1 Introduction

The Yaqui Valley is located on the northwest coast of Mexico, in Sonora State. Its total area is 225,000 hectares (ha), and it is characterized by a semiarid climate with a mean annual precipitation of 320 mm, a temperature range of 9.6°C (minimum) to 27.3°C (maximum: January-April) and an evapotranspiration of 2000 mm/a (Ahrens et al. 2008). Approximately 49% of the total land area is dedicated to agriculture. The principal crops cultivated in Yaqui Valley are wheat (177,719 ha), maize (13,734 ha), safflower (13,272), chickpea (9,103), and a small area dedicated to spices, such as oregano with an area of 3.4 ha. (Villasenor 2012). Oregano is a versatile plant with a world-wide distribution, the principal species being Origanum vulgare, native to Europe, and Lipia graveolens, native to Mexico.

The global production of oregano is estimated to total 13607.771 tons/year; Mexico is responsible for 20% of the total production, mainly for export, making it the second largest oregano-exporting country after Turkey (Garcia 2012). Between 2006 and 2008, Mexican oregano sales increased to $2 million dollars (CONAFOR 2009). There is increased interest in oregano in Mexico due to its use in the food processing industry and also due to its recently discovered antibacterial, antifungal, antiparasitic, antimicrobial and antioxidant properties (Rivero-Cruz et al. 2011). The principal oregano-producing states in Mexico are: Chihuahua, Durango, Tamaulipas, Coahuila, Jalisco, Zacatecas, Querétaro, Hidalgo and Baja California Sur (Villavicencio et al. 2007). In addition, the Yaqui Valley provides a small percentage of the national production, with oregano being cultivated at a small scale compared to wheat and maize production.

Agricultural practices affect the basis of their own future through soil damage, salinization, excessive water extraction and the reduction of agricultural genetic diversity (FAO 2004). Additionally, abiotic parameters such
as rising temperatures, salinity and pesticide resistance, directly rely on crop production and maintenance. Ashour and Al-Najar (2013) evaluated the effect of temperature increases on olive, palm, grape, citrus and guava, and determined that at a higher temperature, water and soil salinity increased; this could have been associated with a higher demand for irrigation. Furthermore, when salinity is raised in agricultural soils, productivity decreases by approximately 35% (Lavado 2008), and it is estimated that temperatures could increase by about three degrees by 2050 (Rowlands et al. 2012). Fungicide resistance of plant pathogens, on the other hand, can be an important factor in disease management (Fernandez 2003) affecting root colonization and growth (Channabasaya et al. 2015). Due to the growth of the world’s population, food demand is increasing and so is the need to find suitable alternatives for crop fertilization in order to satisfy this demand. One of these alternatives is the use of organic fertilizers that incorporate plant growth promoting bacteria (PGPB), improving plant growth and health (Rodriguez et al. 2006).

Under these conditions, the use of PGPB could be a promising alternative in order to diminish fungicide dependency, reduce environmental impacts and enhance crop production (Garcia 2012). The objective of this study was to estimate microbial diversity in two soil samples, oregano cultivation and native soil, and to evaluate them under three scenarios: temperature, salinity and pesticide resistance, and to determine their potential as PGPB using metabolic tests.

2 Materials and methods

Sampling site: Oregano cultivation and native soil samples were collected at two different sites: a) Cócorit, Sonora (27.57837N, -109.958887W) and b) Corral station in Cajeme, Sonora (27.623670N, -109.965773W), respectively. Following the methodology reported by Ferraris (2016), one sample to a depth of 30 cm was taken at each site and then kept in bags for less than 24 hours before use.

Isolation of bacteria: In order to assess bacterial diversity, isolation of microbial communities was conducted using serial dilutions (1:10) method up to $10^{-6}$. Then 0.1 mL of the $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilutions were inoculated on petri dishes containing nutrient agar (NA). Inoculated petri dishes were incubated for 24 h at 28°C. Then colonies were isolated based on their micro- and macroscopic traits (colony morphology, color, growth and gram stain).

Temperature stress assay: The temperature resistance of strains was determined qualitatively by the growth of strains inoculated into Petri dishes containing nutrient agar as culture medium. Inoculated Petri dishes were incubated at 28°C and 32°C for 24 hours. The assay was performed using two independent replicates.

Salinity resistance assay: The salinity resistance of strains was determined qualitatively by the growth of strains inoculated into Petri dishes containing nutrient agar and supplemented with sodium chloride (30% equivalent to 40.8 dS/m). Inoculated Petri dishes were incubated at 28°C for seven days.

