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Effect of *Bacillus* sp. on P uptake in *Vicia sativa*, *Vicia ervilia*, *Medicago sativa* grown in greenhouse conditions

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Abstract. *Bacillus* sp. MK4 isolate was isolated from Şanlıurfa soils, Turkey. MK4 isolate was biochemically characterized. MK4 isolate was evaluated in vitro for plant promoting traits like ammonia (NH_3) production, antifungal activity, phosphate solubilization. In addition, the effect of *Bacillus* sp. MK4 on P uptake by *Vicia sativa, Vicia ervilia* and *Medicago sativa* under greenhouse conditions was studied. Seed inoculation of this bacterial isolate resulted in significant increases in shoot and root weight of *Vicia sativa, Vicia ervilia, Medicago sativa* with or without added P source.

Key words. *Vicia sativa* – *Vicia ervilia* – *Medicago sativa* – *Bacillus* sp. – Inoculation.

Effet de* Bacillus *sp. sur l'absorption de P dans* Vicia sativa, Vicia ervilia *et* Medicago sativa *cultivées dans de conditions de serre

Résumé. Bacillus *sp. MK4 a été isolé à partir de sols de Şanlıurfa, en Turquie. L' isolat MK4 a été caractérisé biochimiquement. MK4 isolat a été évaluée in vitro pour des traits importants pour la promotion des la croissances des plantes, tels que la production d'ammoniac (NH3), l'activité antifongique, et la solubilisation du phosphate. En plus, l'effet de* Bacillus *sp. MK4 sur l'absorption de P par* Vicia sativa*,* Vicia ervilia *et* Medicago sativa*, dans des conditions de serre a été étudiée. L'inoculation des semences avec cet isolat bactérien a entraîné des augmentations significatives dans des pousses et le poids des racines de* Vicia sativa*,* Vicia ervilia *et* Medicago sativa*, avec ou sans source de P ajoutée.*

Mots-clés. Vicia sativa – Vicia ervilia – Medicago sativa – Bacillus *sp.* – *Inoculation.*

I – Introduction

Plant growth bacteria are of great agronomic importance. They produce metabolite such as plant growth regulators that directly promote growth and nutrient uptake by plants (Krey *et al.,* 2011). Solubility of phosphorus is very low in most of the agricultural soil of the world (Kaçar and Katkat, 2008). Application of phosphate fertilizers is therefore essential to increase crop yields. However, availability of phosphorus is a serious problem because it is fixed in the soil (Schiling *et al.,* 1998). The use of phosphate solubilizing microorganisms has been previously tested (Lugtenberg and Kamilova, 2009).

The rhizosphere of cereal crops was found to harbor a great number of phosphate solubilizing bacteria (Krey *et al.,* 2011). The bacterial genus *Bacillus* group plays important roles in the agricultural ecosystems (Lugtenberg and Kamilova, 2009). Specificially it is involved in disease suppression in many crops (Krey *et al.,* 2011; Khalafallah *et al.,* 1982; Kumar and Narula, 1999) and enhanced plant growth and development (Kumar and Norula, 1999; Lugtenberg and Kamilova, 2009). The main objectives of this research were to isolate the *Bacillus* isolates from rhizosphere soil and determine their effect on P uptake of *Vicia sativa, Vicia ervilia* and *Medicago sativa.*

II – Materials and methods

Bacillus sp. B14, B9, B21 and MK4 were isolated from the rhizosphere soil of maize grown in vicinity of Şanlıurfa, Turkey.

NH_3 production: Isolates were tested for the production of ammonia in peptone water. Nessler`s reagent (0.5ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappucino and Sherman, 1992).

Phosphate solubilization: All isolates were tested on Pikovskaya`s agar plates for phosphate solubilization as described by Haripsavad and Niranjana (2006).

Antifungal assay: Bacterial isolates were tested on growth plates on peptone agar. Isolates were streaked on the test plates perpendicular to pathogen fungi. The isolates were tested for antagonistic activity as described by Idriss *et al.,* 2007.

Plant and soil: The soil for pot experiment was collected from a non-fertilized field site near Osmanbey, Şanlıurfa. The soil chemical and physical properties are presented in Table 1.

