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Modes of plant-growth promotion of H_2-oxidizing rhizobacteria

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INTRODUCTION

Rhizobia trigger the formation of N_2-fixing nodules on the roots of legumes. H_2 gas is an energy-rich by-product of symbiotic nitrogen fixation. Dong and Layzell[2] first reported that the H_2 liberated from root nodules can promote the growth of microorganisms in the root zone, as well as plant growth. The study provides information on the plant growth promoting effect of a collection of 10 H_2-oxidizing bacteria isolated from the rhizosphere of lentil growing in semiarid Saskatchewan. We tested the hypothesis that these lentil rhizosphere-competent bacteria can promote plant growth through various mechanisms.

MATERIALS AND METHODS

1) Characterization of plant growth promoting (PGP) trait: We tested 10 H_2-oxidizing bacteria isolated from lentil rhizosphere growing in semiarid Saskatchewan, for phosphate-solubilizing capacity, siderophore production activity, indole acetic acid production, and ACC deaminase activity. The positive controls for the assays were from Dr. Louise Nelson, UBC, Kelowna, BC .

❑ **Screening for phosphate-solubilization capacity : The bacteria were inoculated onto K_2HPO_4-amended glucose-yeast (GY) agar medium, with an insoluble, opaque layer of calcium phosphate (Popavath et al. 2008). The experiment had a completely randomized design with six repetitions. Strain 2-106 was a positive control for comparison. Six replicates of all treatments were incubated for 5 days at 24°C in a completely randomized design (CRD). A zone of clearing around the bacterial colony indicated a positive result.**

❑ **Screening for siderophore production : The bacteria were tested using the universal siderophore assay with Chrome Azurol S (CAS) and hexadecyltrimethyl ammonium bromide as indicators (Schwyn and Neilands 1987). Strain 2-106 was a positive control for comparison. Six replicates of all treatments were incubated for 5 days at 24°C in a CRD. Siderophore production was indicated by the medium color changing from blue to orange.**

❑ **Screening for indole acetic acid (IAA) production : The bacteria were assayed based on the method of Patten and Glick (2002) . The bacteria were inoculated on LB agar medium. Each inoculated plate was overlaid with sterile Whatman no. 1 filter paper. Strain 5-51 was a positive control. Six replicates of all treatments were incubated for 3 days at 24°C in a completely randomized design (CRD). After incubation, the filter paper was removed, and soaked in Salkowski's reagent at room temperature. A red halo indicated a positive result.**

❑ **Screening for ACC deaminase activity : The bacteria were inoculated onto DF minimal salt medium (Dworkin and Foster, 1958) containing ACC as sole nitrogen source. Strain 6-8 was a positive control. Ten replicates of all treatments were incubated for 2 days at 24°C in a CRD. Growth in this medium indicates ACC deaminase activity (Husen et al. 2011).**

2) Assay for growth promotion

❑ **Greenhouse assay on lentil : An experiment was conducted in growth pouches, in the greenhouse, to test the effect of the bacteria on root nodule formation. Ten bacteria plus a negative control were applied on red lentil cultivar CDC Maxim in a CRD with six replicates. The protocol of Maimaiti and Valentine (2007) was used with some modification. After 3 weeks of growth, the number of nodules per plant was recorded.**

➢ **Statistical analysis:** The treatment effects were statistically analyzed by ANOVA in JMP 6, using Tukey's HSD test for means comparisons.

RESULTS

(1)

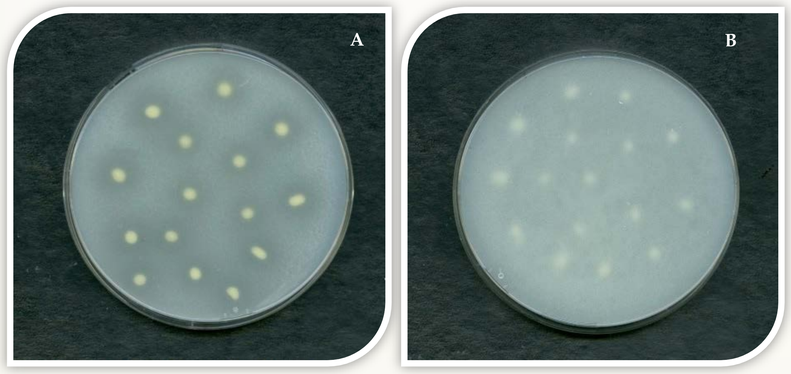

Fig.1 Phosphate solubilizing bacteria cultured in petri dish. The zone of clearance is seen around the colonies with the strain *L1* (A). No clear zone is seen around the colonies of the strain *L11 (B).*

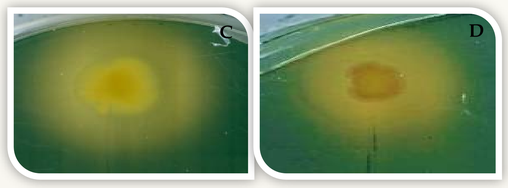

Fig. 2 Orange halos around the colonies of the strain L11 (C) and strain L22 (D) indicate the ability of those strains to produce siderophores.

Table 1 Level of different modes of action of H_2-oxidizing rhizobacterial isolates: phosphate solubilization, siderophore production, IAA and ACC deaminase activity.

