The ISME Journal (2008) 2, 1066–1076

www.nature.com/ismej

ORIGINAL ARTICLE

Isolation of *Streptomyces* sp. PCB7, the first microorganism demonstrating high-affinity uptake of tropospheric H_2

Philippe Constant[1,2], Laurier Poissant[2] and Richard Villemur[1]
[1]*INRS-Institut Armand-Frappier, Laval, Québec, Canada and* [2]*Direction des sciences et de la technologie, Environnement Canada, Montréal, Québec, Canada*

Microbial-mediated soil uptake accounts for ~80% of the global tropospheric dihydrogen (H_2) sinks. Studies conducted over the last three decades provide indirect evidences that H_2 soil uptake is mediated by free soil hydrogenases or by unknown microorganisms that have a high affinity for H_2. The exact nature of these hypothetical free soil enzymes or of H_2-consuming microorganisms remains elusive because the activity has never been observed in pure culture. Here, we present the first aerobic microorganism able to consume tropospheric H_2 at ambient levels. A dynamic microcosm chamber was developed to enrich a microbial consortium with a high affinity for H_2, from which selected bacterial and fungal strains were isolated and tested for H_2 uptake. Strain PCB7 had a H_2 consumption activity that followed a Michaelis–Menten kinetics, with an apparent K_m of 11 p.p.m.v. and a H_2 threshold concentration <0.100 p.p.m.v., corresponding to the high-affinity uptake of tropospheric H_2 observed in soil. 16S ribosomal RNA gene sequences showed that strain PCB7 is highly related to several *Streptomyces* species. H_2 consumption occurred during the sporulation period of the bacterium. Addition of nickel increased the activity, suggesting that the enzymes involved in H_2 consumption belong to the NiFe uptake class of hydrogenases. Because this is the first microorganism showing a high-affinity uptake of tropospheric H_2, we anticipate that *Streptomyces* sp. PCB7 will become a model organism for the understanding of the environmental factors influencing H_2 soil uptake.
The ISME Journal (2008) **2,** 1066–1076; doi:10.1038/ismej.2008.59; published online 12 June 2008
Subject Category: geomicrobiology and microbial contributions to geochemical cycles
Keywords: tropospheric hydrogen; high-affinity uptake; *Streptomyces*; soil

Introduction

Tropospheric H_2 concentration is approximately 0.530 p.p.m.v., and its global sources and sinks are generally assumed to be near equilibrium (Khalil and Rasmussen, 1990; Novelli *et al.*, 1999; Simmonds *et al.*, 2000). On an annual basis, 107 ± 15 Tg H_2 ($1\,Tg = 10^{12}$ g) originating from natural and anthropogenic processes are emitted to the troposphere (Rhee *et al.*, 2006). Once in the troposphere, H_2 has a lifetime of 1.4–2.0 years (Novelli *et al.*, 1999; Rhee *et al.*, 2006), before being oxidized by hydroxyl radicals ($OH^{\cdot}$) or consumed by a microbial-mediated soil uptake. Because of its reactivity toward $OH^{\cdot}$, H_2 is considered an indirect greenhouse gas. Because H_2 and methane (CH_4) have similar $OH^{\cdot}$-mediated oxidation rate, a modification of the H_2 emission factor would exert an indirect influence on CH_4 tropospheric burden (Derwent *et al.*, 2001; Schultz *et al.*, 2003; Warwick *et al.*, 2004). $OH^{\cdot}$ oxidation represents <20% of the global tropospheric H_2 sinks that are largely dominated by microbial-mediated H_2 soil uptake. Recent estimates reveal that soil uptake removes 88 ± 11 Tg H_2 $year^{-1}$ from the troposphere (Rhee *et al.*, 2006). Despite their importance for tropospheric H_2 cycling, H_2 consumption reactions occurring in soils are yet to be defined.

Microbial-mediated H_2 soil uptake is a first-order reaction operating in the presence of oxygen within the upper layers of the ground (Conrad and Seiler, 1981; Conrad *et al.*, 1983c). Biological nature of H_2 soil uptake was confirmed by observing that soil heat sterilization, treatments with sodium azide (NaN_3) or fumigation with chloroform caused a significant loss of the activity (Conrad and Seiler, 1981). However, residual H_2 uptake activity persists even following soil chloroform or toluene fumigations, and this activity is often attributed to free soil hydrogenases (Conrad and Seiler, 1981; King,

Correspondence: P Constant, INRS-Institut Armand-Frappier, 531 Boulevard des Prairies, Laval, Québec, Canada H7V 1B7.
E-mail: philippeconstant@hotmail.com
Received 6 March 2008; revised 13 May 2008; accepted 15 May 2008; published online 12 June 2008

2003a). According to these observations, enzymatic fractions partially purified from soils oxidized H_2 at ambient concentrations and exhibited a typical biphasic kinetics (Guo and Conrad, 2008). Biphasic kinetics of H_2 soil uptake is characterized by two distinct activities: the *high-affinity activity* ($K_m < 100$ p.p.m.v.) and the *low-affinity activity* ($K_m \sim 1000$ p.p.m.v.) (Schuler and Conrad, 1990; Häring and Conrad, 1994). The exact nature of H_2 high-affinity free soil hydrogenases or of H_2-consuming microorganisms remains elusive. To date, only aerobic microorganisms with low affinity for H_2 have been characterized. Among them, facultative chemolithoautotrophs knallgas bacteria consume H_2 to obtain energy under specific conditions such as substrate limitation (Aragno, 1998). In the context of H_2 biogeochemical cycle, the importance of these microorganisms relies on their ability to oxidize H_2 diffusing from legume nodules (La Favre and Focht, 1983; Maimaiti *et al.*, 2007). Knallgas bacteria are not involved in the consumption of tropospheric H_2 because of their low affinity for H_2 (Conrad *et al.*, 1983b).

Because of the inevitable enrichment of low-affinity H_2-oxidizing microorganisms in the presence of high H_2 concentrations (Stein *et al.*, 2005; Maimaiti *et al.*, 2007), we developed an original approach, namely the '*dynamic microcosm chamber*' (DMC), to enrich a microbial consortium that has a high affinity for H_2. Soil samples were incubated inside the DMC wherein the headspace was continuously renewed with ambient air, while a trace gas analyzer provided real-time H_2 and CO_2 emission or production rates. Instead of studying microbial populations from selected soils demonstrating H_2 uptake activity, our strategy consisted in monitoring H_2 uptake activity of sterile soil samples during their colonization by soil microbial extracts' biomass. By combining the measurements of H_2 and CO_2 net exchange (NE) rates with the monitoring of the microbial population by molecular and conventional cultivation methods, it became possible to link the development of the H_2 consumption activity with the emergence of candidate H_2-oxidizing microorganisms in the soil.

