JOURNAL OF PURE AND APPLIED MICROBIOLOGY, August 2014. Vol. **8**(4), p. 2591-2594

Phytohormones Production and Phosphate Solubilization Capacities of *Acinetobacter* sp., Strains Isolation from Mexicali Valley

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(Received: 02 May 2014; accepted: 24 June 2014)

Studies about indigenous microorganism's diversity in extreme environments in *Baja california*, mainly Mexicali valley are scarce. Therefore, in the present study the objective was investigated the phytohormones production and phosphate solubilization capacities of four *Acinetobacter* strain, *ICA*01, *ICA*02Ba, *ICA*03Bs and *ICA*04Ma, isolated from *Prosopis glandulosa* rhizospere) in Mexicali valley. The result showed that the maximum P solubilization was recorded by the *ICA04Ma* (11.03 mg/L) with a maximum drop in the pH to 4.3. Followed by *the ICA*03*Bs* (9.28 mg/l), *ICA*02*Ba* (8.24 mg/L) and *ICA*01 (6.5 mg/L) with a maximum drop in the pH to 6.3, 5.7 and 6.7, respectively. On the other hand, the IAA production was significantly higher (Pd"0.05) in *A.calcoaceticus ICA02Ba* (46μg/mL) compared with the others strains that no showed changes significant. The production of gibberelins by *A. calcoaceticus* strains in liquid medium showed that *ICA02Ba* produced significantly amount of GA_3 (4.3 mgL^{-1}), followed by the *ICA*04*Ma* (3.2 mgL^{-1}), *ICA*01 (3.6 mgL^{-1}mg/L) and *ICA*03*Bs* Mc *strains* (3.6 mgL^{-1}) after 72 h of incubation. Finally, *A. calcoaceticus* strains could be used for further field trials to improve agricultural productivity under arid conditions.

Key words: Mexicali valley, Mesquite, Microorganism, Phytohormones, Phosphorus.

The Mexicali Valley (semi-arid area) is an important agricultural region in the northwest of Mexico. The soils in this zone are characterized for the presence of low available water capacity and organic carbon content, vulnerability to wind erosion, and high soluble salts accumulation. On the other hand, the presence of temperature up to 50ºC with average rainfall less than 200 mm, make conditions more severe for the life forms present in these ambient. These situations cause negative effects in the absorption of nutrients (*eg*., phosphorus) by the plants (Munns and Tester, 2008). Phosphorous (P) is an essential nutrient used to build cell molecules like phospholipids, nucleic acids and it is involved in energy transformation (ATP). In the plants the P is acquired across of soil solution as phosphate anions. Nevertheless phosphate anions are particularly reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the pH of soil (Bhattacharya *et al*., 2013). On the other hand, the presence of microorganism in the rhizosphere can significantly influence the nutrient supply of plants by competing for mineral nutrients and by mediating the turnover and mineralization of organic compounds (Oliveira *et al*., 2009). Therefore, the use of rhizospheric microorganisms

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represents an option economically and environmentally friendly for to enhance solubilization of phosphate in the arid and semi-arid area and to provide sufficient quantities for plant nutrition. The P-solubilizing microorganisms (PSM) can solubilize and mineralize P from inorganic and organic pools of total soil P, and may be used as inoculants to increase P-availability to plants (Richardson, 2001). The indigenous microorganisms capable of phosphorus solubilization have a superior adaptability to the environment than the introduced strains. In this sense, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superiour adaptability to the environment (Vassilev *et al*., 2006).

Several Phosphate solubilizing bacteria have been studied extensively (Collavino *et al*., 2010; Charana and Yoon, 2013). Nevertheless, studies about indigenous microorganism's diversity in extreme environments in *Baja California*, mainly Mexicali valley are scarce. Therefore, in the present study the objective was investigated the phytohormones production and phosphate solubilization capacities of *Acinetobacter* strains isolation from mesquite tree (*Prosopis glandulosa*) rhizospere in Mexicali valley.

MATERIALS AND METHODS

The experiments were carried out using four *Acinetobacter* strain: *ICA*01, *ICA*02Ba, *ICA*03Bs and *ICA*04Ma, previously deposited in *GenBank* with the accession numbers: KJ190162, KJ190163, KJ190164 and KJ190165, respectively.

