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SOIL SALINITY SHIFTS CULTIVABLE MICROBIAL COMMUNITIES OF WHEAT (*Triticum turgidum* subsp. *durum*) RHIZOSPHERE IN THE YAQUI VALLEY, MEXICO

Arlett Leticia **Ibarra-Villarreal**[1], Jonathan **Rojas-Padilla**[1], Luis Abraham **Chaparro-Encinas**[2], Alondra María **Díaz-Rodríguez**[1], Valeria **Valenzuela-Ruiz**[1], Angélica **Herrera-Sepúlveda**[3], Fannie Isela **Parra-Cota**[4], Sergio **de los Santos-Villalobos**[1*]

[1] Instituto Tecnológico de Sonora. Calle 5 de Febrero 818 Sur, Col. Centro, Ciudad Obregón, Sonora, Mexico. C. P. 85000.
[2] Universidad Autónoma Agraria Antonio Narro. Unidad Laguna. Periférico Raúl López Sánchez, Valle Verde, Torreón, Coahuila, Mexico. C. P. 27054.
[3] Tecnológico Nacional de México. Instituto Tecnológico del Valle del Yaqui. Av. Tecnológico, Block 611, Bacum, Sonora, Mexico. C. P. 85276.
[4] Insituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Norman E. Borlaug. Calle Norman E. Borlaug km 12, Ciudad Obregón, Sonora, Mexico. C. P. 85000.
* Author for correspondence: sergio.delossantos@itson.edu.mx

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ABSTRACT

Saline soils are a common issue in commercial fields using intensive and irrigated agricultural practices under arid or semi-arid climates, such as the Yaqui Valley in Mexico, one of the most important wheat-producing regions worldwide. This study aimed to assess the cultivable microbial diversity (having plant growth-promoting attributes) associated with wheat rhizosphere in commercial fields, under a saline soil gradient in the Yaqui Valley. Thus, seven wheat commercial zones were studied according to their electrical conductivity (0.9 to 6.4 dS cm^{-1}). The isolation of microorganisms was carried out by dependent culture techniques, sequencing of the 16S (bacteria) and 5.8S (fungi) rRNA genes , and plant growth-promoting attributes were evaluated: phosphate solubilization (Pikovskaya medium), siderophore production (Chrome Azurol S medium), indole production (Salkowsky reagent), and hemolytic activity (Petri dishes 5 % Sheep Blood). Wheat commercial zones with 1.6 dS m^{-1} showed the highest fungal (2.59 x 10^6 CFU g^{-1} dry soil) and bacterial (2.88 x 10^7 CFU g^{-1} dry soil) populations, being *Rhizopus* and *Bacillus* the most abundant and well-distributed microbial genera, respectively. The impact of saline soils on the microbial metabolic background was not species- or genus-specific; it was at a strain-specific level. In addition, 52 % of fungal and 83 % of bacterial strains showed the ability to produce γ-hemolysis, suggesting it is not harmful to humans and animals. This work provides a microbial culture collection where their members have shown several beneficial traits that potentially increase the yield and quality of wheat growing under saline soil conditions.

Keywords plant growth-promoting microorganisms, *Bacillus*, *Rhizopus*, electrical conductivity, taxonomic affiliation.

INTRODUCTION

The Yaqui Valley is located in the south of the Sonora State in Mexico and is the main wheat producer state in this country (Matson and Jewett, 2012). This valley contributes 47 % of the national wheat production (1.7 x 10^6 Mg $year^{-1}$), which is produced in 173 000 ha (SIAP, 2018). However, this crop is highly sensitive to environmental conditions, such as extreme temperature, water reduction, and saline soils (Porter and Semenov, 2005).

Salinity in agricultural soils is recognized as a major constraint for food production. It has been estimated that 20 % of cultivated fields around the world are affected by this abiotic condition (Flowers, 2004). Saline soils are a common issue in commercial fields using intensive and irrigated agricultural practices under arid or semi-arid climates, *i.e.* the Yaqui Valley (approximately 12 % of its agricultural soils are classified as saline (> 4 dS m^{-1})) (Cortés-Jimenez *et al*., 2009; Pulido-Madrigal *et al*., 2010). Thus, it has been reported that wheat yield is reduced by ~ 65 % under moderately saline soils (Shafi *et al*., 2010), at a rate of 7 % per 1 dS m^{-1} in increment (Wang *et al*., 2015). Soil salinity affects almost all aspects of plant development including germination, vegetative growth, and yield (Foolad, 2004), due to hyperosmotic stress that causes a build-up of salt in roots, which decreases their ability to uptake water and generates oxidative stress (Annunziata *et al*., 2017).

Salinity also regulates the abundance, diversity, and function of soil microbial communities, due to a negative osmotic potential in microbial cells that generates plasmolysis and loss of cellular activity (Wang *et al*., 2017), as well as specific ion toxicity (Ibekwe *et al*., 2017). This microbial unbalance in agroecosystems leads to high economic and environmental costs due to a reduction in crop yield, fertilizer volatilization, generation of greenhouse gases, and contamination of groundwater (Cortés-Jimenez *et al*., 2009). Therefore, microbiota represent a sustainable alternative contributing to food security since these microorganisms carry out several vital ecosystemic services, such as i) social and ecological sustainability, ii) adaptation and mitigation of climate change, iii) biotechnological resources for humanity, iv) biogeochemical cycles, and v) increase in agricultural production (de los Santos-Villalobos *et al*., 2018b).

Microbial communities can interact with crops (Valenzuela-Aragón *et al*., 2019), regulating their growth and productivity through different mechanisms such as phytohormone production, fixation of atmospheric nitrogen, siderophore production, mineral nutrients solubilization, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, excretion of antibiotics, toxins, lytic enzymes, and induced systemic resistance (Orhan, 2016; Díaz-Rodríguez *et al*., 2019; Robles-Montoya *et al*., 2019; Villa-Rodríguez *et al*., 2019; Rojas-Padilla *et al*., 2020), which are named Plant Growth-Promoting Microorganisms (PGPM) (Valenzuela-Aragón *et al*., 2019; Valenzuela-Ruiz *et al*., 2019). Under salinity conditions, the protective effect of PGPM consists of reducing the production of ethylene, increasing the concentrations of phytohormones such as abscisic acid, auxins, gibberellins, and cytokinins, giving protection against reactive oxygen species (ROS), producing compatible solutes, solubilize phosphates, produce

exopolysaccharides and control phytopathogens (Numan *et al*., 2018; Egamberdieva *et al*., 2019).