Materials and methods

DMC

The DMC consisted of a 500 ml heat-sterilized capped glass bottle equipped with two air sampling ports: the '*ambient sample port*' provided air samples collected outside the DMC, whereas the '*microcosm sample port*' provided air samples from inside the DMC (Figure 1). Formation of a negative pressure inside the DMC was avoided by the presence of a '*vent*' equipped with a 0.20 μm filter to circumvent the contamination of the DMC by airborne microorganisms. A second 0.20 μm filter was installed on the '*ambient sample port*' to ensure the same airflow restriction at both air sampling lines. A sequenced air sampling inside and outside the DMC was obtained with a three-way solenoid valve controlled by a datalogger (CR23X Campbell Scientific) with a 0–12 V output signal (0 V: air sample in ambient air and 12 V: air sample inside the DMC). The air was circulating in the sampling network through the trace gas analyzer's pumping system (RGA5, see next section), operated continuously with a flow rate of 40 $cm^3 min^{-1}$. Inside the DMC, the headspace had a hydraulic retention time of 18 min. Because the analytical method is automated and has a response time of 10 min (see next section), H_2 concentration gradients ($[H_2]_{(DMC)} - [H_2]_{(amb)}$) were calculated every 20 min (72 readings

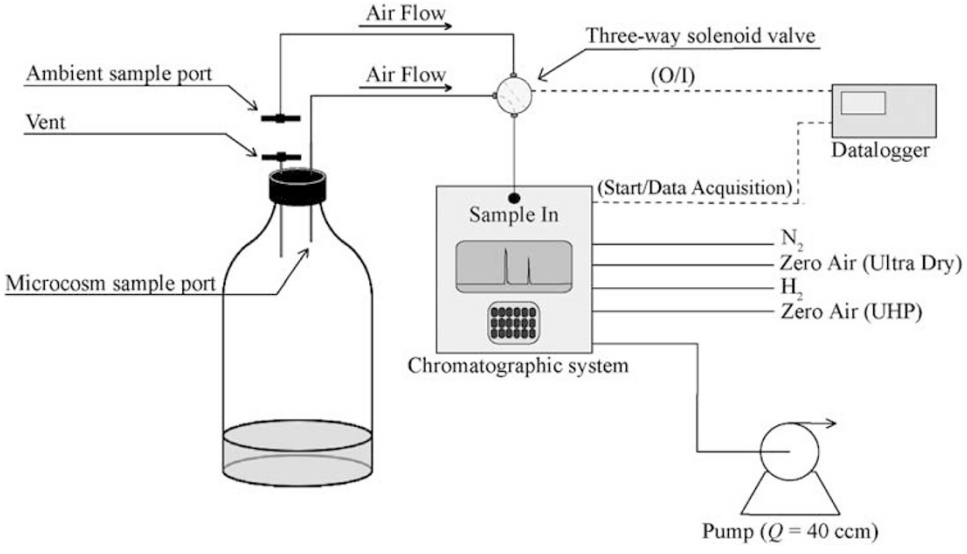

Figure 1 Diagram of the dynamic microcosm chamber (DMC) used to measure the real-time H_2 and CO_2 net exchange (NE) rates of heat-sterilized soils during their colonization by microbial soil extracts' biomass.

a day). H_2 utilization or production rates of soils, namely H_2 NE rates ($\mu g_{(H_2)}\, kg^{-1}_{(soil)}\, h^{-1}$), were calculated following Equation (1).

$$H_{2(NE\,rate)} = \frac{[H_2]_{(DMC)} - [H_2]_{(amb)}}{W_{(soil)}} \times Q \qquad (1)$$

$[H_2]_{(DMC)}$ and $[H_2]_{(amb)}$ are the H_2 concentrations measured inside and outside the DMC ($\mu g\, m^{-3}$), respectively, Q is the air sampling flow rate ($m^3\, h^{-1}$) and $W_{(soil)}$ is the dry weight of the soil inside the DMC (kg). Negative values mean that the soil sample acts as a net sink for H_2, whereas positive values are associated with net emissions. CO_2 NE rates were also measured and were utilized as indicators of microbial respiration and metabolic activity during the colonization of heat-sterilized soils.

Given that the air delivered inside the DMC originated from the laboratory, H_2 and CO_2 concentrations ($[H_2]_{amb}$ and $[CO_2]_{amb}$), respectively, varied between 300–600 p.p.b.v. and 275–360 p.p.m.v. These concentrations are similar to levels measured in the environment (Tans *et al.*, 1989; Novelli *et al.*, 1999). H_2 ambient concentrations were not significantly different between each set of experiments (assessed by Student's *t*-tests with $\alpha = 0.05$). In the case of atypical H_2 concentrations (outliers representing less than 1% of the entire data set), derived NE rates were eliminated to avoid drawbacks in the experiments.

Trace gas analyzer

Chromatographic RGA5 system (Trace Analytical, Sparks Glencoe, MD, USA) was employed for H_2 and CO_2 concentration measurements. Two detectors were mounted in parallel: the reductive gas detector for H_2 and the flame ionization detector for CO_2. RGA5 analyzer was supplied by four different carrier gases (BOC Gas, Montréal, QC, Canada). For the general operation of the instrument (for example, valve movements), ultra dry zero air (60–80 p.s.i.g.) was used. Carrier gas consisted of UHP nitrogen (30–70 p.s.i.g.) and for the flame ionization detector, UHP hydrogen (20–40 p.s.i.g.) and UHP zero air (20–50 p.s.i.g.) were supplied. The analyzer was calibrated before and after each experiment using a standard gas mixture (815 p.p.b.v. H_2, 190.5 p.p.b.v. CO and 380.3 p.p.m.v. CO_2; Scott Marrin Inc., Riverside, CA, USA) certified from the National Oceanic and Atmospheric Administration (NOAA; Climate Monitoring and Diagnostics Laboratory). Difference between the calibration factors obtained between the beginning and the end of the experiments was always $<5\%$. Analyzer response time was 9 min but analyses were conducted every 10 min, synchronized by a datalogger (CR23X, Campbell Scientific) programmed to send a 'start signal' to the analyzer (Figure 1). The datalogger also recorded H_2 and CO_2 concentrations measured by the analyzer. Data were retrieved from the datalogger with a portable computer using PC208w 3.2 version software (Campbell Scientific).

Soil preparation

Heat-sterilized soil samples were inoculated with active (no added antimicrobial agents) or killed (heat sterilized, NaN_3) soil microbial extracts. Soil utilized for all the experiments originated from St Anicet (Québec, Canada), a rural area where the annual cycle of the H_2 surface to air exchanges has been well documented (Constant *et al.*, 2008). At the sampling site, vegetation (mainly grass) was removed from an area of approximately 1 m^2 before the soil's first 10 cm layer was collected. The soil sample was then thoroughly mixed and conserved at 4 °C in a 12-l plastic container until its utilization.

Before each set of experiments, 60 g of soil (on a dry basis) was heat sterilized (30 min at 121 °C and 1 bar, two times separated by a 24 h time interval). Once sterilized, soil samples were conserved at room temperature and utilized no more than 2 days later. Soil microbial extracts were also prepared before each set of experiments by mixing 100 g of soil in 1000 ml of sterile sodium pyrophosphate solution (0.1%) for 1 h. Soil particles were then separated from microorganisms by centrifugation (1000 *g*, 15 min). Microorganisms in the supernatant were then concentrated by a second centrifugation (10 000 *g*, 15 min) and the pellet was suspended in 50 ml of a sterile 40% soil extract. The latter consisted of 400 g of soil suspended in 1000 l of water that was heat sterilized (30 min at 121 °C and 1 bar, two times separated by a 24-h time interval) and centrifuged to remove soil particles (1000 *g*, 10 min).

For each set of experiments, the soil microbial extract was incubated 12 h at room temperature under agitation. Following the incubation, 4.5 ml of this conditioned soil microbial extract was transferred into the heat sterilized soil. Sterile water was then added to the inoculated soil to adjust soil water content to 20%. Soil was subsequently aseptically homogenized with a heat-sterilized metal spatula and transferred into the DMC to monitor real-time H_2 and CO_2 NE rates as well as microbial communities' structure over a 10-day period.