Estimation of phosphate solubilization and pH

The phosphate solubilizing potential and pH variations of four strains isolates in liquid medium were estimated in 100 ml PVK (Pikovskaya's agar medium) broth containing 0.5%, containing insoluble Tricalcium phosphate (TCP), adjusted to 7.0, containing 1 mL aliquot of each strain, respectively (OD_{600} of 2.0) at 26±2°C for 3 days with agitation (130 rev min^{-1}). After incubation period, 5.0 ml of each strain were taken, centrifuged, and pH of the supernatant was recorded with a pH meter equipped with glass electrode.Soluble phosphate was quantified using the QuantiChrom phosphate assay kit (BioAssay Systems, Hayward CA). The optical density was read at 620 nm on a spectrophotometer (Genesys 10S) Thermo Scientific to determine the intensity of the coloured complex that is formed by the Malachite green method (Ekman and Jager, 1983).

Determination of auxins produced by *A. calcoaceticus* strains

The amounts of Indole acetic acid (IAA) produced by each *Acinetobacter* strain, was evaluated using the Salkowski's method (Mohite, 2013). Each strain ($3x10^9$ CFU mL^{-1}) were grown in 10 mL of nutrient broth, supplemented with L-tryptophan (100 mg mL^{-1}) and incubated at 26±2°C for 24 days. Thereafter, the cultures of each strain were centrifuged at 6000 x *g* for 10 min. After one milliliter of the supernatant was mixed with 2 ml of Salkowski reagent (50 mL, 35% perchloric acid; 1 mL of 0.5 molar $FeCl_3$).Then it was kept for 60 min in dark room temperature and the light absorption measured in 540 nanometer of wave length by spectrophotometer device (Biomate 3 Thermo Scientific). Indole acetic acid (IAA) concentration was estimated by preparing calibration curve using indole acetic acid (IAA, Spectrum) as standard (0-100 $mgml^{-1}$). Each experiment was performed in triplicates and the mean values were considered.

Determination of gibberellins produced by *A. calcoaceticus* strains

All strains ($3x10^9$ CFU mL^{-1}) were grown in 25 mL of nutrient broth for triplicate at 26°C in the dark with shaking for 24 h. Culture supernatants were recovered after centrifugation at 6,000 × *g* for 10 min. Fifteen milliliter of supernatant was mixed with 2 ml of zinc-acetate reagent (21.9 g of zinc acetate in 80 ml of *distilled water + 1 ml of glacial acetic acid and volume* was *made up to 100 ml with distilled water*) *was added*. After 2 minutes 2 ml of potassium ferrocyanide (10.6% in distilled water) was added and was centrifuged at low speed (2000 rpm) for 15 minutes. To 5 ml of supernatant 5 ml of 30% HCI was added and mixture was incubated at 20°C for 75 min in dark room temperature. Absorbance was read at 254 nm using a Biomate 3 (Thermo Scientific) UV/VIS spectrophotometer (blank 5 ml of 5% HCI was used). Gibberellins (GA) Concentration was estimated by preparing calibration curve using gibberellic acid (GA_3, Hi-Media) as standard(100-1000 µg mL^{-1}).

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed

by Duncan's multiple range test (DMRT) using the STATISTICA software package. The values are mean±*SD* for three samples in each group. *P* d"0.05 were considered as significant.

RESULTS AND DISCUSSION

Estimation of phosphate solubilization and pH

The capacities of phosphate solubilization by plant-associated microorganism species have been reported in diverse studies (Chen *et al*., 2006; Ahuja *et al*., 2007). Nevertheless, very few studies have reported strains of *Acinetobacter* as phosphate solubilizing plant-associated bacteria (Bottini *et al*., 2004; Fan *et al*., 2011). In the present study our result showed that the solubilization of TCP in the liquid medium by the four *A.calcoaceticus* strains was accompanied by a significant drop in pH after 72 h (Figure 1). The maximum P solubilization was recorded by the *ICA04Ma* (11.03 mg/L) with a maximum drop in the pH to 4.3. Followed by *the ICA*03*Bs* (9.28 mg/l), *ICA*02*Ba* (8.24 mg/L) and *ICA*01 (6.5 mg/L) with a maximum drop in the pH to 6.3, 5.7 and 6.7, respectively. The decreased of pH suggested the production of low molecular weight organic acids and major efficient process of phosphate solubilization by *Acinetobacter* strains. In this sense, our results is similar to reported for Kang *et al*. (2009) that evaluated the solubilization potential of *Acinetobacter* sp (isolate SE370) and concluded that these microorganism can promote the plant development by converting insoluble soil P to soluble form which are readily available to the roots system.