Currently, PGPM has been used to mitigate the effect of soil salinity on crops (Qin *et al*., 2016; Sharma *et al*., 2016). For example, Upadhyay and Singh (2014) showed that the salinity-tolerant bacteria (*Bacillus pumilus*, *Bacillus aquimaris*, *Bacillus arsinicus*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas mendocina,* and *Arthrobacter* sp.) inoculation to wheat fields increased bulk density (1.32 to 1.55 g cm^{-3}), organic carbon (1.3 to 3.8 %), particulate density (1.42 to 1.6 g cm^{-3}) and water-holding capacity (8.2 to 12 %), while sodium content (33 to 10 ppm), electrical conductivity (4.8 to 1.6 dS m^{-1}) and pH (8.1 to 6.8) decreased. Furthermore, the inoculation of plant growth-promoting bacteria (*Bacillus*, *Halobacillus*, *Thalassobacillus*, *Oceanobacillus*, *Halomonas*, *Staphylococcus*, and *Zhihengliuella*) to wheat under salt stress (200 mM NaCl) significantly increased (compared to the un-inoculated treatment) plant height and root length, as well as the total weight of plants as compared to the un-inoculated plants (Orhan, 2016). This work aimed to assess the cultivable microbial (bacteria and fungi) diversity having plant growth-promoting attributes associated with wheat rhizosphere in commercial fields, under a saline soil gradient in the Yaqui Valley, Mexico. This study provides the first insight, from the beginning of the Yaqui Valley (since the 1930s), into the salt-tolerant plant growth-promoting microorganisms associated with wheat and their potential role as a saline stress-mitigating agent in this crop.

MATERIALS AND METHODS

Sampling and nutritional analysis

Seven wheat commercial zones in the Yaqui Valley, Mexico (26° 45′ - 27° 33′ N latitude and 109° 30′- 110° 37′ W longitude) were analyzed in this study. For 10 years, all studied agricultural zones received a similar amount and source of synthetic fertilizers according to wheat nutritional requirements (N, 263 kg ha^{-1}; P, 120 kg ha^{-1}; and K, 60 kg ha^{-1}). Additionally, all studied zones had the same conventional tillage (subsoiling, fallow, leveling), irrigation (4 times, 13 cm irrigation per time), and planting density (180 kg ha^{-1}) (Cortés-Jimenez *et al*., 2009). The soil samples were collected (30 cm depth) according to SAGARPA (2011) and SENASICA (2015), using a "zig-zag" sampling method, obtaining three composite (21 individual samples) soil samples for each wheat commercial zones. Samples collected for physical and chemical analyses were transferred to paper bags to dry (60 °C), and then analyzed according to NOM-021-SEMARNAT-2000; while those collected for microbiological analysis were placed into moist chambers and transported in a cooler at 4 °C (de los Santos-Villalobos *et al*., 2013).

Microbial isolation

The isolation of microorganisms was carried out by dependent culture techniques, where 10.0 g of each soil sample was placed in a 250 mL Erlenmeyer flask containing

90 mL of sterile (121 °C and 15 psi for 15 min) distilled water and homogenized in a rotatory shaker for 1 h at 150 rpm. Serial dilutions (1:10) were prepared until 10^{-4} for bacteria and 10^{-3} for fungi isolation. Then, 100 µL of each serial dilution was poured on Petri dishes containing Nutrient Agar (NA) supplemented with 80 ppm of terbinafine for bacteria isolation, Potato Dextrose Agar (PDA) supplemented with 80 ppm of nalidixic acid for fungi isolation, and incubated for 3 days at 28 °C. Colony Forming Units (CFU) were used to estimate the microbial population in each wheat commercial zones (Córdova-Bautista *et al*., 2009; Villa-Rodríguez *et al*., 2016).

All bacterial isolates were preserved in Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA) (www.itson.mx/COLMENA) (de los Santos-Villalobos *et al*., 2018b; de los Santos-Villalobos *et al*., 2021).

Taxonomic affiliation of isolated microorganisms

Genomic DNA from each obtained isolate was extracted according to Valenzuela-Aragon *et al*. (2019). The 16S rRNA gene was used to affiliate bacterial strains (primers FD1 (5′-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′) and RD1 (5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3′), and the 5.8S rRNA gene for fungal strains (primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)) (de los Santos-Villalobos *et al*., 2013). Ribosomal genes amplification was carried out by using a 50 µL Polymerase Chain Reaction (PCR) mixture, containing 100 ng genomic DNA as a template, 0.2 µmol of forward and reverse primers, and 4 U MyTaq DNA polymerase. The PCR condition consisted of a denaturation step at 94 °C (3 min), 35 cycles of denaturation at 94°C (30 s), followed by annealing at 55 °C (30 s), and extension at 72 °C (1 min), and a final extension step at 72 °C (10 min). The amplicons were verified by electrophoresis on agarose (2 %) gel and sequenced by the Sanger platform. The obtained sequences were edited by using FINCH TV software (Geospiza, Inc., Seattle, WA, USA; http://www.geospiza.com), and analyzed by the NCBI BLASTn tool (Altschul *et al*., 1990), considering the lowest Expect value (E value), and the highest identity percentage (Max ident). The DNA sequences were deposited in the GenBank database and accession numbers were assigned (Table 1).

Table 1. Taxonomic assignment of the 78 microorganisms isolated from the seven ha-wheat commercial zones in the Yaqui Valley, Mexico.

Strain	NCBI accession number	Genus	Species	Reference strain accession number	Query cover (%)	Identity (% sequence length)
TRQ15A	OM047158	*Bacillus*	*thuringiensis*	FJ377887.1	94 %	97.35 %
TRQ15	MK493677	*Bacillus*	*megaterium*	MN626631.1	97 %	99.42 %
TRQ16	OM047159	*Cupriavidus*	*alkaliphilus*	MT935666.1	99 %	99.60 %
TRQ17	OM047160	*Stenotrophomonas*	*indicatrix*	MW303508.1	100 %	98.97 %
TRQ18	MK493678	*Sphingomonas*	sp.	MZ960296.1	100 %	99.77 %
TRQ19	MK493679	*Brevundimonas*	*vesicularis*	MK248082.1	100 %	99.88 %