Isolation of bacteria and fungi and analysis of their H_2 uptake activity

Two samples were collected. The first one was an aliquot of the active microbial extract, whereas the second sample consisted of soil collected from the DMC after 10 days of incubation. Both of them were serially diluted (10^0–10^{-6}) in saline water and then inoculated on different cultivation media. Czapek Solution Agar (Difco, Detroit, MI, USA) enriched with Bacto yeast extract (5 $g\, l^{-1}$), Rose Bengal Agar (Difco) and Sabouraud Dextrose Agar (Difco) were used for fungi isolation, whereas R2A Agar (Difco) and 40% soil extract agar were utilized for bacteria. All the incubations were performed at room

temperature for 1 week. Twenty-nine bacterial and four fungal isolates were chosen based on their morphology. They were further classified based on the restriction endonuclease digestion profiles (Sambrook and Russell, 1989) and the PCR-denaturing gradient gel electrophoresis (DGGE) migration profiles of their PCR-amplified 16S ribosomal RNA (rRNA) gene sequences (see below). One colony of the chosen isolates was picked, dispersed in sterile saline, then inoculated in heat-sterilized soil and incubated inside the DMC for a 10-day period to verify its ability to consume ambient H_2. Enumeration of viable bacteria and fungi was performed every 48 h by counting the colony forming units (CFU per gram soil) of diluted soil suspensions inoculated on specific nutrient media. R2A Agar (Difco) was used for bacteria, while Czapek Solution Agar (Difco) enriched with Bacto yeast extract ($5\,g\,l^{-1}$) was used for fungi. Student's *t*-test was conducted on a daily basis to confirm if H_2 concentrations measured inside and outside the DMC were equivalent (H_0: $\mu_{DMC} = \mu_{amb}$) or not (H_1: $\mu_{DMC} \neq \mu_{amb}$). This analysis was performed to assess the statistical significance of H_2 NE rates observed for the isolated strains.

By following the same procedure, 10^7 spores of *S. lividans* 1326 (wild-type strain from the collection of microorganisms of the John Innes Institute) prepared as described by Hopwood *et al.* (1985) were also inoculated in sterile soil and incubated 10 days in the DMC to assess its H_2 consumption activity.

DNA extraction

Active soil microbial extract (500 µl) and soil collected from the DMC (~500 mg) were mixed with 350 mg of glass beads (0.25–0.50 mm in diameter), 500 µl TEP buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 1% polyvinylpolypyrrolidone), 50 µl 20% SDS and 500 µl phenol. The samples were then vortexed for 1 min and conserved at −80 °C for later nucleic acid extraction. The samples were transferred into a FastPrep instrument (Q-Biogene, Carlsbad, CA, USA) for two series of mechanical cells disruption of 20 s at speed 4.0 separated by a 5-min incubation on ice. The tubes were centrifuged (15 min, 16 000 *g*) and 300 µl of the aqueous phase was collected and kept on ice. Following the addition of 300 µl TEP and 50 µl 20% SDS, a second series of mechanical cells disruption, centrifugation and aqueous-phase collection were performed on the residual samples. The corresponding aqueous-phase aliquots were combined, extracted with 600 µl chloroform/isoamylic alcohol (24:1) and centrifuged (5 min, 16 000 *g*). After collecting 500 µl of the aqueous phase, 167 µl of 10 M $AcNH_4$ was added, incubated for 20 min on ice and centrifuged (15 min, 16 000 *g*). The supernatant was collected and nucleic acids were precipitated at room temperature for 2 h by adding 1 ml of 30% PEG-NaCl (polyethylene glycol 1.6 M NaCl) to the supernatant. The tubes were centrifuged (15 min, 16 000 *g*) after which the nucleic acid pellets were washed with 1 ml of 70% ethanol (2 min, 16 000 *g*) and dissolved in 100 µl sterile water.

Total DNA from the bacterial and fungal isolates was extracted as described before but TEP was replaced by TEN buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 150 mM NaCl).

PCR

PCR was performed in 50 µl reaction volumes containing the following concentrations or total amounts: 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTP, 10 pM of each primer, 20 µg of bovine serum albumin, 2.5 U of *Taq* polymerase and 10 ng (isolates) or 100 ng (soil and soil microbial extract samples) DNA. Amplifications were done in a DNA thermal cycler (Geneamp PCR System 2700; Applied Biosystems, Streetsville, ON, Canada) at 94 °C for 5 min, 55 °C for 5 min, followed by 35 cycles at 72 °C for 45 s, 94 °C for 45 s, 55 °C for 45 s and a final extension period of 10 min at 72 °C.

16S rRNA genes were amplified using the PA (5′-AG AGTTTGATCMTGGCTCAG-3′) and PH (5′-AAGG AGGTGATCCARCCGCA-3′) primer pair that correspond to the 8–27 and 1521–1541 positions in *Escherichia coli* 16S rRNA gene sequence (Edwards *et al.*, 1989). For PCR-DGGE experiments, the variable V3 region of 16S rRNA gene was amplified by using 341f-GC clamp (5′-CGCCCGCCGCGCGC GGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGG AGGCAGCAG-3′) and 534r (5′-ATTACCGCGGCTG CTGG-3′) primers (Muyzer *et al.*, 1993). For the fungal isolates, the V7 and V8 (partial) variable regions of the 18S rRNA genes were amplified using the NU-ssu-1196-5′ (5′-GGAAACTCACCAGGTCCA GA-3′) and NU-ssu-1536-3′-GC clamp (5′-CGCC CGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGG GGATTGCAATGCYCTATCCCCA-3′) primers (Borneman and Hartin, 2000).

16S and 18S rRNA gene sequences were compared with gene databases with BLASTN (http://www.ncbi.nlm.nih.gov/) and FASTA (http://www.ebi.ac.uk/fasta33/) programs. Accession number of the 16S rRNA gene sequence of strain PCB7 is EU532017.

DGGE

DGGE was carried out using the D-code system (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) with PCR products (500 ng) separated inside 8% polyacrylamide gels (acrylamide/*N*,*N*′-methylene bisacrylamide 37.5:1) containing a linear denaturing gradient (100% denaturing gel contained 7 M urea and 40% deionized formamide). Electrophoresis was carried out using TAE running buffer (40 mM Tris-HCl, 40 mM acetic acid, 1 mM EDTA, pH 8.0) for 16 h at 100 V and 60 °C (Labbé *et al.*, 2003). The gels

were stained with ethidium bromide (10 mg/ml) and the bands were visualized with ultraviolet exposure. When needed, DNA fragments were isolated from the polyacrylamide gels by using 'the crush and soak method' (Sambrook and Russell, 1989) and then amplified by PCR before sequencing.

Testing the affinity of strain PCB7 for H_2
Strain PCB7 spores were prepared as described by Hopwood *et al*. (1985). R2A Agar (Difco), M9 minimal salts agar (42 mM Na_2HPO_4, 24 mM KH_2PO_4, 9 mM NaCl, 19 mM NH_4Cl, 0.2 mM $MgSO_4$, 2.0% glucose, 1.5% agar) and soya flour–mannitol agar (Hobbs *et al*., 1989) plates were inoculated with 10^7 of these spores and incubated for 10 days at room temperature. Petri dishes covered by spore mats were transferred to enclosed systems consisting of a bottom-free 1000 ml glass bottle mounted on a plastic support. A rubber gasket sealed the bottle at the bottom of the plastic support. The enclosed system was equipped with a septum used for air sample collection and for the injection of specified amounts of H_2. H_2 NE rates were then calculated after addition of specified amounts of H_2 in the static headspace ($[H_2]_{initial}$: 0.8, 2.8, 3.1, 8.6, 8.9, 20 and 35 p.p.m.v.). Over a 1-h time period, air samples (10 ml) were collected from inside the enclosed system every 10 min and then injected through the sample injection port of the RGA5 trace gas analyzer. H_2 consumption rates (p.p.m.v. H_2 per minute) were calculated by integrating the decreasing slopes of H_2 concentrations measured. The reproducibility of the H_2 and CO_2 analyses was assessed by repeated manual injections of the certified standard gas. The standard deviations were $<5\%$ for H_2 and CO_2 ($n=6$). No H_2/CO_2 production or consumption was observed for blank experiments.