Determination of auxins and gibberellins produced by *A. calcoaceticus* strains

On the other hand, in the present study the phytohormones production by the *A. calcoaceticus* strains showed that all differed in their capacity to produce IAA and GA_3 (Figure 2). After 72 h of growth, the IAA production was significantly higher (Pd"0.05) in *A.calcoaceticus ICA02Ba* (46µg/mL) compared with the others strains that no showed changes significant in IAA concentrations. On the other hand, the production of gibberelins by *A.calcoaceticus* strains in liquid medium showed that *ICA02Ba* produced significantly amount of GA_3 (4.3 mgL^{-1}), followed by the *ICA*04*Ma* (3.2 $mg\ L^{-1}$), *ICA*01 (3.6 $mg\ L^{-1}$mg/ L) and *ICA*03*Bs* Mc *strains* (3.6 $mg\ L^{-1}$) after 72 h of incubation. The production of IAA and gibberellins has been confirmed in numerous bacteria genera (Thuler *et al*., 2003; Bottini *et al*., 2004).

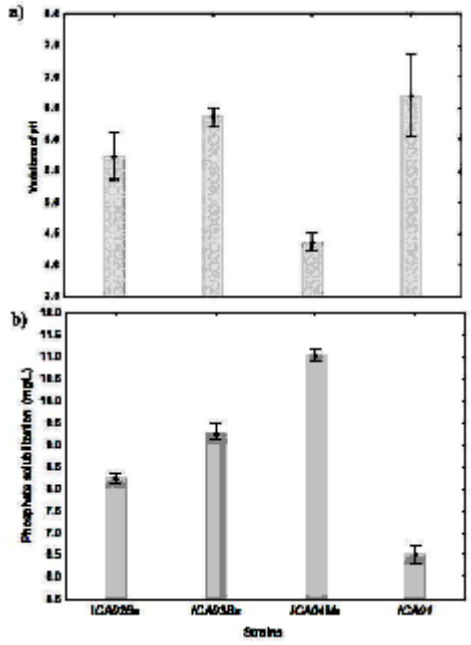

Fig. 1. Change in the solubilization of TCP and pH in the liquid medium by four *Acinetobacter* sp., strains isolation from Mexicali valley after 72 h of incubation

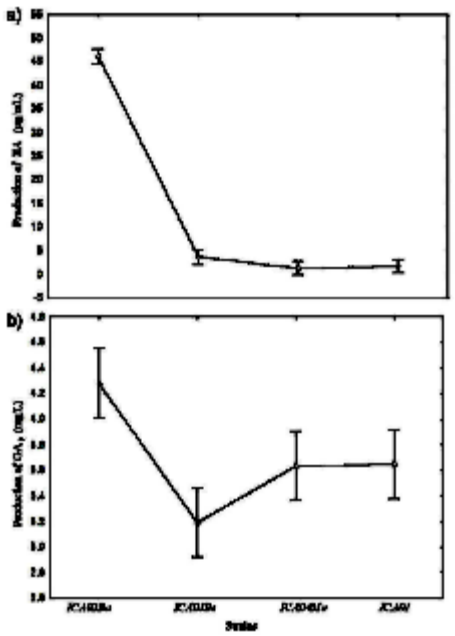

Fig. 2. Production of IAA (a) and gibberellin (b) by four *Acinetobacter* sp., strains isolation from Mexicali valley after 72 h of incubation

Nevertheless, the evaluation of auxins and gibberellins production by microorganisms has been evaluated by independent ways (Suzuki and Oyaizu,2003; Kang *et al*., 2009). In this sense, the presence of IAA and GA_3 in *A.calcoaceticus* strains suggests the existence of IAA and GA biosynthesis pathway. It is in accordance with result of UmaMaheswari *et al*. (2013) who reported the production of GA, IAA and cytokinin by strains of bacterial endophytes.

CONCLUSION

Finally, the four strains evaluated in the present study were show to produce IAA and GA which can affect plant growth. Therefore, further investigation can be made to evaluate the effect of *A.calcoaceticus* strains in crop growth.

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