Table 1. Soil some chemical properties

EC	pH	% CaCO3	P_2O_5 (kg da^{-1})	K_2O (kg da^{-1})	Organic matter %
1.65	7.66	20.8	34.5	207.5	22.1

Total nitrogen content was determined by the Kjeldahl method (Bremner, 1965). Potassium was determined by the Flame photometric method (Riehm,1985). Soil pH volue was measured by pH meter. *Vicia sativa, V.ervilia* and *Medicago sativa* seeds were obtained from the Ege University, Faculty of Agriculture.

Pots experiment: The inoculation treatment was set up in randomized design with four replicates.The day before sowing pots were filled with 1000 g soil. Ten seeds of *V.sativa, V.ervilia* and *Medicago sativa* were sown per pot. Isolates were grown in Nutrient broth. Treatments for the greenhouse experiments were: Untreated control, bacterial isolate and P, P treatment. The crops were harvested 60 days after sowing. Plant parameters studied were P uptake (mg $plant^{-1}$), root and shoot biomass (mg $plant^{-1}$).

III – Results and discussion

B14, B9, B21 and MK4 were identified as *Bacillus* sp. on the basis of morphological and biochemical tests and also on the basis of description given in Bergey`s manual of systematic Bacteriology (Holt *et al*.,1994). B14, B9, B21 and MK4 are Gram positive. They are also motile. MK4 isolate gives positive tests in nitrate and ammonia production. *Fusarium* species are the most destructive pathogen in most agricultural crops in all around the world (Idriss *et al.,* 2007). The antagonistic properties of *Bacillus* isolates against *F.moniliforme*, *F.solani* and *F.oxysporium* were shown in Fig. 1.

The metabolites produced by phytopathogenic fungi have been postulated as responsible for the increased plant growth and nutrient uptake (Haripsavad and Niranjana, 2006; Krey *et al.,* 2011). As *Pseudomonas* sp., *Bacillus* sp. increase the uptake of P, they may also increase the plant growth. In this study, it was observed the production of clear zone in Pikovskaya`s medium obtained in MK4 isolate. And this also indicated that the P uptake was improved by MK4 isolate.

Isolate MK4 inoculation affected the growth of tested plants. The inoculation of *V.sativa* with MK4 increased the shoot dry weights by 50% as compared to the control (Fig. 2). The

inoculation of *V.ervilia* with MK4 increased the root dry weight 66.7% as compared to the control (Fig. 3).

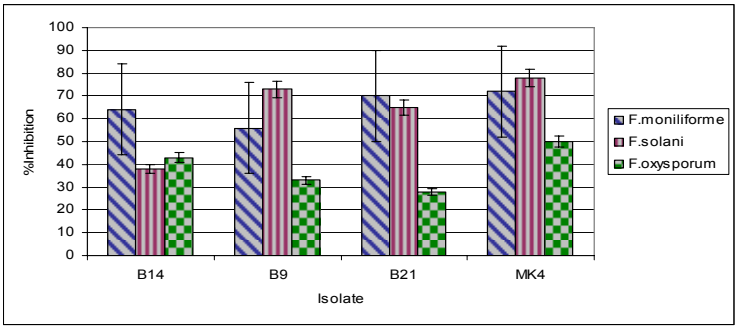

Fig. 1. Inhibition of pathogenic fungi by isolates (%).

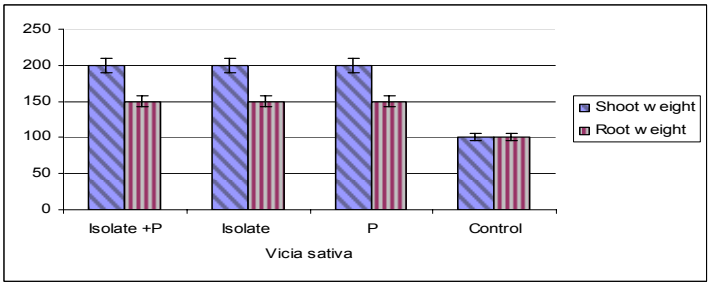

Fig. 2. The influence of isolate MK4 on shoot and root weight (mg plant $^{-1}$) on *Vicia sativa* (p< 0.01).