$NiCl_2$ assays
H_2 consumption activity of strain PCB7 was determined in triplicate on non-amended and nickel-amended R2A Agar. In the case of nickel-amended media, R2A Agar (Difco) formulation was modified by adding 10 μM $NiCl_2$. Non-amended and $NiCl_2$-amended media were inoculated with 10^7 spores of the strain PCB7 and incubated 10 days at room temperature. Petri dishes covered by spore mat were then transferred to enclosed systems described in the previous section. The headspace (720 ml) of the enclosure was subsequently flushed with ambient air and the H_2 concentrations of the static headspace were monitored over a 1-h period to calculate the H_2 uptake rates as described before. As H_2 consumption activity distribution did not follow a normal distribution, non-parametric Wilcoxon–Mann–Whitney test ($\alpha=0.05$) was performed to confirm if activities observed on non-amended and on $NiCl_2$-amended R2A Agar were equivalent (H_0: $\mu_{\text{non-amended}}=\mu_{\text{nickel}}$) or not ($H_1$: $\mu_{\text{non-amended}}\neq\mu_{\text{nickel}}$).

Results

Enrichment of a consortium having a high affinity for H_2
The DMC is based on the respirometry principle. Sterile soil samples were inoculated with active or killed soil microbial extracts and were subsequently incubated under a dynamic headspace, continuously renewed with ambient air. During soil colonization, an automated analyzer provided real-time H_2 and CO_2 NE rates while soil samples were collected on a daily basis to monitor the evolution of the microbial populations. Development of H_2 soil uptake activity occurred only in the presence of active biomass as no significant H_2 exchanges were observed when the soil was inoculated with heat-sterilized or NaN_3-inactivated biomass (Figure 2a). In the DMC inoculated with active microbial extract, H_2 NE rates reached $-0.5\,\mu g\,kg^{-1}\,h^{-1}$ at day 4 and remained relatively stable until the end of the incubation period (Figure 2a). Monitoring the CO_2 NE rate provided indications on the microbial metabolic state inside the DMC. CO_2 net production was only detected in the presence of active biomass (Figure 2b). As no nutrients were added into the soil during the incubation period, CO_2 net production decreased but remained positive until the end of the experiment (Figure 2b). The establishment of bacterial biota in the sterile soil was also monitored by PCR-DGGE. Migration profiles evolved over the first 3 days and then remained unchanged until the end of the incubation period (Figure 2c).

Isolation of bacteria and fungi from the DMC
Examination of soil particles from the DMC revealed that H_2 consumption activity correlated with the appearance of a white mycelium (Figure 3a). This mycelium was sampled and inoculated on different cultivation media to isolate microorganisms and investigate their H_2 consumption potential. Eight bacterial (designated PCB1 to PCB8) and two fungal (designated PCM1 and PCM2) isolate types were obtained. Analysis of the PCR-amplified 16S and 18S rRNA gene sequences revealed that the isolates belonged to β-Proteobacteria, γ-Proteobacteria and Actinobacteridae for the bacterial strains and to Ascomycetes for the fungi (Table 1). Fungal and bacterial strains were inoculated in sterile soil samples and transferred to the DMC. Even if biomass was similar for each set of experiments ($0.1–1.0\times10^9$ CFU g^{-1} after 10 days of incubation), the ability to consume ambient H_2 was restricted to *Streptomyces* sp. PCB7 (Table 1). H_2 NE rates calculated for the other isolated strains were negligible as H_2 concentrations measured inside and outside the DMC were not significantly different (*t*-test, $\alpha>0.05$). *Streptomyces* spp. growth follows a complex series of differentiation steps showing similarities with those of filamentous fungi. As observed in the DMC inoculated with the active microbial extract (Figure 3a), strain PCB7 also

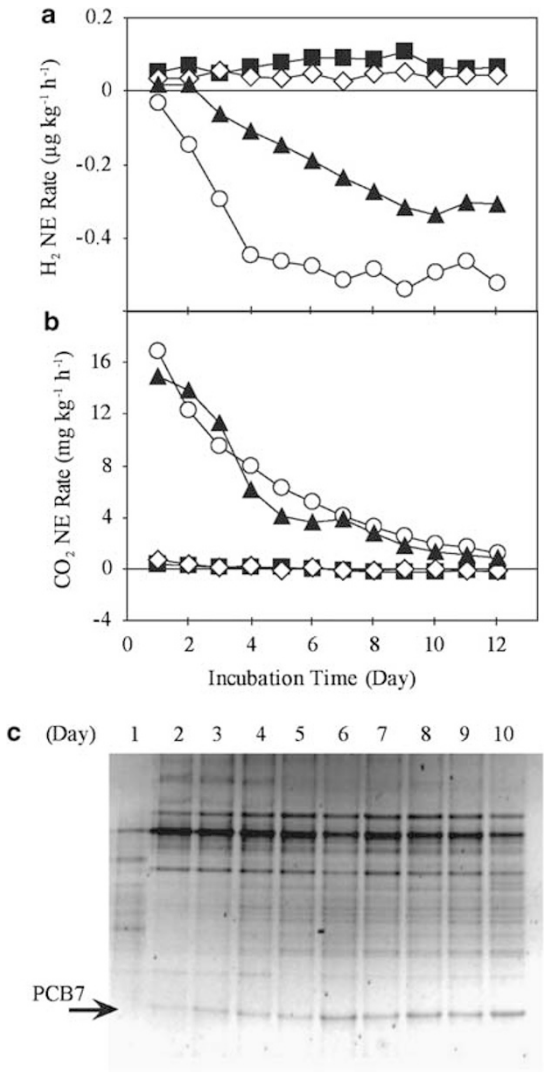

Figure 2 Time series for (**a**) H_2 and (**b**) CO_2 net exchange (NE) rates measured during the colonization of sterile soil by active biomass (circles), heat-sterilized (squares) and NaN_3 (diamonds)-inactivated biomass, or by a cell suspension of *Streptomyces* sp. PCB7 (triangles). Each point represents an average of 72 measurements (standard deviations were $<0.1\,\mu g\,kg^{-1}\,h^{-1}$ for H_2 and $<0.9\,mg\,kg^{-1}\,h^{-1}$ for CO_2). (**c**) PCR-denaturing gradient gel electrophoresis (DGGE) profile of the 16S rRNA gene sequences of DNA extracted from soil samples collected daily (day 1–10) inside the dynamic microcosm chamber (DMC) during the colonization of the sterile soil with the active biomass. The arrow indicates the band corresponding to *Streptomyces* sp. PCB7 (assessed by co-migration, and by extracting, followed by PCR reamplification and DNA sequencing of the corresponding band).

formed a white mycelium on soil particles. H_2 consumption activity of strain PCB7 followed a reproducible pattern represented at Figure 2a. For the first 3 days following strain PCB7 inoculation in sterile soil, its viable cell counts increased by two log units (up to 10^8 CFU $g^{-1}_{(dw)}$), and then remained