Table 1. Continue

Strain	NCBI accession number	Genus	Species	Reference strain accession number	Query cover (%)	Identity (% sequence length)
TRQ20A	OM047161	*Pseudomonas*	*putida*	CP050951.1	91 %	99.39 %
TRQ21	OM047162	*Streptomyces*	*galilaeus*	CP023703.1	100 %	100 %
TRQ22	OM047163	*Streptomyces*	*galilaeus*	CP023703.1	100 %	100 %
TRQ61	MK493703	*Cupriavidus*	*alkaliphilus*	MT935666.1	100 %	96.37 %
TRQ62A	OM047164	*Bacillus*	*megaterium*	MT525296.1	100 %	100 %
TRQ63	OM047165	*Bacillus*	*thuringiensis*	CP050183.1	100 %	100 %
TRQ64	MK493705	*Cupriavidus*	*taiwanensis*	LT991977.1	100 %	98.69 %
TRQ65	MN587961	*Bacillus*	*paralicheniformis*	MN396257.1	100 %	90.91 %
TRQ66A	ON103400	*Bacillus*	*paramycoides*	MT875316.1	100 %	99.77 %
TRQ67A	OM047166	*Pseudomonas*	sp.	DQ977702.1	100 %	97.35 %
TRQ24	MK493680	*Microbacterium*	*arborescens*	JN644505.1	99 %	99.20 %
TRQ25	MK493681	*Acinetobacter*	*lactucae*	CP053391.1	99 %	99.61 %
TRM13	MK493715	*Delftia*	sp.	MT101746.1	100 %	100 %
TRQ27A	OM047167	*Stenotrophomonas*	*maltophilia*	KP241046.1	100 %	99.88 %
TRQ28A	MN133867	*Bacillus*	*megaterium*	MH168997.1	100 %	99.76 %
TRQ29	MK493682	*Delftia*	*tsuruhatensis*	MT271888.1	100 %	100 %
TRQ30A	OM047168	*Curtobacterium*	*pusillum*	MT487611.1	100 %	98.18 %
TRQ69	ON011078	*Bacillus*	*thuringiensis*	CP020754.1	100 %	88.20 %
TRQ70	MK493708	*Paenibacillus*	*lautus*	LC588571.1	99 %	96.71 %
TRQ71	MK493709	*Microbacterium*	*oxydans*	JQ660100.1	100 %	99.01 %
TRQ72	OM047169	*Stenotrophomonas*	sp.	MG818736.1	100 %	99.91 %
TRQ73	MK493710	*Bordetella*	*hinzii*	LC521276.1	84 %	98.22 %
TRQ74	MK493711	*Staphylococcus*	*sciuri*	MK015796.1	100 %	94.12 %
TRQ32	MK493683	*Bacillus*	*sonorensis*	JX237852.1	99 %	99.89 %
TRQ33	OM967032	*Cupriavidus*	*taiwanensis*	LT984801.1	96 %	98.63 %
TRQ34	MK493685	*Pseudomonas*	*putida*	MT271890.1	97 %	95.88 %
TRQ35A	OM047170	*Pseudomonas*	*putida*	MT271890.1	100 %	99.69 %
TRQ36	MK493686	*Bacillus*	*licheniformis*	KR909301.1	99 %	99.26 %
TRQ37A	OM047171	*Cupriavidus*	*neocaledonicus*	LT984806.1	87 %	94.10 %
TRQ38	MK493687	*Stenotrophomonas*	*maltophilia*	LC106036.1	99 %	97.93 %
TRQ39	MK493688	*Stenotrophomonas*	*maltophilia*	MK841317.1	99 %	99.49 %
TRQ76A	OM047172	*Acinetobacter*	*calcoaceticus*	MT197389.1	99 %	99.50 %
TRQ40	OM047173	*Microbacterium*	*paraoxydans*	MT279337.1	100 %	100 %
TRQ41	OM047174	*Achromobacter*	*xylosoxidans*	MK855127.1	100 %	99.89 %
TRQ42	OM047175	*Lysinibacillus*	sp.	MH683160.1	100 %	99.49 %
TRQ43	MK493689	*Achromobacter*	*xylosoxidans*	MK855127.1	100 %	100 %
TRQ44	MK493690	*Rhizobium*	*pusense*	CP053857.1	100 %	99.89 %
TRQ46	MK493691	*Stenotrophomonas*	*maltophilia*	KJ499779.1	98 %	94.91 %
TRQ47	MK493692	*Bacillus*	*subtilis*	JX126864.1	99 %	95.12 %
TRQ77	MK493713	*Stenotrophomonas*	*maltophilia*	MN209840.1	100 %	88.94 %
TRQ78	OM047176	*Achromobacter*	*xylosoxidans*	MK855127.1	100 %	99.78 %
TRQ48	MK493693	*Bacillus*	*subtilis*	JN555584.1	100 %	100 %
TRQ49	MK493754	*Bacillus*	*cereus*	HQ684014.1	99 %	99.87 %
TRQ51	MK493694	*Pseudomonas*	*putida*	CP045551.1	100 %	99.56 %
TRQ52	MK493695	*Pseudomonas*	*frederiksbergensis*	MN865449.1	100 %	99.89 %
TRQ90	OM047177	*Pseudomonas*	*chlororaphis*	KP784660.1	99 %	99.72 %
TRQ55A	MK493697	*Achromobacter*	sp.	LC133693.2	100 %	99.86 %