Fungicide resistance assay. The ability of the strains to tolerate the fungicide Chlorothalonil 720 S, was determined qualitatively by the growth of strains inoculated into Petri dishes containing nutrient agar as culture medium and supplemented with 4.3 g L-1 of the fungicide, equivalent to twice the dose commonly used to treat wheat seed (3 L/ton of seed) according to Mexican Official Standard NOM-001-FITO-1995 for the prevention of Karnal bunt in wheat. Inoculated Petri dishes were incubated at 28°C for seven days.

Plant Growth promoting assay-metabolic characterization: In order to determine if the isolated strains exhibit plant growth promoting traits, metabolic characterization was done, including siderophores production, phosphate solubilization and indoleacetic acid (IAA) production.

Siderophores production: Isolated microorganisms were inoculated in Chrome Azurol S (CAS) petri dishes (Schwyn and Neilands 1987) and incubated at 28°C for five days, to observe a change of color from blue to orange in the colonies producing siderophores.

Phosphate solubilization: Microorganisms were inoculated in Pikovskaya (PVK) medium containing tricalcium phosphate (Ca$_3$HPO$_4$) as the sole source of phosphate, incubated at 28°C for seven days (Cherif-Silini et al. 2016). After incubation, the phosphate solubilization was qualitatively analyzed.

Indoleacetic acid (IAA) production: Microorganisms were inoculated in DF medium supplemented with 1 g/L tryptophan, and incubated at 28°C for 72 h. The cultures were centrifuged at 5000 rpm for 20 min; 1mL supernatant was mixed with 2 mL Salkowski reagent (50 mL perchloric acid and 1 mL 35% FeCl$_3$, 0.5 M). The Optical density was measured at 530 nm. Concentrations of IAA were
determined using a calibration curve prepared from an IAA solution in the range 0 to $10^5$ M (Cherif-Silini et al. 2016).

3 Results

The bacterial population was $5.9 \times 10^6$ and $3.8 \times 10^6$ CFU/g dry soil, for the native and oregano soil respectively. A total of twenty-four bacterial and one Actinobacterial strains were isolated (Table 1). Based on the morphological characterization of bacterial strains, 50% were classified as gram-positive, about 50% presented coccoid morphology and 50% bacillary morphology. From the 25 isolated microorganisms, 10 were from the agricultural soil and 15 from the native soil.

After cultivation at two temperatures, strains showed optimal growth at 28°C and 32°C (Table 1); however, at 32°C, the strains grew faster and colony color variation was present in two strains, VNS13 and VNS4 (Figure 1). Just one strain (OSM12) isolated from agricultural soil did not resist the temperature stress.

In this study, eight microorganisms were tolerant to 30% of NaCl (Table 1), four of the microorganisms isolated from agricultural soil, and four from native soil. Only the strains isolated from native soil were able to grow in culture medium supplemented with 5.4 g L Chlorothalonil (Table 1).

In regards to metabolic characterization, only the strain VNS14, isolated from native soil, produced siderophores, however, all native soil strains showed great efficiency in phosphate solubilization. Nonetheless, microorganisms from agricultural soil showed excellent results in both tests, 20% produced siderophores (Figure 2) and 70% solubilized phosphates (Figure 3). Production of IAA was higher for native soil microorganisms, ranging from 40 to 65 ppm, with lower production in agricultural soils (1 to 15 ppm). The strain VNS8, from native soil, produced 65.346 ppm, being the highest concentration of IAA obtained (Table 1).

4 Discussion

The high productivity of the Yaqui Valley is closely associated with intensive agricultural practices and poor water management for irrigation (Matson et al. 2005; Lamz et al. 2013). However, these practices generate a high fertilizer dependency, increased cost of production, soil salinization, the contamination of soil with persistent pesticides and the disruption of soil microbial communities. The latter are potentially associated with
the crop and affect its eco-functional roles such as nutrient cycling, soil organic matter decomposition, and soil formation (Matson et al. 2005). In addition, microbial soil populations from the Yaqui Valley are constantly under stress due to the edaphoclimatic conditions of the region. Because global temperatures are predicted to increase by at least four degrees Celsius by 2100 (Martins 2014), the isolation of microorganisms tolerant to high temperatures is necessary. In this study, a total of 24 strains tolerated an increase of 4°C, and two strains (VNS13 and VNS4) experienced variation in color. The production of dark pigments has been reported to be a strategy for survival in unfavorable environments (Urzì et al. 1991). Ramos and Zuniga (2007) found similar results with microorganisms isolated from lima bean cultivation, at temperatures of 8°C, 21°C, 27.5°C and 37°C, observing that microorganisms grew better at the highest temperatures. The isolation of the thermotolerant strains strengthens their potential to be an excellent source of PGPB to cope with temperature stress in plants.

