Soil Biology & Biochemistry 41 (2009) 2178–2186

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Efficient mineral weathering is a distinctive functional trait of the bacterial genus *Collimonas*

S. Uroz [a,*], C. Calvaruso [b], M.P. Turpault [b], A. Sarniguet [c], W. de Boer [d], J.H.J. Leveau [d,e], P. Frey-Klett [a]

[a] INRA-UHP, UMR1136, INRA/Nancy Université, "Interactions Arbres Micro-organismes", Centre INRA de Nancy, 54280 Champenoux, France
[b] INRA, UR1138 "Biogéochimie des Ecosystèmes Forestiers", Centre INRA de Nancy, 54280 Champenoux, France
[c] INRA-Agrocampus Rennes, UMR1099 'Biologie des Organismes et des Populations appliquée à la Protection des Plantes', 35 653 Le Rheu Cedex, France
[d] Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, Heteren, The Netherlands
[e] University of California, Department of Plant Pathology, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 13 May 2009
Received in revised form
6 July 2009
Accepted 31 July 2009
Available online 14 August 2009

Keywords:
Mineral weathering
Collimonas
Mechanism
Biotite
Mycorrhizosphere
Nutrient-poor environment

ABSTRACT

The mineral weathering ability of 45 bacterial strains belonging to the genus *Collimonas* and coming from various terrestrial environments was compared to that of 5 representatives from the closely related genera *Herbaspirillum* and *Janthinobacterium*. Using glucose as the sole carbon source in a microplate assay for quantifying the release of iron and protons from biotite, all *Collimonas* strains proved to be very efficient weathering agents, in contrast to the *Herbaspirillum* and *Janthinobacterium* strains. The weathering phenotype was also evident during growth of collimonads on mannitol and trehalose, but not on gluconic acid. All *Collimonas* strains were able to solubilize inorganic phosphorus and produce gluconic acid from glucose, suggesting that acidification is one of the main mechanisms used by these bacteria for mineral weathering. The production of siderophores may also be involved, but this trait, measured as the ability of collimonads to mobilize iron, was shared with *Herbaspirillum* and *Janthinobacterium* strains. These findings are discussed in an ecological context that recognizes collimonads as mycophagous (fungal-eating) and efficient mineral weathering bacteria and suggests that this ability has evolved as an adaptation to nutrient-poor conditions, possibly as part of a mutualistic relationship with mycorrhizal fungi.

1. Introduction

Mineral weathering, by geochemical and biological means, plays a fundamental role in the environment by shaping the landscape and influencing soil fertility and water quality. Moreover, it controls the availability of inorganic nutrients for living organisms. For instance, in nutrient-poor soils, microorganisms with mineral weathering ability play a key role in plant nutrition (Marschner, 1995; Calvaruso et al., 2006). However a lot remains to be learned about their diversity and their distribution.

In temperate forest ecosystems, most trees live in close association with ectomycorrhizal fungi. These symbiotic fungi connect the tree roots to the soil nutrient resources via a true hyphal pipeline and the production of weathering organic acid molecules (Landeweert et al., 2001; Van Breemen et al., 2000). They exert selective pressure on soil bacterial communities (Frey et al., 1997) in their vicinity, the mycorrhizosphere, for bacterial strains efficient in mineral weathering (Frey-Klett et al., 2005; Calvaruso et al., 2007; Uroz et al., 2007). Interestingly, among a collection of 32 mycorrhizosphere bacterial isolates that are very efficient at mineral weathering using glucose as sole carbon source, six *Collimonas* strains have recently been identified (Uroz et al., 2007). This genus was previously described as being able to grow at the expense of living fungal hyphae (mycophagy) and to hydrolyze chitin (De Boer et al., 2004, 2005). It was also shown to be specifically associated with arbuscular mycorrhizal plants of *Medicago truncatula* and was detected in lichen-dominated surface soils and with bryophytes (Aspray et al., 2005; Männistö and Häggblom, 2006; Offre et al., 2007; Opelt and Berg, 2004). Thus the genus *Collimonas* has so far mainly been recognized as a fungal-associated genus (Leveau et al., in press).