Table 1. Continue

Strain	NCBI accession number	Genus	Species	Reference strain accession number	Query cover (%)	Identity (% sequence length)
TRQ56	MK493698	*Bacillus*	*cereus*	JQ659737.1	99 %	98.73 %
TRQ57A	MK493699	*Pseudomonas*	*extremorientalis*	MT348509.1	99 %	99.77 %
TRQ58	MK493700	*Stenotrophomonas*	*maltophilia*	MT124564.1	100 %	97.25 %
TRQ59A	MN133852	*Bacillus*	*amyloliquefaciens*	CP054479.1	100 %	100 %
TRQ60	MK493702	*Pseudomonas*	*chlororaphis*	MT540543.1	99 %	98.23 %
TRQ79	OM964575	*Taifanglania*	sp.	KT163397.1	98 %	99.62 %
TRQ81	OM964576	*Rhizopus*	*oryzae*	MT603963.1	100 %	100 %
TRQ82	OM964577	*Rhizopus*	*oryzae*	MW147622.1	78 %	98 %
TRQ83	OM964578	*Aspergillus*	*nidulans*	MH237626.1	99 %	99.07 %
TRQ84	OM964579	*Penicillium*	*citrinum*	MK281570.1	99 %	99.22 %
TRQ85	OM964580	*Trichoderma*	sp.	MK871126.1	100 %	99.62 %
TRQ86	OM964581	*Mortierella*	*alpina*	FJ025187.1	98 %	98.90 %
TRQ87	OM964582	*Penicillium*	*rubidurum*	HQ608058.1	98 %	98.90 %
TRQ88	OM964583	*Albifimbria*	*verrucaria*	MH001947.1	99 %	100 %
TRQ89	OM964584	*Aspergillus*	*tubingensis*	MF135503.1	99 %	99.82 %
TRQ90	OM964585	*Penicillium*	*rubidurum*	HQ608058.1	98 %	99.08 %
TRQ91	OM964586	*Fusarium*	*chlamydosporum*	MK212931.1	100 %	100 %
TRQ92	OM964587	*Aspergillus*	*flavus*	MK742795.1	97 %	99.64 %
TRQ93	OM964588	*Trichoderma*	sp.	MK871126.1	100 %	99.62 %
TRQ94	OM964589	*Aspergillus*	*flavus*	MH345952.1	98 %	98.82 %
TRQ95	OM964590	*Clonostachys*	*rosea*	MG748667.1	100 %	98.81 %
TRQ96	OM964591	*Rhizopus*	*oryzae*	MT603963.1	100 %	99.83 %
TRQ97	OM964592	*Rhizopus*	*oryzae*	MT603964.1	100 %	100 %
TRQ98	OM964593	*Mortierella*	*alpina*	MT453274.1	100 %	99.68 %
TRQ99	OM964594	*Rhizopus*	*oryzae*	MT603963.1	100 %	100 %

Plant growth-promoting traits in isolated microbial strains

Phosphate solubilization

This trait was assayed on Pikovskaya medium supplemented with bromophenol blue ($C_{19}H_{10}Br_4O_5S$), and tricalcium phosphate as an insoluble phosphate source (Pikovskaya, 1948). The composition of the Pikovskaya medium was: 10 g L^{-1} glucose; 5 g L^{-1} $Ca_3(PO_4)_2$; 0.5 g L^{-1} $(NH_4)_2SO_4$; 0.2 g L^{-1} NaCl; 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$; 0.2 g L^{-1} KCl; 0.5 g L^{-1} yeast extract; 0.002 g L^{-1} $MnSO_4 \cdot H_2O$; 0.002 g L^{-1} $FeSO_4 \cdot 7H_2O$; and 15 g L^{-1} Agar. The presence of a white/transparent halo around the inoculated ($1x10^3$ CFU or spores) microbial strain was observed after 7 days of incubation at 28 °C.

Siderophore production

The ability of the studied microorganisms to produce siderophores was quantified as described by de los Santos-Villalobos *et al*. (2012), using the CAS (Chrome Azurol S) medium. This medium was prepared by carefully combining four solutions, having

the following composition. Solution 1: 10 mL 1 mM $FeCl_3$ (dissolved in 1 mM HCl) and 50 mL CAS (1.21 mg mL^{-1}) were added to 40 mL of CTAB (1.82 mg mL^{-1}). Solution 2: 30.24 g PIPES was dissolved in 750 mL salt solution (0.3 g KH_2PO_4, 0.5 g NaCl, and 1 g NH_4Cl), the pH was adjusted to 6.8 with KOH at 50 %, and the volume was adjusted to 800 mL, and then 15 g agar was added. Solution 3: 2 g glucose, 2 g mannitol, 493 mg $MgSO_4$, 11 mg $CaCl_2$, 1.17 mg $MnSO_4$, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4$, 1.2 mg $ZnSO_4$, and 1 mg Na_2MoO_4 were dissolved in 70 mL of water. Solution 4: filtered 30 mL of 10 % casamino acids. The presence of a colored halo around the inoculated ($1x10^3$ CFU or spores) microbial strain was observed after 10 days of incubation at 28° C.

The siderophore production and phosphate solubilization by the studied microbial strains was quantified using the index (Rojas-Padilla *et al*., 2020):

SI or *PI* = H_1 / H_2
SI = solubilization index
PI = production index
H_1 = diameter of halo including the microbial colony (mm)
H_2 = diameter of the microbial colony (mm)

Indole production

Each microbial strain was inoculated at a concentration of $1x10^3$ CFU or spores of in a 250 mL Erlenmeyer flasks containing 90 mL of sterile Nutritive Broth (NB) supplemented with tryptophan (100 mg L^{-1}) at 28 °C for 5 days and 120 rpm. After the incubation period, the microbial culture was centrifuged at 8000 rpm for 10 min, the supernatant was mixed in a 1:2 volume ratio with Salkowsky reagent and incubated for 30 min in the dark at room temperature (Glickmann and Dessaux, 1995). The quantification of indoles produced by microbial strains was analyzed at 540 nm.

Hemolytic blood assay

This assay was carried out using a volume of 10 µL of each microbial strain at $1x10^6$ CFU or spores mL^{-1}, which were inoculated onto Petri dishes containing Columbia Agar supplemented with 5 % Sheep Blood. After 3 days of incubation at 28 °C, hemolytic activity was categorized as reported by Villa-Rodríguez *et al*. (2019). Partial or α-hemolysis was represented by a color change to dark green, while β-hemolysis was observed by a clear zone around the microbial colony (indicating erythrocyte breakage), and non-alteration over the medium (γ-hemolysis) indicated no damage to erythrocytes.

Statistical analysis

Data (n = 5) were analyzed by one-way analyses of variance (ANOVA) test, and the Tukey-Kramer test ($p \leq 0.05$), using the Statgraphics Plus software v5.1.

RESULTS AND DISCUSSION

Texture, soil organic matter, and pH values showed no significant differences among studied wheat commercial zones. The texture observed for soils collected in all studied zones was clay, which has been previously reported in 45 % of soils in the Yaqui Valley (Cortés-Jimenez *et al*., 2009; Verhulst *et al*., 2011). The soil organic matter content ranged from 1.4 % to 1.5 % (with no significant differences), which suggests a medium biological activity in this soil (Bhat *et al*., 2017). The pH value in the study zones was slightly alkaline, ranging from 7.8 to 8.1 (with no significant differences), which has been previously reported for agricultural soil located in arid or semi-arid zones (such as the Yaqui Valley), where the low humidity and the limited washing of salts and carbonates increase the soil pH value (Corrales-Maldonado *et al*., 2014). In addition, the chemical analysis of soils collected in all studied zones showed the following mean (with no significant differences) values (ppm): N: 11.5, P: 12.5, Mg: 1067.5, K: 3433, Na: 540, Fe: 10.3, Mn: 12.2, Zn: 4, Cu: 0.4 and B: 0.5, which are standard nutrients concentrations for soils having intensive agricultural practices. Finally, the soil electrical conductivity showed a significant difference among all studied zones, showing values from 0.9 to 6.4 dS m^{-1}, *i.e.* 0.9 ± 0.1 (zone 1), 1.6 ± 0.3 (zone 2), 2.4 ± 0.2 (zone 3), 3.5 ± 0.4 (zone 4), 4.5 ± 0.1 (zone 5), 5.2 ± 0.1 (zone 6), and 6.4 ± 0.1 1.6 dS m^{-1} (zone 7), which may be related to the land topography, climatic conditions, soil erosion, tillage, irrigation with poor quality water, and the excessive use of synthetic agro-inputs (Yan *et al*. 2015; Medina-García, 2016).