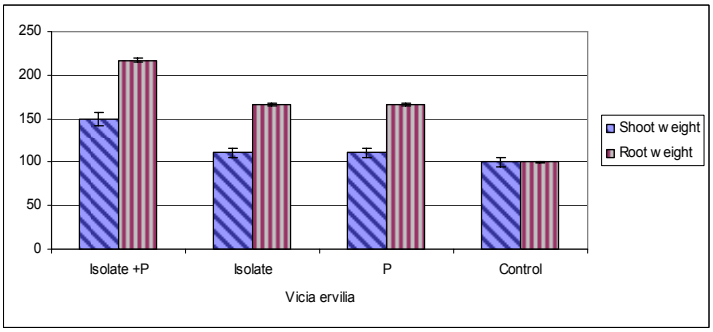

Fig. 3. The influence of isolate MK4 on shoot and root weight (mg plant $^{-1}$) on *Vicia ervilia* (p< 0.01).

Shoot and root growth increased more than control in *V.sativa* and *Medicago sativa* with isolate + P treatment (Fig. 4).

Seed inoculation with various bacterial isolates was reported to improved yield and nutrient uptake of various crops (Linu *et al.*, 2009; Lugtenberg and Kamilova, 2009). The inoculation of

Vicia ervilia, *Vicia sativa* and *Medicago sativa* grown with bacterial isolate MK4 increased the P uptake 58.4 %, 24.5 % and 37.1 %, respectively as compared to the control (Fig. 5). Similar results were reported by Kumar and Narula (1999), Linu *et al.* (2009), Krey *et al.* (2011), *Pseudomonas* sp. and *Bacillus* sp. isolates significantly enhancing plant growth and nutrient uptake.

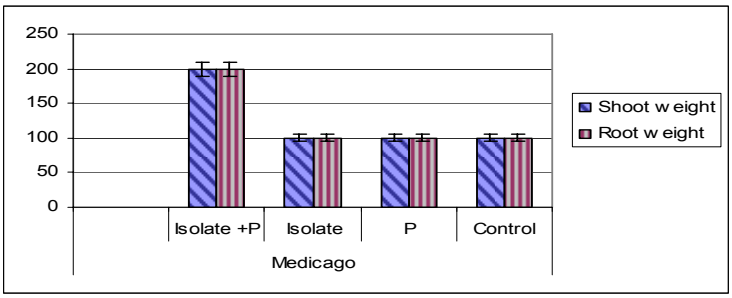

Fig. 4. The influence of isolate MK4 on shoot and root weight (mg plant^{-1}) on Medicago sativa (p< 0.01).

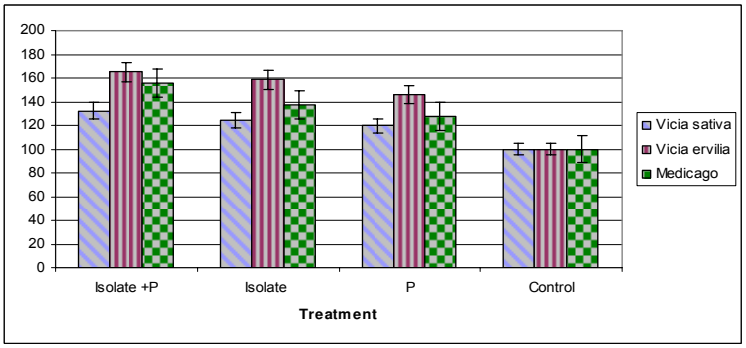

Fig. 5. The influence of isolate MK4 on P uptake (mg plant^{-1}) of *V.sativa*, *V.ervilia* and *Medicago sativa* (p< 0.01).

IV – Conclusion

Four isolates isolated from rhizospheric soil of maize were characterized as *Bacillus* sp. *Bacillus* sp. MK4 isolate showed beneficial effects on *Vicia sativa, V.ervilia* and *Medicago sativa* growth in greenhouse experiment. The favourable effect of the inoculation on plant growth may be due to growth promoting subtances by isolates.

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