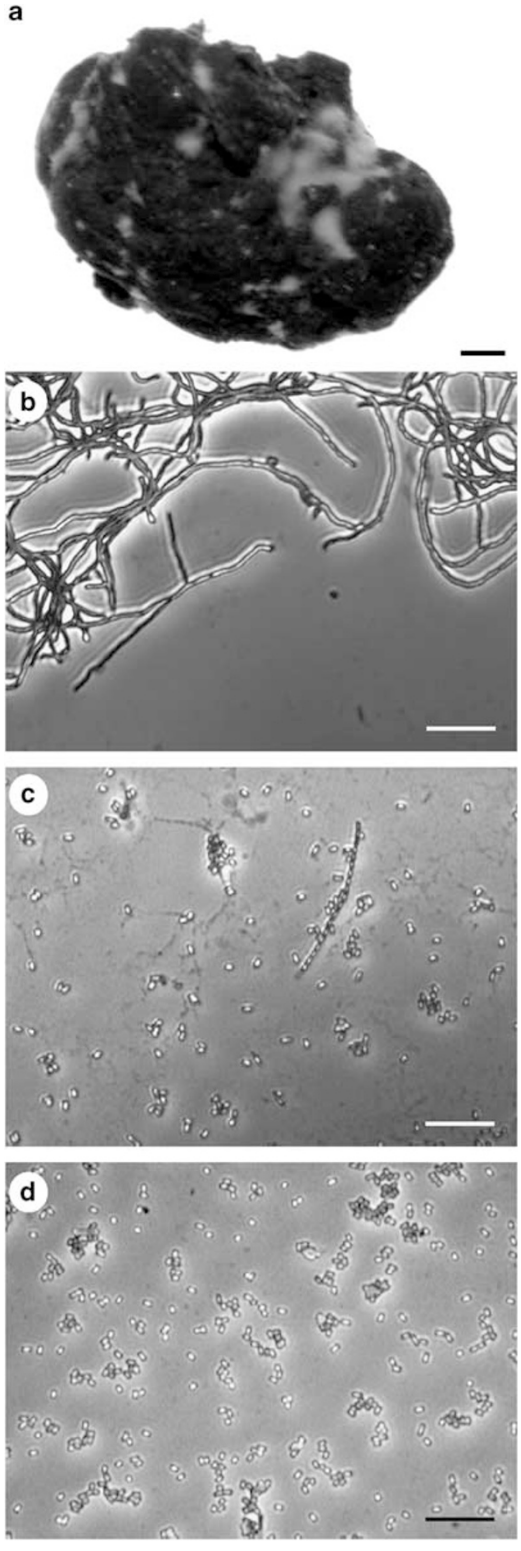

Figure 3 (**a**) Representative soil particles covered with white mycelium after 10 days of incubation inside the DMC. (**b–d**) Microscopic observations of the developmental stages of *Streptomyces* sp. PCB7 inoculated on R2A Agar are also shown. (**b**) At 24 h following its inoculation, strain PCB7 was growing as a substrate mycelium that penetrated into the media. (**c**) After 48 h, strain PCB7 was producing structures known as aerial mycelium consisting of hyphae that were protruding into the air. At that time, chains of cells that were metamorphosing into spores were visible. (**d**) After 72 h, gray pigmentation of the aerial mycelium associated with the maturation of the spores was visible and ambient H_2 consumption began to be significant (H_2 consumption rate: 5.0 ± 1.2 p.p.b.v. min^{-1}). Scale bar = 1 mm (**a**), 10 µm (**b–d**).

Table 1 H_2 consumption activity of the cultivable bacteria and fungi

Strains	*Affiliation (accession number)*	*Identity (%)*[a]	*H_2 NE rates ($\mu g\,kg^{-1}\,h^{-1}$)*
PCB1	*Flavobacterium saccharophilum* (AM230491)	96	0.027 (0.013)
PCB2	*Pseudomonas fluorescens* (AY512614)	96	−0.018 (0.029)
PCB3	*Variovorax paradoxus* (EU169152)	98	0.0036 (0.028)
PCB4	*Flavobacterium frigidimaris* (AB183888)	95	0.065 (0.15)
PCB5	*Agromyces cerinus* (AM410680)	88	0.021 (0.014)
PCB6	*Arthrobacter ramosus* (X80742)	94	−0.020 (0.028)
PCB7	***Streptomyces* sp.**[b]	**>99**	**−0.19 (0.13)**
PCB8	*Rhodococcus erythropolis* (EU479709)	98	−0.08 (0.08)
PCM1	*Penicillium* sp.[b]	100	0.015 (0.036)
PCM2	*Fusarium solani* (EF621487)	94	−0.010 (0.042)

Abbreviations: DMC, dynamic microcosm chamber; NE, net exchange.
[a]Percentage of identity with the most related 16S or 18S rRNA gene sequence at the species level. Sequences from uncultured or unaffiliated species were not considered. Affiliations were based on the almost completed 16S rRNA gene sequences, but with 306 nucleotides for the 18S rRNA gene sequences.
[b]Sequences from more than one *Streptomyces* and *Penicillium* species were highly identical to the PCB7 and PCM1 sequences, respectively.
H_2 NE rates were observed for the bacterial and fungal strains isolated from the DMC. Measurements were performed during 10 days (standard deviations are given in parentheses).
Bold characters to emphasis on strain PCB7 (the strain characterized in this study).

essentially unchanged until the end of the incubation period. H_2 consumption activity was significant at day 3 when spore-forming aerial mycelium began to be observable on soil particles and reached $\sim 0.3\,\mu g\,kg^{-1}\,h^{-1}$ after 10 days of incubation.

PCR-DGGE analyses confirmed the enrichment of strain PCB7 in the DMC inoculated with active microbial extract. Relative intensity of the DNA band corresponding to strain PCB7 increased after 3 days of incubation while H_2 consumption activity began to be significant (Figure 2c). 16S rRNA gene sequence of *Streptomyces* sp. PCB7 showed >99% identity to that of several *Streptomyces* species, including *S. lividans*. *S. lividans* 1326 has been inoculated in sterile soil and incubated for 10 days in the DMC, but no significant H_2 consumption activity was observed.

Characterization of the H_2 consumption by strain PCB7
Strain PCB7 grew well on R2A, M9 minimal salts and soya flour–mannitol agar, but only the R2A Agar medium sustained its H_2 consumption activity. Nickel amendment to R2A Agar media doubled the H_2 consumption activity of strain PCB7 that was 5.7 ± 1.1 and 12 ± 2.6 p.p.b.v. min^{-1}, respectively, after 10 days of incubation on non-amended and $NiCl_2$-amended R2A Agar. As observed with *Streptomyces* spp., strain PCB7 grew following well-defined series of differentiation. Growth began with substrate mycelium development and was followed by the formation of aerial mycelia and then, sporulation. H_2 uptake was neither observed at the growing phase of the substrate mycelia (Figure 3b) nor during the formation of the sporulating aerial mycelia (Figure 3c). The activity was only detected at day 3, corresponding to the terminal stage of sporulation (Figure 3d).

H_2 consumption rate of strain PCB7 was determined as a function of increasing H_2 concentrations. The activity followed a typical Michaelis–Menten kinetics, with an apparent K_m of 11 p.p.m.v. (Figure 4a). Exposure of the spore mat to ambient H_2 concentration allowed the determination of strain PCB7 H_2 threshold concentration. H_2 threshold refers to the minimal H_2 concentration below which no more H_2 consumption was observed when strain PCB7 was incubated under a static headspace. Under these conditions, strain PCB7 reduced the H_2 levels from 0.400 p.p.m.v. to a minimal H_2 concentration <0.100 p.p.m.v. in less than 1 h (Figure 4b).

Discussion

Microbial-mediated soil uptake is the main sink for tropospheric H_2 (Rhee *et al.*, 2006). This reaction is operating in the presence of oxygen within the upper layers of the ground, but the microorganisms responsible for the activity remain to be identified (Conrad, 1996). Aerobic H_2 consumption by soil microorganisms exposed to concentrations 2–3 orders of magnitude higher than typical tropospheric H_2 level has been extensively studied. These investigations presented important findings concerning the fertilization effect of the knallgas bacteria and their role in the consumption of H_2 produced as nitrogen fixation by-products (for example, Maimaiti *et al.*, 2007), but provided no information about the microorganisms that have high affinity for H_2. In this article, we report a novel approach to study H_2 soil uptake under ambient concentration. The strategy combined the simultaneous monitoring of H_2 NE rate with microbial population during the colonization of a soil sample exposed to ambient atmosphere. By considering that soils with poor organic matter content sustain the highest H_2 soil uptake (King, 2003a; King and Weber, 2008), neither carbon nor nutrient amendments were applied to the soil during the incubation