High electrical conductivities negatively affect the physical and chemical properties of soils, as well as their microbial population and diversity (Orhan, 2016), due to an osmotic regulation (Yan *et al*., 2015). Plants interact with ~ $1x10^9$ microbial cells g^{-1} dry soil and $1x10^5$ microbial species g^{-1} dry soil, showing the ability to transform the soil organic matter and nutrients, and regulate plant and phytopathogen growth (Grover *et al*., 2011; Dohrmann *et al*., 2012). Consequently, microorganisms have an important role in agroecosystems, and studying the modulation of those microbial communities by saline soils is a daunting prospect since salt stress represents one of the most common constraints in agriculture (Yan *et al*., 2015). Various PGPM have been reported to mitigate osmotic stress on plants in saline soils (Kumar and Verma, 2018). In order to improve plant growth under salt stress conditions, PGPM have developed diverse mechanisms, including regulation of water flow by changing root architecture, modulation of K+ and Na+ ions uptake, exopolysaccharides production, production of VOC and osmoprotectants synthesis, up-regulating antioxidant defense enzymes, production of ACC deaminase production, and the expression of stress-responsive genes, among others (Gupta *et al*., 2022). For example, the inoculation of *B. paralicheniformis* TRQ65, a salt-tolerant strain, reduces the negative salt effect on wheat, increasing the shoot height by 24.5 % and the root length by 136 % compared to the un-inoculated treatment (Ibarra-Villarreal *et al*., 2021). Besides, some studies have shown that this strain contains genes involved in tolerance to salinity stress conditions, such as OsmY, OpuCC, OpuCB, and OpuCA (Valenzuela-Ruiz *et al*., 2019).

In this work, wheat commercial zones having 1.6 dS m^{-1} showed a fungal population (2.59 x 10^6 CFU g^{-1} dry soil) higher than the other study zones (Figure 1A). In addition, no fungal strains from zones having 3.5, 4.5, and 5.2 dS m^{-1} were isolated (Figure 1A). Soils having 1.6 dS m^{-1} are considered non-saline (very low abiotic stress) (Bronicka *et al*., 2007). The most important metabolic strategy for fungi to tolerate this low level of salinity level is the use of compatible solutes, which generate a balance in the environmental osmotic pressure by the accumulation of organic molecules (glycerol) that help to maintain low intracellular salt concentrations (Perl *et al*., 2017). However, the efficiency of this metabolic strategy depends on the genetic background of each fungal strain. Thus, based on the 5.8S rRNA gene sequencing, the most abundant fungal genera were *Rhizopus*, *Aspergillus*, and *Penicillium* (Figure 1B). *Rhizopus* has a high variability of lifestyles and habitats due to its capacity to use a wide range of carbon sources and tolerance to osmotic and thermal stress (Meussen *et al*., 2012; Kaerger *et al*., 2015). In addition, it has been reported as a biocontrol agent against *Fusarium*, the causal agent of *Fusarium* head blight (FHB) of wheat (Mullenborn *et al*., 2008). On the other hand, *Penicillium* is one of the most common fungi found in extreme (salinity, temperature, water, and pH) environments, where its function in nature is the decomposition of organic materials (Yadav *et al*., 2018). *Aspergillus* is found in spoiled foods, stored grains, nuts, and spices. This genus generally grows at higher temperatures or lower water activities and produces spores that often are more resistant to light and chemicals than *Penicillium* (Pitt and Hocking, 2009).

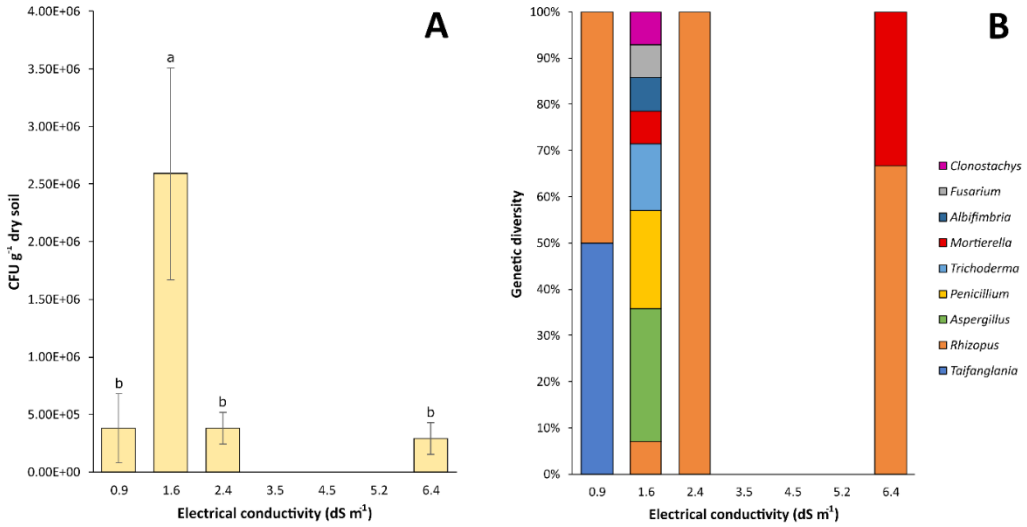

Figure 1. Cultivable fungal communities found in seven wheat commercial zones in the Yaqui Valley, under different levels of soil electrical conductivity. A: Cultivable fungi population; B: genetic diversity of the obtained fungal strains (based on the 5.8S rRNA gene sequencing). Different letters indicate significant difference using the Tukey-Kramer test ($p \leq 0.05$)].

