Hydrogen-Oxidizing Bacteria from Lentil Field

Chao Yang, Chantal Hamel, Yantai Gan

Semiarid Prairie Agricultural Research Centre, AAFC, Swift Current, SK, Canada

Key Words: Hydrogen-Oxidizing, bacteria, lentil

Introduction

Some H2-oxidizing bacteria associated to N2-fixing soybean grown under subhumid climate were shown to possess plant growth promoting (PGP) effects1. In this study, twenty four H2-oxidizing bacteria were isolated from the rhizosphere of fourteen lentil (Lens culinaris) cultivars growing in semiarid Saskatchewan, by using selective-medium based culture, and their hydrogen uptake capabilities were tested by Qubit Open Flow Gas Exchange System. Objectives: (1) Demonstrate the existence of H2-oxidizing bacteria in field-grown lentil under semiarid conditions, and quantify their hydrogen gas uptake capabilities; (2) test the plant growth promoting (PGP) capabilities of positive H2-oxidizing bacteria strains.

Materials & methods

Soil sampling: In August of 2012, rhizosphere soil samples were taken from field plots gorwing 14 lentil varieties in Swift Current, SK. Five plants were dug from each plot using a shovel, bulk soil was shaken off, and rhizosphere soil was carefully brushed down and sieved through 2 mm. All soil samples were pool together and mixed well to yield one soil sample and kept in a sealed plastic bag at -20°C for further analysis.

Incubation and Isolation: The incubation and isolation procedure followed the protocol of Maimaiti[2] with some modification. Rhizosphere soil sample was incubated in air containing 0.3% H_2 gas for a month, then serially diluted (10^{-3} - 10^{-10}) and plated onto mineral salt agar medium (MSA), and incubated under the same gas condition for 3 weeks.

Assessment of H_2 uptake: After incubation, the bacterial colonies that grew on the MSA were transferred onto 5 ml of MSA slants in sealed tubes under the same top gas as described above for another 3 weeks. A sterilized MSA slant topped with the same gas but un-inoculated was used as the negative control. Each colony and negative control had three replicates. Gas samples (0.1 ml) from each test tube were analyzed by Qubit Flow Gas Exchange System (Fig. 1) equipped with a H_2 sensor. The concentration of H_2 in each tube was recorded by the computer connected to the Qubit system.

PGP capability test: The Greenhouse assay was used to test the PGP capabilities of these 13 positive H_2-oxidizing bacteria strains. Positive stains were used to inoculate durum wheat and

red lentil, respectively. Four replicates were applied for each treatment. Non-inoculated plants were grown as control.

Data analysis: The data collected were analyzed by student t-tests to compare the amounts of consumed hydrogen gas by the negative control and the bacterial isolates (5% of probability level using JMP).

Results

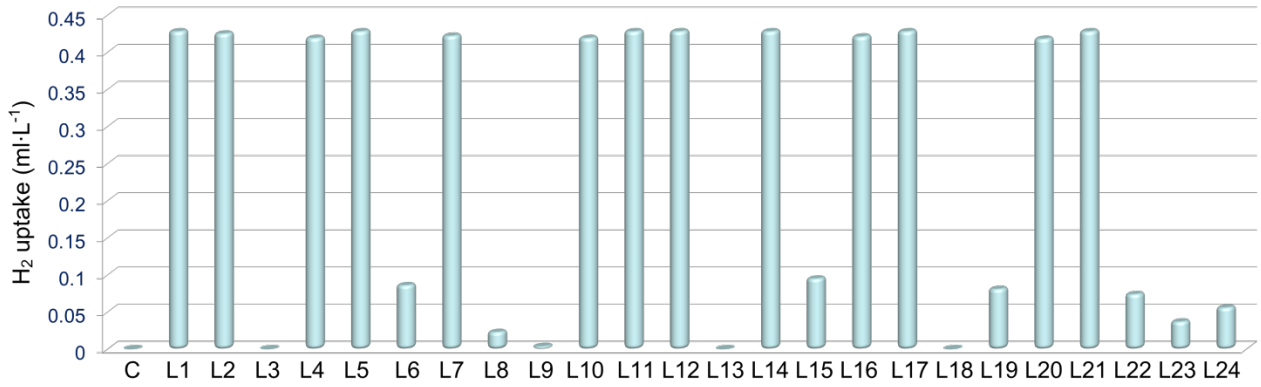

Figure 1. H2 consumption by 24 bacterial isolates. Bars with a star are significantly higher than the negative control according to student t-tests.

C: negative control. L1~L24: 24 bacterial isolates.

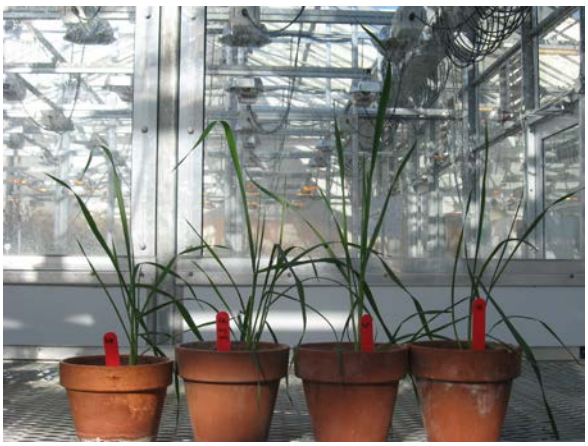

Figure 2. Inoculation effects of positive H2-oxidizing bacteria strains on durum wheat

Figure 3. Inoculation effects of positive H2-oxidizing bacteria strains on red lentil

Conclusions

This study demonstrates the existence of H2-oxidizing bacteria in lentil rhizosphere soil, in semiarid environment. We quantified the H2 uptake capabilities of 13 positive H2-oxidizing

bacteria from lentil field. Greenhouse assay results proved that these lentil associated H2-oxidizing bacteria have potential plant growth promoting capabilities to their associated plants.

References

1. Dong, Z., Wu, L., Kettlewell, B., Caldwell, C. D. and Layzell, D. B. 2003. Hydrogen fertilization of soils - Is this a benefit of legumes in rotation? Plant, Cell and Environment 26(11):1875-1879.

2. Maimaiti, J., Zhang, Y., Yang, J., Cen, Y. P., Layzell, D. B., Peoples, M. and Dong, Z. 2007. Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. Environmental Microbiology 9(2):435-444.