In Mexico, soil salinization affects 3.2% of its territory. This process is an increasingly evident reality, with one of the main causes being chemical degradation, mainly in arid zones, particularly in irrigated soils where the application of fertilizers and industrial residues have favored salinity (Mata-Fernandez et al. 2014). According to the salinity stress assay, microorganisms with high salinity tolerance were found in soils with different land use in

| Strain | GS  | TA  | ST  | SP  | PS  | FT  | IAAP |
|--------|-----|-----|-----|-----|-----|-----|------|
| OSM1   | -   | NG  | +   |   -| +   |   -| Low  |
| OSM10  | +   | NG  |   -|   -| +   |   -| Low  |
| OSM11  | +   | NG  | +   |   -|   -|   -| Low  |
| OSM12  | -   | LG  |   -|   -|   -|   -| Low  |
| OSM13  | -   | NG  |   -|   -| +   |   -| Low  |
| OSM14  | +   | NG  | +   | +  |   +|   -| Low  |
| OSM15  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM16  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM17  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM18  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM19  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM20  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM21  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM22  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM23  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM24  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM25  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM26  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM27  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM28  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM29  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM30  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM31  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM32  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM33  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM34  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM35  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM36  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM37  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM38  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM39  | +   | NG  | +   | +  | +  |   -| Low  |
| OSM40  | +   | NG  | +   | +  | +  |   -| Low  |

(*) Showed resistance to the assay. (-) Didnt show resistance to the assay. (NG) similar growth at 28°C. (FG) faster growth than at 28°C (LG) didn't resist the stress. OSM Prefix: microorganism isolated from agricultural soil. VNS Prefix: microorganism isolated from native soil. (Low) ≥ 30ppm, (High) < 30ppm. GS (Gram Stain), TA (Temperature Assay), ST (Salinity Test), SP (Siderophore Production), PS (Phosphate Solubilisation), FT (Fungicide Test), IAAP (Indoleacetic Acid Production).
the present study. Similar results were found in Brazil by Bastos et al. (2000) in virgin forest soil and a second location adjacent to the forest area subjected to slash-and-burning activities. The salt-tolerant microorganisms isolated in the present study could become a promising option for their use in bioremediation processes for industrial effluents or contaminated soils based on their co-adaptation.

The composition and activity of soil microbial populations can be affected by biotic and abiotic factors, including anthropogenic activities that modify soil chemical structure and composition, the introduction of organisms, climate and edaphic bearings (Nadal 2016). Although chlorothalonil is not used in the cultivation of oregano, it was evaluated due to the soil had a previous crop rotation applying this chemical fungicide for fungi control. We observed that it is not restricted to fungal control, as it eliminated about 68% of isolated bacteria, showing a significant impact on soil microbial populations. Similar results were found by Xiaqiang et al. (2007), who demonstrated inhibition of microorganisms when Chlorothalonil was added to a 75% concentration of the medium, thus the evaluation of the impact of pesticides on microbial soil, should be the subject of future studies.

Metabolic assays proved that microorganisms collected from different soils had the capacity to solubilize phosphates and produce siderophores, as evaluated in qualitative terms, helping the plant to obtain insoluble nutrients found in the soil. In another study, IAA production was closely associated with an increase in root hair and plant growth in general (Lara Mantilla, 2011). The microorganisms isolated from both soil samples in the present study produced a range of 10.0 to 65.0 ppm of IAA, showing higher concentrations than those presented by Lara et al. (2011), who evaluated the IAA production of isolated genus of Azotobacter sp, Azospirillum sp, and Bacillus sp. In this study the strains OSM1, OSM2, OSM9 and OSM14 produced good responses to stress and to metabolic assays, but the strain OSM14 was the most outstanding of the collection.

5 Conclusion

The results presented in this study showed that native soil microorganisms produced better results in fungicide tolerance and IAA production assays than those from agricultural soil; however in regards to temperature stress, salinity resistance, siderophores production and phosphate solubilization, agricultural soil microorganisms performed better. It was demonstrated that microorganisms isolated from agricultural soil responded better to abiotic stress and metabolic assays, promising to be great potential growth-promoting bacteria. In this study the strains OSM1, OSM2, OSM9 and VNS6 produced good responses to stress and to metabolic assays, but the strain OSM14 was the most outstanding of the collection.

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