.0062

De León-González F, Celada-Tornel E, Hidalgo-Moreno CI, Etchevers-Barra JD, Gutiérrez-Castorena MC, Flores-Macías A. 2006. Root-soil adhesion as affected by crop species in a volcanic sandy soil of Mexico. *Soil & Tillage Research* **90**: 77-83. DOI: https://doi.org/10.1016/j.still.2005.08.007

Francioli D, Schulz E, Buscot F, Reitz T. 2018. Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. *Microbial Ecology* **75**: 216-227. DOI: https://doi.org/10.1007/s00248-017-1012-0

Fuentes-Ponce M, Moreno-Espíndola IP, Sánchez-Rodríguez LM, Ferrara-Guerrero MJ, López-Ordaz R. 2016. Dehydrogenase and mycorrhizal colonization: Tools for monitoring agrosystem soil quality. *Applied Soil Ecology* **100**: 144-153. DOI: https://doi.org/10.1016/j.apsoil.2015.12.011

Garcia C, Roldan A, Hernandez T. 2005. Ability of different plant species to promote microbiological processes in semiarid soil. *Geoderma* **124**: 193-202. DOI: https://doi.org/10.1016/j.geoderma.2004.04.013

García C, Gil F, Hernández T, Trasar C. 2003. *Técnicas de Análisis de Parámetros Bioquímicos en Suelos: Medida de Actividades Enzimáticas y Biomasa microbiana*. Madrid: Mundi-Prensa. ISBN: 9788484761549

Gianfreda L. 2015. Enzymes of importance to rhizosphere processes. *Journal of Soil Science and Plant Nutrition* **2**: 283-306. DOI: https://doi.org/10.4067/S0718-95162015005000022

Hernández T, Garcia E, García C. 2015. A strategy for marginal semiarid degraded soil restoration: a sole addition of compost at a high rate. A five-year field experiments. *Soil Biology & Biochemistry* **89**: 61-71. DOI: https://doi.org/10.1016/j.soilbio.2015.06.023

Kapoor R, Mukerji KG. 2006. Rhizosphere microbial community dynamics. *In*: Mukerji KG, Manoharachary C, Singh J. Eds. *Microbial Activity in the Rhizosphere,* pp. 55-66, Springer-Verlag, Berlin Heidelberg. ISBN: 978-3-540-29182-4

Kuddus SM, Ahmad RIZ. 2013. Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. *Journal of Genetic Engineering and Biotechnology* **11**: 39-46. DOI: https://doi.org/10.1016/j.jgeb.2013.03.001

Laguerre G, Allard M-R, Revoy F, Amarger N. 1994. Rapid identification of rhizobia by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes. *Applied and Environmental Microbiology* **60**: 56-63.

Lesuffleur F, Paynel F, Bataillé M-P, Le Deunff E, Cliquet JB. 2007. Root amino acid exudation: measurement of high efflux rates of glycine and serine from six different plant species. *Plant and Soil* **294**: 235-246. DOI: https://doi.org/10.1007/s11104-007-9249-x

Moreno-Espíndola IP, Ferrara-Guerrero MJ, De León-González F, Rivera-Becerril F, González-Halphen

D. 2013. Comunidad bacteriana cultivable asociada a la rizocoraza de *Amaranthus hypochondriacus*. *Terra Latinoamericana* **31**: 57-69.

Moreno-Espíndola IP, Rivera-Becerril F, Ferrara-Guerrero MJ, De León-González F. 2007. Role of root-hairs and hyphae in adhesion of sand particles. *Soil Biology and Biochemistry* **39**: 2520-2526. DOI: https://doi.org/10.1016/j.soilbio.2007.04.021

Othman AA, Amer WM, Fayez M, Hegazi NA. 2004. Rhizosheath of Sinai desert plants is a potential repository for associative diazotrophs. *Microbiological Research* **159**: 285-293. DOI: https://doi.org/10.1016/j.micres.2004.05.004