Similarly, wheat commercial zones having 1.6 dS m^{-1} showed a higher bacterial population (2.88 x 10^7 CFU g^{-1} dry soil) than the other zones evaluated (Figure 2A). The most abundant and well-distributed (in all study zones) genus was *Bacillus* (Figure 2B), which has a greater genetic and metabolic background involved in the production of antibiotics, antimicrobial and antifungal metabolites, siderophores, lytic enzymes, toxins, induction of systemic resistance in plants, and adaptability to extreme environmental conditions (Luna-Martínez *et al*., 2013; Villarreal-Delgado *et al*., 2018; de los Santos-Villalobos *et al*., 2019; Robles-Montoya *et al*., 2019; Valenzuela-Ruiz *et al*., 2019; Villa-Rodríguez *et al*., 2019). *Bacillus* is the most widely distributed bacteria in soils by its ability to form spores and tolerate a wide range of environmental conditions (heat, radiation, chemicals, and pH) (Parvathi *et al*., 2009).

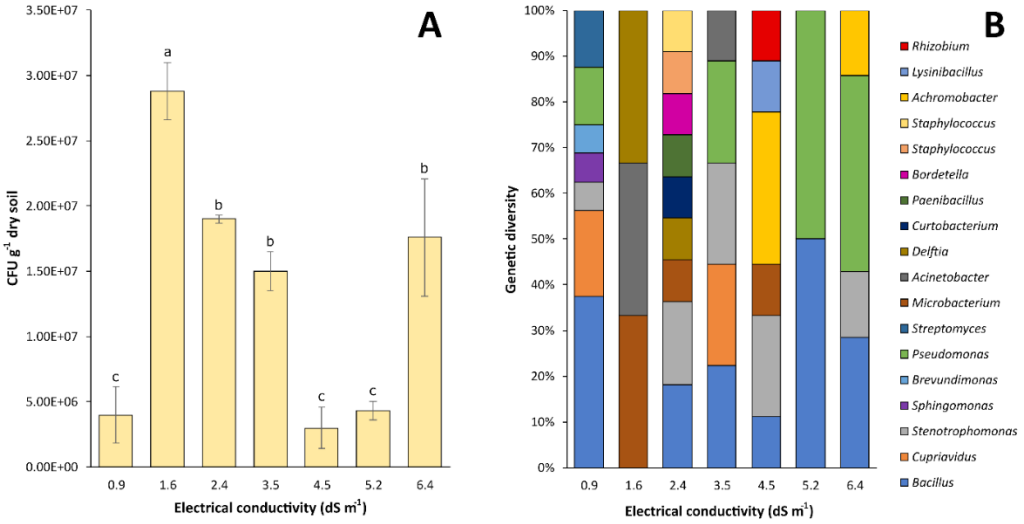

Figure 2. Cultivable bacterial communities in seven wheat commercial zones in the Yaqui Valley, under different levels of soil electrical conductivity. A: Cultivable bacteria population; B: genetic diversity of the obtained bacterial strains (based on the 16S rRNA gene sequencing). Different letters indicate significant difference using the Tukey-Kramer test ($p \leq 0.05$).

In all studied zones the bacterial population was higher than the fungal population (Figure 1A, Figure 2A). Similarly, Yan *et al.* (2015) have reported an increment in the bacteria/fungi ratio in saline soil. The higher abundance of bacteria compared to other microorganisms in saline soils can be explained by their faster growth, and their ability to use a wide range of carbon and nitrogen substrates (Calvo-Vélez *et al*., 2008). In addition, bacteria have several adaptation strategies to combat saline conditions, such as i) the accumulation of solutes (KCl) in the cytoplasm to compensate for the osmotic pressure of the external environment (Ramírez *et al*. 2006), and ii) the salt exclusion from the cytoplasm to synthesize and/or accumulate solutes that do not interfere with the enzymatic activity (Oren, 2008).

To identify promising PGPM, the obtained bacterial and fungal strains were *in vitro* characterized for plant growth-promoting traits, such as phosphate solubilization, siderophore, and indole production. In this study, 36 % of bacterial strains showed the ability to solubilize insoluble phosphates. Such bacteria belong to the genus *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Paenibacillus*, and *Stenotrophomonas* (Table 2). It has been reported that a high diversity of bacterial species can solubilize insoluble inorganic phosphates, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatites, and phosphoric rock (Goldstein, 1986), which have been reported as plant growth-promoting bacteria (El-Azouni, 2008; Ahemad and Kibret, 2014).

Table 2. Metabolic traits of bacterial strains isolated from the study zones in the Yaqui Valley, grouped by soil electrical conductivity.

Electrical conductivity (dS m^{-1})	Strain	Genus	Species	Phosphorus solubilization (PI)*	Siderophores production (SI)*	Indole production (μg mL^{-1})*	Hemolysis
0.9	TRQ15A	*Bacillus*	*thuringiensis*	-- ~	--	14.0 ± 1.7 d	γ
	TRQ15	*Bacillus*	*megaterium*	1.4 ± 0.1 ab	--	9.3 ± 0.9 d	γ
	TRQ16	*Cupriavidus*	*alkaliphilus*	--	--	1.8 ± 0.2 e	β
	TRQ17	*Stenotrophomonas*	*indicatrix*	--	1.1 ± 0.0 a	1.2 ± 0.1 e	γ
	TRQ18	*Sphingomonas*	sp.	--	--	3.0 ± 0.7 e	γ
	TRQ19	*Brevundimonas*	*vesicularis*	--	--	3.6 ± 0.9 e	γ
	TRQ20A	*Pseudomonas*	*putida*	--	--	5.3 ± 0.5 de	α
	TRQ21	*Streptomyces*	*galilaeus*	1.2 ± 0.0 b	--	38.9 ± 7.1 c	α
	TRQ22	*Streptomyces*	*galilaeus*	1.4 ± 0.0 b	--	17.4 ± 2.1 d	γ
	TRQ61	*Cupriavidus*	*alkaliphilus*	--	--	1.5 ± 0.1 e	γ
	TRQ62A	*Bacillus*	*megaterium*	--	--	3.2 ± 0.1 e	γ
	TRQ63	*Bacillus*	*thuringiensis*	--	--	5.6 ± 0.2 e	β
	TRQ64	*Cupriavidus*	*taiwanensis*	--	--	1.5 ± 0.1 e	γ
	TRQ65	*Bacillus*	*paralicheniformis*	1.4 ± 0.1 b	--	39.3 ± 1.7 c	γ
	TRQ66A	*Bacillus*	*paramycoides*	--	1.5 ± 0.1 a	46.1 ± 2.6 ab	γ
	TRQ67A	*Pseudomonas*	sp.	--	1.1 ± 0.1 a	2.7 ± 0.2 e	γ
1.6	TRQ24	*Microbacterium*	*arborescens*	--	--	2.1 ± 0.1 e	β
	TRQ25	*Acinetobacter*	*lactucae*	1.3 ± 0.1 b	--	52.7 ± 2.9 ab	γ
	TRM13	*Delftia*	sp.	--	--	2.1 ± 0.2 e	γ
2.4	TRQ27A	*Stenotrophomonas*	*maltophilia*	1.3 ± 0.1 b	--	6.0 ± 0.2 e	γ
	TRQ28A	*Bacillus*	*megaterium*	1.3 ± 0.0 b	--	2.2 ± 0.1 e	γ
	TRQ29	*Delftia*	*tsuruhatensis*	1.2 ± 0.1 b	1.5 ± 0.0 a	72.1 ± 4.1 a	γ
	TRQ30A	*Curtobacterium*	*pusillum*	1.6 ± 0.0 b	--	2.2 ± 0.2 e	γ
	TRQ69	*Bacillus*	*thuringiensis*	1.2 ± 0.0 b	--	1.5 ± 0.1 e	γ
	TRQ70	*Paenibacillus*	*lautus*	--	1.1 ± 0.0 a	2.1 ± 0.0 e	γ
	TRQ71	*Microbacterium*	*oxydans*	--	--	4.6 ± 0.2 e	γ
	TRQ72	*Stenotrophomonas*	sp.	--	--	8.6 ± 0.1 d	γ
	TRQ73	*Bordetella*	*hinzii*	--	--	1.9 ± 0.0 e	γ
	TRQ74	*Staphylococcus*	*sciuri*	--	--	8.6 ± 0.2 d	γ

