Contribution of plant-associated microorganisms as global sinks of atmospheric hydrogen

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Hydrogen (H_2) is an important constituent of the atmosphere, with a typical mixing ratio of 0.530 parts per million by volume (ppmv). Rising H_2 emissions under a future H_2-based economy are concerned to increase the atmospheric burden of H_2, resulting to the indirect influence of the lifetime of greenhouse gas CH_4, an alteration of temperature and ozone loss in the stratosphere. Thus, mitigation of H_2 emission is of critical importance for atmospheric chemistry. The most part (~80%) of tropospheric H_2 is consumed by microorganisms in soil. A recent literature survey of H_2 flux measurements unveiled that soil H_2 uptake is responsible for the loss of 40 to 90 Tg yr^{-1}. Recently, high-affinity H_2-oxidizing bacteria possessing novel hydrogenase have been found as important contributors to the soil H_2 uptake. Although previous experiments using molecular tritium reported the occurrence of significant H_2 uptake activity in vegetation, there has been no report on the identification and diversity of the responsible microorganisms. This study aimed to verify the existence of plant-associated bacteria possessing the ability to consume atmospheric H_2.

We first investigated the presence of *hhyL* gene in various plant species. The *hhyL* gene, which encodes for the large subunit of the novel group of hydrogenase, has been generally used as a functional biomarker to evaluate the distribution, taxonomic diversity, and abundance of high-affinity H_2-oxidizing bacteria. In total, 42 *hhyL* gene sequences were successfully detected in all tested herbaceous plants, indicating a wide distribution of high-affinity H_2-oxidizing bacteria in plants. It is noteworthy that the abundance levels of *hhyL* gene detected in plants were comparable to those detected in soil. High-affinity H_2-oxidizing bacteria were isolated from inside herbaceous plant tissues. Among 145 isolates, 7 *Streptomyces* strains were shown to possess *hhyL* gene. The H_2 uptake activity was evaluated by gas chromatography. All the isolates reduced H_2 concentration to less than 0.530 ppmv, demonstrating the ability to consume H_2 at ambient level. Sterile plant seedlings were inoculated with selected isolates to verify their ability to penetrate and disseminate in plant tissues and scavenge atmospheric H_2 in plant. After four weeks of seedling inoculation, an internalization of the bacteria in plant tissues was visualized by fluorescence *in situ* hybridization imaging. H_2 oxidation rates measured in plant fractions ranged from 1079 to 3472 pmol $g_{(dw)}^{-1}$ h^{-1}. These rates are comparable to the previously observed activity of atmospheric tritium uptake in other plants. Importantly, atmospheric H_2 is not oxidized in aseptically grown plants, clearly showing that plant-associated bacteria was responsible for H_2 loss. H_2 uptake activity per bacterial cell was comparable between plant and soil, demonstrating that both environments are favorable for the microbial-mediated H_2 uptake.

In conclusion, this study demonstrated the occurrence of plant-associated high-affinity H_2 -oxidizing bacteria and their ability to consume atmospheric H_2 on plant surface or inside plant tissues. From a global perspective, herbaceous and woody plant biomass represent approximately 64 Pg, and 736 Pg, respectively. Considering that high-affinity H_2-oxidizing bacteria may be present and active in these plants, the contribution of plant-associated bacteria deserves more attention to better understand the global cycling of atmospheric H_2.

Keywords: biogeochemistry, microbial ecology, tropospheric H2 cycle, vegetation