Pal SS. 1998. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant and Soil* **198**: 169-177. DOI: https://doi.org/10.1023/A:1004318814385

Parra-Cota FI, Peña-Cabriales JJ, de los Santos-Villalobos S, Martínez-Gallardo NA, Délano-Frier JP. 2014. *Burkholderia ambifaria* and *B. caribensis* promote growth and increase yield in grain amaranth (*Amaranthus cruentus* and *A. hypochondriacus*) by improving plant nitrogen uptake. *PLOS ONE* **9**: e88094. DOI: https://doi.org/10.1371/journal.pone.0088094

Philippot L, Kuffner M, Chèneby D, Depret G, Laguerre G, Martin-Laurent F. 2006. Genetic structure and activity of the nitrate-reducers community in the rhizosphere of different cultivars of maize. *Plant and Soil* **287**: 177-186. DOI: https://doi.org/10.1007/s11104-006-9063-x

Schütz A, Golbik R, Tittmann K, Svergun DI, Koch MHJ, Hübner G, König S. 2003. Studies on structure–function relationships of indolepyruvate decarboxylase from *Enterobacter cloacae*, a key enzyme of the indole acetic acid pathway. *European Journal of Biochemistry* **270**: 2322-2331. DOI: https://doi.org/10.1046/j.1432-1033.2003.03602.x

Scotti R, Bonanomi G, Scelza R, Zoina A, Rao MA. 2015. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *Journal of Soil Science and Plant Nutrition* **15**: 333-352. DOI: https://doi.org/10.4067/S0718-95162015005000031

Segura-Castruita MA, Gutiérrez-Castorena MC, Ortiz-Solorio CA, Sánchez-Guzmán P. 2005. Régimen de humedad y clasificación de suelos pomáceos del Valle Puebla-Tlaxcala. *Terra Latinoamericana* **23**: 13-20.

Soil Survey Staff. 2003. *Soil taxonomy.* Natural Resources Conservation Service, Washington.

Spohn M, Kuzyakov Y. 2013. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with ^{14}C imaging. *Soil Biology and Biochemistry* **67**: 106-113. DOI: https://doi.org/10.1016/j.soilbio.2013.08.015

Vaishampayan PA, Kanekar PP, Dhakephalkar PK. 2007. Isolation and characterization of *Arthrobacter* sp. strain MCM B-436, an atrazine-degrading bacterium, from rhizospheric soil. *International Biodeterioration & Biodegradation* **60:** 273-278. DOI: https://doi.org/10.1016/j.ibiod.2007.05.001

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**: 703-707. DOI: https://doi.org/10.1016/0038-0717(87)90052-6

Walkley A, Black IA. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37:** 29-38. DOI: https://doi.org/10.1097/00010694-193401000-00003

Yang P-X, Ma L, Chen M-H, Xi J-Q, He F, Duan C-Q, Mo M-H, Fang D-H, Duan Y-Q, Yang F-X. 2012. Phosphate solubilizing ability and phylogenetic diversity of bacteria from P-rich soils around Dianchi Lake drainage area of China. *Pedosphere* **22**: 707-716. DOI: https://doi.org/10.1016/S1002-0160(12)60056-3

Yaroslavtsev AM, Manucharova NA, Stepanov AL, Zvyagintsev DG, Sudnitsyn II. 2009. Microbial destruction of chitin in soils under different moisture conditions. *Eurasian Soil Science* **42**: 797-806. DOI: https://doi.org/10.1134/S1064229309070114

Yasir M, Aslam Z, Kim SW, Lee S-W, Jeon CO, Chung YR. 2009. Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. *Bioresource Technology* **100**: 4396-4403. DOI: https://doi.org/10.1016/j.biortech.2009.04.015

York LM, Carminati A, Mooney SJ, Ritz K, Bennett MJ. 2016. The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. *Journal of Experimental Botany* **67**: 3629-3643. DOI: https://doi.org/10.1093/jxb/erw108