Table 2. Continue

Electrical conductivity (dS m^{-1})	Strain	Genus	Species	Phosphorus solubilization (PI)*	Siderophores production (SI)*	Indole production (µg mL^{-1})*	Hemolysis
3.5	TRQ32	*Bacillus*	*sonorensis*	--	--	1.6 ± 0.1 e	γ
	TRQ33	*Cupriavidus*	*taiwanensis*	--	--	1.6 ± 0.1 e	γ
	TRQ34	*Pseudomonas*	*putida*	--	1.1 ± 0.0 a	4.2 ± 0.2 e	γ
	TRQ35A	*Pseudomonas*	*putida*	1.8 ± 0.2 a	--	3.9 ± 0.3 e	γ
	TRQ36	*Bacillus*	*licheniformis*	1.3 ± 0.1 b	--	2.1 ± 0.2 e	γ
	TRQ37A	*Cupriavidus*	*neocaledonicus*	--	--	2.2 ± 0.1 e	γ
	TRQ38	*Stenotrophomonas*	*maltophilia*	--	1.7 ± 0.1 a	3.3 ± 0.0 e	γ
	TRQ39	*Stenotrophomonas*	*maltophilia*	--	--	3.1 ± 0.1 e	γ
	TRQ76A	*Acinetobacter*	*calcoaceticus*	1.5 ± 0.1 ab	--	2.6 ± 0.1 e	γ
4.5	TRQ40	*Microbacterium*	*paraoxydans*	--	--	13.4 ± 1.0 d	γ
	TRQ41	*Achromobacter*	*xylosoxidans*	1.5 ± 0.1 ab	--	3.0 ± 0.0 e	γ
	TRQ42	*Lysinibacillus*	sp.	--	--	12.2 ± 1.7 d	α
	TRQ43	*Achromobacter*	*xylosoxidans*	1.3 ± 0.0 b	--	1.5 ± 0.17 e	γ
	TRQ44	*Rhizobium*	*pusense*	1.3 ± 0.1 b	1.0 ± 0.0 a	5.2 ± 0.5 e	γ
	TRQ46	*Stenotrophomonas*	*maltophilia*	--	--	1.9 ± 0.1 e	γ
	TRQ47	*Bacillus*	*subtilis*	--	--	37.8 ± 1.1 c	γ
	TRQ77	*Stenotrophomonas*	*maltophilia*	1.3 ± 0.1 b	1.6 ± 0.0 a	1.4 ± 0.0 e	γ
	TRQ78	*Achromobacter*	*xylosoxidans*	--	--	5.3 ± 0.2 e	γ
5.2	TRQ48	*Bacillus*	*subtilis*	--	1.8 ± 0.8 a	2.3 ± 0.1 e	γ
	TRQ49	*Bacillus*	*cereus*	1.3 ± 0.1 b	--	3.2 ± 0.0 e	β
	TRQ51	*Pseudomonas*	*putida*	--	--	4.5 ± 0.1 e	α
	TRQ52	*Pseudomonas*	*frederiksbergensis*	1.4 ± 0.0 ab	--	3.7 ± 0.1 e	γ
6.4	TRQ90	*Pseudomonas*	*chlororaphis*	--	1.2 ± 0.0 a	3.1 ± 0.1 e	β
	TRQ55A	*Achromobacter*	sp.	--	--	2.5 ± 0.0 e	γ
	TRQ56	*Bacillus*	*cereus*	--	1.2 ± 0.0 a	48.9 ± 2.5 ab	γ
	TRQ57A	*Pseudomonas*	*extremorientalis*	1.6 ± 0.1 ab	--	2.1 ± 0.1 e	γ
	TRQ58	*Stenotrophomonas*	*maltophilia*	1.3 ± 0.1 b	1.3 ± 0.0 a	7.8 ± 0.2 de	γ
	TRQ59A	*Bacillus*	*amyloliquefaciens*	1.4 ± 0.1 ab	--	2.5 ± 0.3 e	β
	TRQ60	*Pseudomonas*	*chlororaphis*	--	2.3 ± 0.1 a	2.1 ± 0.1 e	γ

*Different letter indicates significant difference using the Tukey-Kramer test ($p \leq 0.05$).
(--): negative test.

Moreover, 23 % of fungal strains were able to solubilize phosphorus, *i.e.*, *Taifanglania, Penicillium, Rhizopus*, and *Aspergillus* (Table 3). These genera have been reported as phosphate-solubilizing dominants in the rhizosphere (Alam *et al*., 2002; Elias *et al*., 2016). The higher phosphate-solubilizing bacterium and fungus were *Pseudomonas putida* TRQ35A (1.83), and *Albifimbria verrucaria* TRQ88 (2.4), respectively. Phosphate solubilization is an important microbial growth promotion mechanism in alkaline soils (Hernández-Leal *et al*., 2011) because phosphorus is insoluble under this condition, but it is involved in several microbial and plant functions, such as respiration, photosynthesis, macromolecular biosynthesis, energy transfer, and signal transduction (Gupta *et al*., 2015).