Code Isolate	Isolate identity	P Solubilize (mm^2)	Siderophore (mm^2)	IAA	ACC
Control	Strain 2-106 Strain 5-51 Strain 6-8	10.3b	34.15a	+	+
L1	*Variovorax sp.*	13.9a	35.43a	+	-
L4	*Variovorax sp.*	-	25.73b	-	-
L7	*Rhodococcus sp.*	-	26.96b	-	-
L10	*Mycobacterium sp.*	-	23.55b	-	+
L11	*Mycobacterium sp.*	-	33.83a	+	+
L14	*Variovorax sp.*	-	-	-	-
L17	*Variovorax sp.*	-	26.48b	+	+
L20	*Acinetobacter sp.*	-	32.95a	-	+
L21	*Acinetobacter sp.*	-	32.53a	+	+
L22	*Curtobacterium sp.*	-	33.53a	-	+

Symbol (+): positive activity for ACC deaminase and IAA, (-) no activity for ACC deaminase and IAA.

A zone of clearing around the bacterial colony indicated phosphate solubilization. Only one bacterial strain was able to solubilize calcium phosphate (Table 1 and Fig. 1).

Nine bacterial strains produced siderophore, on the basis of change in colour of the CAS medium from blue to orange (Table 1 and Fig. 2).

Four bacterial strains were able to synthesize IAA from tryptophan. They form a red halo on the paper filter surrounding the colony in Salkowski's reagent (Table 1).

Six bacterial strains also showed positive activity for ACC deaminase when they were able to growth on DF minimal medium containing ACC (Table 1).

(2)

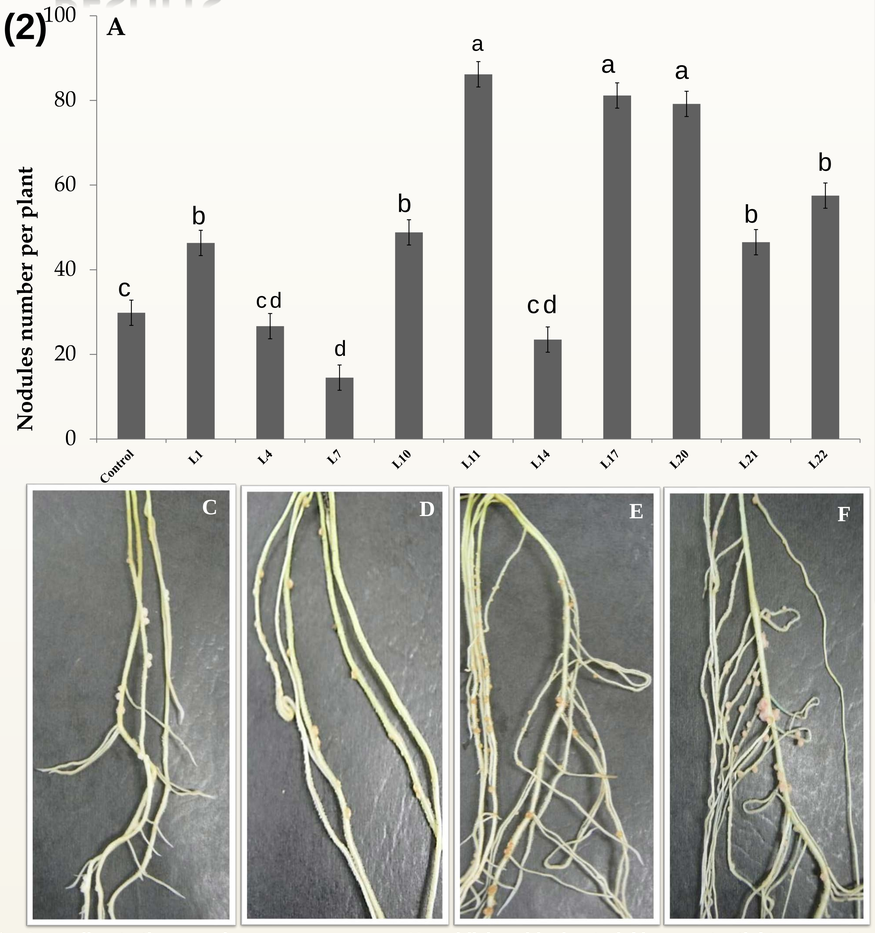

Fig.4 Effect of the ten H_2-oxidizing rhizobacterial isolates on the number of root nodules per lentil plant three weeks after inoculation assay in the greenhouse. (A) Bars with the different letters are significantly different at α = 0.05 (Tukey HSD, *n* = 6). (B) Lentil was inoculated with *Rhizobium leguminosarum* (Rh), and nodulation of the roots of lentil inoculated with (Rh) plus H_2-oxidizing rhizobacterial isolates. (C) Lentil inoculated with *Rhizobium leguminosarum* (Rh), and nodulation of the roots of lentil inoculated with (Rh) plus H_2-oxidizing rhizobacterial isolates (D) *L14*, (E) *L20*, and (F) *L11*.

➢ **According to the greenhouse assays, seven H_2-oxidizing rhizobacterial have potential to promote shoot-root biomass and root nodules number per plant (Fig. 4)**

CONCLUSIONS

The bacterial strains L1, L10, L11, L17, L20, L21 and L22 may have the characteristics to promote plant growth through different mechanisms (P-Solubilize, Siderophore production, IAA and ACC activity) + **Greenhouse trials showed that many H_2-oxidizing rhizobacterial isolates improved the growth of lentil plants, by stimulating root nodulation. The strains L11, L17 and L20 best promoted root nodulation.** = **The effect of some select bacteria to promote plant growth is mainly due to phytohormones and enzymes production.**

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