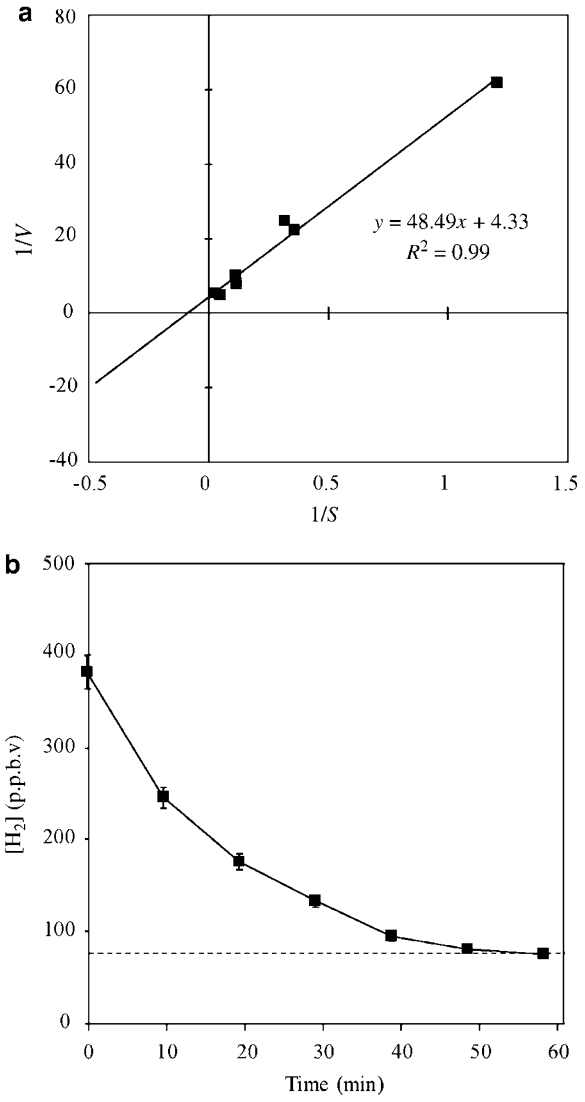

Figure 4 (**a**) Measurement of H_2 consumption rate (V; p.p.m.v. min^{-1}) as a function of the initial H_2 concentration (S; p.p.m.v.) shown by a Lineweaver–Burke plot. The K_m was determined at the x axis intercept ($-1/K_m$). (**b**) Typical trends of the H_2 headspace concentrations when a spore mat of strain PCB7 was transferred in an enclosed system. Dotted line represents the H_2 threshold ([H_2] approximately 80 p.p.b.v.).

period. This strategy enabled the isolation of *Streptomyces* sp. PCB7, the first isolated aerobic bacterium consuming tropospheric H_2.

Strain PCB7 was the only one of our isolate collection capable of ambient H_2 consumption. However, due to the difference between the H_2 NE rates of the active microbial extract (H_2 NE rate of $-0.50\,\mu g\,kg^{-1}\,h^{-1}$) and of strain PCB7 ($H_2$ NE rate of $-0.30\,\mu g\,kg^{-1}\,h^{-1}$), other microorganisms could be involved in the H_2 consumption activity. The inability of the other isolates to consume ambient H_2 (Table 1) suggests that they did not contribute to the H_2 uptake observed in the DMC. DGGE migration profiles representing bacterial (Figure 2c) and fungal (data not shown) diversity of the soil inoculated with the active microbial extract revealed the presence of approximately 15 and 6 bands, respectively. Because only eight bacterial and two fungal isolate types were obtained, it is reasonable to assume that other H_2 consumers were present among the uncultured bacteria and fungi.

16S rRNA gene sequence of *Streptomyces* sp. PCB7 was >99% identical to that of several *Streptomyces* species, but no data are currently available regarding their specific H_2 consumption activity. We tested one of these species, *S. lividans* 1326, for its ability to consume H_2. No such activity was observed, which suggest that the genes encoding for the enzymes involved in H_2 consumption or those involved in its regulation have been lost due to genetic instability of the chromosome (Volff and Altenbuchner, 1998) or have been acquired by strain PCB7 following horizontal gene transfer in the environment. Soil is the primary reservoir of *Streptomyces* spp. that are typically heterotrophs, but thermophilic chemolithotrophs have also been identified. These aerobic thermophilic species are either facultative chemolithotrophs (Bell *et al.*, 1987; Kim *et al.*, 1998) or obligate chemolithoautotrophs (Gadkari *et al.*, 1990) utilizing H_2 and CO/CO_2 as sole energy and carbon sources under a headspace constituted of >45% H_2. So far, they are the only *Streptomyces* spp. known to use H_2, but their H_2 oxidation activity remains to be characterized. Considering the H_2 consumption activity of strain PCB7 and the chemolithotrophic H_2 oxidation of thermophilic species, further investigations should be performed to verify the H_2 consumption activity of other *Streptomyces* species.

To be considered as a potential tropospheric H_2 consumer, a microorganism should have a threshold that is below 0.530 p.p.m.v., corresponding to the typical tropospheric H_2 concentration, and a high affinity for H_2 (K_m < 100 p.p.m.v.) (Conrad, 1996). H_2 consumption of strain PCB7 followed a typical Michaelis–Mentens kinetics, with an apparent K_m of 11 p.p.m.v. and a threshold concentration of <0.100 p.p.m.v. Because the affinity of strain PCB7 for H_2 has been tested after 10 days of incubation on solid R2A Agar, drawbacks related to H_2 phase transfer limitation or to a growth of the strain during H_2 consumption rate measurements (Robinson and Tiedje, 1982) were considered negligible. Owing to its high affinity toward H_2, strain PCB7 represents the first candidate for the elucidation of microbial-mediated tropospheric H_2 soil uptake. H_2 is utilized by physiologically distinct groups of aerobic and anaerobic microorganisms, but none of them can be considered tropospheric H_2 consumers. Because of their use of H_2 for ATP generation and CO_2 fixation in the presence of oxygen, knallgas bacteria have been previously considered potential tropospheric H_2 consumers. Several knallgas bacteria have been studied, but none of them demonstrated the capacity of consuming tropospheric H_2 because of their low affinity toward H_2 (Table 2). For instance, *Mycobacteria*

Table 2 H_2 affinity of microorganisms catalyzing the H_2 oxidation in aerobic, anoxic or anaerobic conditions

Microorganisms/environmental samples	*Threshold (p.p.m.v.)*	K_m *(p.p.m.v.)*	*References*
Environmental samples (aerobic)			
Microbial soil extract	<0.100	10–50	Conrad (1996)
Particle fraction from a freshwater lake	NA	90	Conrad *et al.* (1983a)
Extracted soil enzymes	NA	5.0	Guo and Conrad (2008)
***Streptomyces* sp. PCB7 (aerobic)**	**<0.100**	**11**	**This study**
Knallgas bacteria (aerobic)			
Bradyrhizobium japonicum	>0.500	1200	Klüber and Conrad (1993)
Paracoccus denitrificans	0.83	1565	Häring and Conrad (1991)
Variovorax sp.; *Flavobacterium* sp.; *Burkholderia* sp.	NA	1000	Maimaiti *et al.* (2007)
Alcaligenes eutrophus	8.0	>1000	Conrad *et al.* (1983b)
Xanthobacter spp.	1.3–6.7	>1000	Conrad *et al.* (1983b)
Mycobacterium smegmatis	1.0	NA	King (2003b)
Other terminal electron-accepting processes			
Halogenated compounds	<0.100	130 ± 65	Smatlak *et al.* (1996); Luijten *et al.* (2004)
Nitrate	<0.83	1565	Häring and Conrad (1991)
Iron (III)	0.400	319	Lovley *et al.* (1989); Klüber and Conrad (1993)
Sulfate	5–25	1336	Kristjansson *et al.* (1982); Lovley and Goodwin (1988)
Carbon dioxide (methanogenesis)	9–13	8000	Kristjansson *et al.* (1982); Lovley and Goodwin (1988)
Carbon dioxide (acetogenesis)	180	6682	Hoehler *et al.* (1998); Krumholz *et al.* (1999)