Table 3. Metabolic traits of fungal strains isolated from the study zones in the Yaqui Valley, grouped by soil electrical conductivity.

Electrical conductivity (dS m^{-1})	Strain	Genus	Species	Phosphorus solubilization (PI)*	Siderophores production (SI)*	Indole production (μg mL^{-1})*	Hemolysis
0.9	TRQ79	*Taifanglania*	sp.	--	--	5.9 ± 1.1 b	γ
	TRQ81	*Rhizopus*	*oryzae*	--	--	3.3 ± 0.5 bc	α
1.6	TRQ82	*Rhizopus*	*oryzae*	--	--	2.8 ± 0.2 c	α
	TRQ83	*Aspergillus*	*nidulans*	1.1 ± 0.0 a	--	2.9 ± 0.0 c	γ
	TRQ84	*Penicillium*	*citrinum*	--	--	4.7 ± 0.9 b	α
	TRQ85	*Trichoderma*	sp.	--	1.2 ± 0.0	3.3 ± 0.3 bc	γ
	TRQ86	*Mortierella*	*alpina*	--	--	3.3 ± 0.4 bc	γ
	TRQ87	*Penicillium*	*rubidurum*	1.2 ± 0.1 a	--	3.5 ± 0.1 bc	γ
	TRQ88	*Albifimbria*	*verrucaria*	2.4 ± 1.3 a	--	6.7 ± 0.9 b	α
	TRQ89	*Aspergillus*	*tubingensis*	--	--	7.0 ± 0.9 b	γ
	TRQ90	*Penicillium*	*rubidurum*	1.3 ± 0.0 a	--	1.5 ± 0.0 c	β
	TRQ91	*Fusarium*	*chlamydosporum*	--	--	4.0 ± 0.1 bc	α
	TRQ92	*Aspergillus*	*flavus*	--	--	4.6 ± 0.3 bc	β
	TRQ93	*Trichoderma*	sp.	--	--	11.0 ± 3.3 a	γ
	TRQ94	*Aspergillus*	*flavus*	--	--	4.6 ± 0.9 bc	γ
	TRQ95	*Clonostachys*	*rosea*	--	--	3.9 ± 0.8 bc	α
2.4	TRQ96	*Rhizopus*	*oryzae*	1.2 ± 0.0 a	--	3.6 ± 0.5 bc	α
6.4	TRQ97	*Rhizopus*	*oryzae*	--	--	8.1 ± 0.9 b	γ
	TRQ98	*Mortierella*	*alpina*	--	--	2.1 ± 0.1 c	γ
	TRQ99	*Rhizopus*	*oryzae*	--	--	3.0 ± 0.0 bc	α

*Different letter indicates significant difference using the Tukey-Kramer test ($p<0.05$).
(--): negative test.

Similar to phosphorus, iron is an essential element for the development of microorganisms and plants, *i.e.*, enzyme cofactor (Gupta *et al*., 2015). Thus, microorganisms produce siderophores -chelating compounds with low molecular weights- that help to promote plant growth and phytopathogen inhibition by iron sequestration (de los Santos-Villalobos *et al*., 2012; de los Santos-Villalobos *et al*., 2018a). The siderophore production was detected in 19 % of the studied bacterial strains, with *Bacillus*, *Pseudomonas*, and *Stenotrophomonas* showing the highest siderophore production; *Pseudomonas chlororaphis* TRQ60 displayed up to 2.3 (Table 2). For fungal strains, only the *Trichoderma* sp. TRQ85 showed the ability to produce siderophores, 1.2 (Table 3).

For indole production, all studied microbial strains were able to produce this phytohormone from 1.2 to 72.1 μg mL^{-1} (using tryptophan as a precursor). *Delftia tsuruhatensis* TRQ29 showed the ability to produce the highest indole concentration, 72.1 μg mL^{-1}, followed by strains of the *Bacillus* genus (~ 50 μg mL^{-1}) (Table 2). Fungal strains showed a low production of this compound, where the highest indole producer

was *Trichoderma* sp. TRQ93, with 11.0 μg mL^{-1} (Table 3). Indoles are the most common plant hormones, which are involved in the regulation of the surface and length of the root; thus, plants inoculated with indole-producing microorganisms have an increased uptake of soil nutrients, cell elongation, cell division, and cell differentiation (Glick, 2012; Kumla *et al*., 2014; Fu *et al*. 2015).

Finally, the hemolysis test helps to know the potential impact of these microbial strains on animal and human health. In this study, 83 % of bacterial strains (Table 2) and 52 % of fungal strains (Table 3) showed the ability to produce γ-hemolysis, which indicates that those microorganisms do not cause any alteration to erythrocytes in the culture medium, and therefore, are not harmful to human and animals. However, the remaining microbial strains showed potential health risks (α- or β-hemolysis), degrading erythrocytes (totally or partially) (Villa-Rodriguez *et al*., 2019). The evolution of microbial hemolysis for the sole purpose of lysing red blood cells *in vivo* to improve growth is highly unlikely. Thus, several specific and detailed assays need to be developed to guarantee that the application of these strains is not a reservoir of human, animal, or plant pathogens or harmful microorganisms, even when they improve soil fertility and food production.

CONCLUSIONS

Salinity negatively impacts the population and diversity of bacteria and fungi in soils from wheat commercial fields in the Yaqui Valley, Mexico. *Bacillus* and *Rhizopus* were the most abundant and well-distributed microbial genera in the gradient of studied saline soils. In addition, the effect of salinity on bacterial and fungal diversity was observed. Their metabolic background was not species- or genus-specific, showing that it was at a strain-specific level. These findings suggest future experimental approaches in the context of important ecological and sustainability issues to be addressed, such as i) the resilience of the Yaqui Valley to saline soils to anticipate the biological soil/environment degradation in terms of functional and/or genetic bacterial diversity; and ii) the development of sustainable alternatives to mitigate the negative impacts of saline soils on wheat yield and quality. Finally, since microbes help plants tolerate salinity, it is important to identify promising plant growth-promoting microorganisms for improving crop productivity under this abiotic stress condition, without a negative impact on animal and human health. Thus, this work provides a microbial culture collection having several beneficial traits that need to be studied in the future for its application to wheat growing under saline soil conditions to increase its yield and quality in the Yaqui Valley (and other agroecosystems having saline soils worldwide) through the use of sustainable agricultural practices.

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