Abbreviation: NA, not available.
Bold characters to emphasis on strain PCB7 (the strain characterized in this study).

smegmatis has been identified as a potential high-affinity H_2 oxidizer. However, there is no evidence showing its consumption of tropospheric H_2 (King, 2003b). In liquid culture, *M. smegmatis* has been shown to reduce H_2 headspace concentrations from 30 p.p.m.v. to a threshold concentration of 1.0 p.p.m.v. (King, 2003b), as reported for other knallgas bacteria, however, known for their low affinity for H_2 if one considers their K_m (Table 2). H_2 generated in anaerobic environments, such as flooded soils and sediments, has a very short lifetime. In these environments, H_2 is maintained at a threshold concentration determined by the energetic yield of the terminal electron-accepting process (Table 2) (Lovley and Goodwin, 1988; Hoehler *et al.*, 1998). For instance, nitrate-, iron- and halogen-respiring bacteria have low H_2 threshold (Table 2), but their importance in tropospheric H_2 soil uptake is currently considered negligible. Juxtaposition of the anaerobic and aerobic zones is limiting the H_2 transfer from the troposphere to anaerobic zones. As aerobic microorganisms have a high affinity for H_2 (Table 2), H_2 originating from the troposphere should be depleted before reaching the anaerobic environments. In the H_2 biogeochemical cycle context, anaerobic H_2 oxidation occurring in sediments and flooded soils is limiting the H_2 emissions to the water column and to the global atmosphere (for example, Schütz *et al.*, 1988).

Previous investigations provided indirect evidences that free soil hydrogenases are responsible for tropospheric H_2 soil uptake activity, but their exact nature remains elusive (Conrad and Seiler, 1981; Guo and Conrad, 2008). Hydrogenases are divided into three classes according to the composition and the structure of their catalytic site. Hydrogenases belonging to the [NiFe] class are generally involved in H_2 uptake, are oxygen tolerant (Shima and Thauer, 2007), and nickel availability has been shown to control the H_2 uptake activity of knallgas bacteria that synthesize these enzymes (Friedrich *et al.*, 1981; Brito *et al.*, 1994; Ureta *et al.*, 2005). Because nickel is involved in the maturation process of these enzymes, its presence in excess enhances the formation of their active form (Böck *et al.*, 2006; Leach and Zamble, 2007). Consequently, the raise of H_2 consumption rate on $NiCl_2$-amended R2A Agar media suggests that strain PCB7's H_2 consumption activity is mediated by enzymes belonging to the NiFe uptake class of hydrogenases.

Streptomyces spp. are well known for their ability to produce a broad variety of enzymes and antibiotics, namely secondary metabolites, during the formation of aerial mycelium and its sporulation. These secondary metabolites are secreted in the environment to protect the growing colony from competing microorganisms in limited nutrient conditions (Chater, 2006). Mature spores of *Streptomyces* spp. are characterized by the development of pigments, some of which being antibiotics (Chater, 1993). Strain PCB7 H_2 uptake activity was only observed at the terminal sporulation stage

(Figure 3d), corresponding to the emergence of gray pigmented spores (data not shown). By considering the data presented, we hypothesize that strain PCB7 synthesizes H_2 high-affinity [NiFe]-hydrogenases that are either utilized for spores' metabolism or are released in the environment during the formation of the aerial mycelia, supporting the proposed concept of free soil hydrogenases (Conrad and Seiler, 1981; Guo and Conrad, 2008).

Exposure of microorganisms to ambient H_2 concentration and the absence of nutrient amendments to the soil provided suitable conditions for the enrichment of a bacterium that has a high affinity for H_2. Other high-affinity tropospheric H_2 consumers would be identified using the innovative approach presented in this article with soil samples collected from different ecosystems. Because the exact nature of the H_2 soil uptake activity is still unknown, purification of the putative high-affinity hydrogenases will be crucial in future investigations. As only low-affinity hydrogenase gene and protein sequences are available in current database, identification of these enzymes is needed to develop specific molecular tools. Afterward, it will become possible to investigate the metabolism and the ecological importance of the microorganisms that consume tropospheric H_2. To conclude, identification of the hydrogenases involved in tropospheric H_2 soil uptake would be valuable for the bioelectrochemical sector to improve the performance of existing biofuel cells (Vincent *et al.*, 2006).

Acknowledgements

PC is grateful to the Fonds québécois de la recherche sur la nature et les technologies and the Fondation Armand-Frappier for his PhD scholarship and thanks Catherine Ormeaux for her technical assistance. We are grateful to the Natural Sciences and Engineering Research Council of Canada (RV) and Environment Canada (LP) for funding. Special thanks are also expressed to Professor Ralf Conrad and Professor Eric Déziel for their critical comments about the results and to Ariane Bisaillon, Josée Castonguay-Vanier and Julie Disnard who kindly revised grammar and style of the article. *Streptomyces lividans* 1326 was kindly provided by Professor Rolf Morosoli (INRS-Institut Armand-Frappier).

References

Aragno M. (1998). The aerobic, hydrogen-oxidizing (knallgas) bacteria. In: Burlage RS (ed). *Techniques in Microbial Ecology*. Oxford University Press: Oxford, pp 79–112.

Bell JM, Falconer C, Colby J, Williams E. (1987). CO metabolism by a thermophilic actinomycete, *Streptomyces* strain G26. *J Gen Microbiol* **133**: 3445–3456.

Böck A, King PW, Blokesch M, Posewitz MC. (2006). Maturation of hydrogenases. *Adv Microb Physiol* **51**: 1–71.

Borneman J, Hartin RJ. (2000). PCR primers that amplify fungal rRNA genes from environmental samples. *Appl Environ Microbiol* **66**: 4356–4360.

Brito B, Palacios J-M, Hidalgo E, Imperial J, Ruiz-Argüeso T. (1994). Nickel availability to pea (*Pisum sativum* L.) plants limits hydrogenase activity of *Rhizobium leguminosarum* bv. viciae bacteroids by affecting the processing of the hydrogenase structural subunits. *J Bacteriol* **176**: 5297–5303.

Chater KF. (1993). Genetics of differentiation in *Streptomyces*. *Annu Rev Microbiol* **47**: 685–711.

Chater KF. (2006). *Streptomyces* inside-out: a new perspective on the bacteria that provide us with antibiotics. *Phil Trans R Soc B* **361**: 761–768.

Conrad R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol Rev* **60**: 609–640.

Conrad R, Aragno M, Seiler W. (1983a). Production and consumption of hydrogen in a eutrophic lake. *Appl Env Microbiol* **45**: 502–510.

Conrad R, Aragno M, Seiler W. (1983b). The inability of hydrogen bacteria to utilize atmospheric hydrogen is due to threshold and affinity for hydrogen. *FEMS Microbiol Lett* **18**: 207–210.

Conrad R, Seiler W. (1981). Decomposition of atmospheric hydrogen by soil microorganisms and soil enzymes. *Soil Biol Biochem* **13**: 43–49.

Conrad R, Weber M, Seiler W. (1983c). Kinetics and electron transport of soil hydrogenases catalyzing the oxidation of atmospheric hydrogen. *Soil Biol Biochem* **15**: 167–173.

Constant P, Poissant L, Villemur R. (2008). Annual hydrogen, carbon monoxide and carbon dioxide concentrations and surface to air exchanges in a rural area (Québec, Canada). *Atm Environ* (in press), advance online publication 10 March 2008, doi:10.1016/j.atmosenv.2008.02.021.

Derwent RG, Collins WJ, Johnson CE, Stevenson DS. (2001). Transient behaviour of tropospheric ozone precursors in a global 3-D CTM and their indirect greenhouse effects. *Climatic Change* **49**: 463–487.

Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**: 7843–7853.

Friedrich B, Heine E, Finck A, Friedrich CG. (1981). Nickel requirement for active hydrogenase formation in *Alcaligenes eutrophus*. *J Bacteriol* **145**: 1144–1149.

Gadkari D, Schricker K, Acker G, Kroppenstedt RM, Meyer O. (1990). *Streptomyces thermoautotrophicus* sp. nov., a thermophilic CO- and H_2-oxidizing obligate chemolithoautotroph. *Appl Environ Microbiol* **56**: 3727–3734.

Guo R, Conrad R. (2008). Extraction and characterization of soil hydrogenases oxidizing atmospheric hydrogen. *Soil Biol Biochem* **40**: 1149–1154.

Häring V, Conrad R. (1991). Kinetics of H_2 oxidation in respiring and denitrifying *Paracoccus denitrificans*. *FEMS Microbiol Lett* **78**: 259–264.

Häring V, Conrad R. (1994). Demonstration of two different H_2-oxidizing activities in soil using an H_2 consumption and a tritium exchange assay. *Biol Fertil Soils* **17**: 125–128.

Hobbs G, Frazer CM, Gardner DCJ, Cullum JA, Oliver SG. (1989). Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol* **31**: 272–277.

Hoehler TM, Alperin MJ, Albert DB, Martens CS. (1998). Thermodynamic control on hydrogen concentrations in anoxic sediments. *Geochim Cosmochim Acta* **62**: 1745–1756.

Hopwood DA, Bibb MJ, Chater KF, Keiser T, Bruton CJ, Keiser HM *et al.* (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. The John Innes Foundation: Norwich, United Kingdom.

Khalil MAK, Rasmussen RA. (1990). Global increase of atmospheric molecular hydrogen. *Nature* **347**: 743–745.

Kim SB, Falconer C, Williams E, Goodfellow M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. *Int J Syst Bacteriol* **48**: 59–68.

King GM. (2003a). Contributions of atmospheric CO and hydrogen uptake to microbial dynamics on recent Hawaiian volcanic deposits. *Appl Environ Microbiol* **69**: 4067–4075.

King GM. (2003b). Uptake of carbon monoxide and hydrogen at environmentally relevant concentrations by mycobacteria. *Appl Environ Microbiol* **69**: 7266–7272.

King GM, Weber CF. (2008). Interactions between bacterial carbon monoxide and hydrogen consumption and plant development on recent volcanic deposits. *ISMEJ* **2**: 195–203.

Klüber HD, Conrad R. (1993). Ferric iron-reducing *Shewanella putrefaciens* and N_2-fixing *Bradyrhizobium japonicum* with uptake hydrogenase are unable to oxidize atmospheric H_2. *FEMS Microbiol Lett* **111**: 337–341.

Kristjansson JK, Schönheit P, Thauer RK. (1982). Different K_s values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Arch Microbiol* **131**: 278–282.

Krumholz LR, Harris SH, Tay ST, Suflita JM. (1999). Characterization of two subsurface H_2-utilizing bacteria, *Desulfomicrobium hypogeium* sp. nov. and *Acetobacterium psammolithicum* sp. nov., and their ecological roles. *Appl Environ Microbiol* **65**: 2300–2306.

Labbé N, Juteau P, Parent S, Villemur R. (2003). Bacterial diversity in a marine methanol-fed denitrification reactor at the Montreal Biodome, Canada. *Microb Ecol* **46**: 12–21.

La Favre JS, Focht DD. (1983). Conservation in soil of H_2 liberated from N_2 fixation by Hup^- nodules. *Appl Environ Microbiol* **46**: 304–311.

Leach MR, Zamble DB. (2007). Metallocenter assembly of the hydrogenase enzymes. *Curr Opin Chem Biol* **11**: 159–165.

Lovley DR, Goodwin S. (1988). Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochim Cosmochim Acta* **52**: 2993–3003.

Lovley DR, Phillips EJP, Lonergan DJ. (1989). Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl Environ Microbiol* **55**: 700–706.

Luijten MLGC, Roelofsen W, Langenhoff AAM, Schraa G, Stams AJM. (2004). Hydrogen threshold concentrations in pure cultures of halorespiring bacteria and at a site polluted with chlorinated ethenes. *Environ Microbiol* **6**: 646–650.

Maimaiti J, Zhang Y, Yang J, Cen Y-P, Layzell DB, Peoples M *et al.* (2007). Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. *Environ Microbiol* **9**: 435–444.

Muyzer G, De Waal EC, Uitterlinden AG. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695–700.

Novelli PC, Lang PM, Masarie KA, Hurst DF, Myers R, Elkins JW. (1999). Molecular hydrogen in the troposphere: global distribution and budget. *J Geophys Res* **104**: 30427–30444.

Rhee TS, Brenninkmeijer CAM, Röckmann T. (2006). The overwhelming role of soils in the global atmospheric hydrogen cycle. *Atmos Chem Phys* **6**: 1611–1625.

Robinson JA, Tiedje JM. (1982). Kinetics of hydrogen consumption by rumen fluid, anaerobic digestor sludge, and sediment. *Appl Environ Microbiol* **44**: 1374–1384.

Sambrook J, Russell DW. (1989). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA.

Schuler S, Conrad R. (1990). Soils contain two different activities for oxidation of hydrogen. *FEMS Microbiol Lett* **73**: 77–83.

Schultz MG, Diehl T, Brasseur GP, Zittel W. (2003). Air pollution and climate-forcing impacts of a global hydrogen economy. *Science* **302**: 624–627.

Schütz H, Conrad R, Goodwin S, Seiler W. (1988). Emission of hydrogen from deep and shallow freshwater environments. *Biogeochemistry* **5**: 295–311.

Shima S, Thauer RK. (2007). A third type of hydrogenase catalyzing H_2 activation. *Chem Rec* **7**: 37–46.

Simmonds PG, Derwent RG, O'Doherty S, Ryall DB, Steele LP, Langenfelds RL *et al.* (2000). Continuous high-frequency observations of hydrogen at the Mace Head baseline atmospheric monitoring station over the 1994–1998 period. *J Geophys Res* **105**: 12105–12121.

Smatlak CR, Gossett JM, Zinder SH. (1996). Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture. *Environ Sci Technol* **30**: 2850–2858.

Stein S, Selesi D, Schilling R, Pattis I, Schmid M, Hartmann A. (2005). Microbial activity and bacterial composition of H_2-treated soils with net CO_2 fixation. *Soil Biol Biochem* **37**: 1938–1945.

Tans PP, Conway TJ, Nakazawa T. (1989). Latitudinal distribution of the sources and sinks of atmospheric carbon dioxide derived from surface observations and an atmospheric transport model. *J Geophys Res* **94**: 5151–5172.

Ureta A-C, Imperial J, Ruiz-Argüeso T, Palacios JM. (2005). *Rhizobium leguminosarum* biovar *viciae* symbiotic hydrogenase activity and processing are limited by the level of nickel in agricultural soils. *Appl Environ Microbiol* **71**: 7603–7606.

Vincent KA, Cracknell JA, Clark JR, Ludwig M, Lenz O, Friedrich B *et al.* (2006). Electricity from low-level H_2 in still air—an ultimate test for an oxygen tolerant hydrogenase. *Chem Comm* **48**: 5033–5035.

Volff J-N, Altenbuchner J. (1998). Genetic instability of the *Streptomyces* chromosome. *Mol Microbiol* **27**: 239–246.

Warwick NJ, Bekki S, Nisbet EG, Pyle JA. (2004). Impact of a hydrogen economy on the stratosphere and troposphere studied in a 2-D model. *Geophys Res Lett* **31**: L05107, doi:10.1029/2003GL019224.