Tolerance to nutrient-poor conditions may be important in the survival and the multiplication of collimonads. *Collimonas* strains have been frequently isolated from low organic, oligotrophic sandy environments e.g. dune soils (De Boer et al., 1998; Höppener-Ogawa et al., 2007). Moreover, Höppener-Ogawa et al. (2007) recently demonstrated by qPCR that the abundance of soils collimonads was significantly higher in the mineral than organic layer of forest soils.

* Corresponding author. Tel.: +33 (0) 3 83 39 41 49; fax: +33 (0) 3 83 39 40 69.
E-mail address: uroz@nancy.inra.fr (S. Uroz).

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doi:10.1016/j.soilbio.2009.07.031

This result suggests that the conditions occurring in the mineral horizon of the soil such as the inorganic nutrient status may influence the abundance of the collimonads. In this context, mineral weathering ability may be a functional and ecological trait of the *Collimonas* genus.

To test this hypothesis, a collection of collimonads was assayed for their mineral weathering ability. Fifty collimonads and closely related strains originating from relatively nutrient-poor environments, where inorganic nutrients are poorly bioavailable, such as forest soils, tundra soils, heathland and coastal dunes were screened for the ability to solubilize phosphorus, to mobilize iron and to weather biotite, a phyllosilicate occurring frequently in soils. The impact of the carbon substrate on the mineral weathering ability was also tested and the data were analyzed statistically.

2. Materials and methods

2.1. Bacterial strains and growth media

A total of 50 bacterial strains from various terrestrial environments was used in this study (Table 1). The majority of the bacterial strains came from lab collections; the *Collimonas* strains from Tundra soils were kindly provided by Dr. Minna Männistö (Finnish Forest Research Institute, Finland). The characteristics of the soils (pH, type of soil texture and/or bedrock, vegetation and location) from which the collimonads were isolated are presented in Table 2. Three reference strains of *Burkholderia* [PN3(3), PML1(4) and PML1(12)] were used as negative and positive controls for the mineral weathering assays as described in Uroz et al. (2007). The strain PN3(3) is unable to weather biotite contrary to the strains PML1(4) and PML1(12) which are efficient. Bacterial strains were grown at 25 °C on 1/10-strength tryptic soy agar (TSA) medium (3 g L^{-1} Tryptic Soy Broth from Difco and 15 g L^{-1} agar). All the bacterial strains were cryopreserved at −80 °C in 20% glycerol.

2.2. Phenotypic characterization of the bacterial isolates

Gram determination was performed using the aminopeptidase test from Sigma on the uncharacterized bacterial strains. Each *Collimonas* strain was also tested for its ability to hydrolyze colloidal chitin on minimal agar medium (5 g L^{-1} NaCl; 1 g L^{-1} KH_2PO_4; 0.1 g L^{-1} yeast extract; 20 g L^{-1} agar and 2 g L^{-1} colloidal chitin) adjusted to pH 6,5. Colloidal chitin was prepared as described by Hsu and Lockwood (1975) from crab shells chitin (Sigma).

2.3. Mineral weathering potential

The mineral weathering potential of the bacterial isolates was quantified as described in Uroz et al. (2007). Briefly, 20 μL of a bacterial inoculum (A_{595nm} = 0.8–1) were inoculated in sterile Multiscreen microplates (MAGVN22, 0.22 μm pore size, Millipore) containing 10 mg of sterile biotite particles (diameter, 200–500 μM, which was convenient for the experimental procedure used) and 180 μL of Bushnell–Hass medium (BHm: KCl, 20 mg L^{-1}; $MgSO_4,7H_2O$, 150 mg L^{-1}; $NaH_2PO_4,2H_2O$, 80 mg L^{-1}; $Na_2HPO_4,2H_2O$, 90 mg L^{-1}; $(NH_4)_2SO_4$, 65 mg L^{-1}; KNO_3, 100 mg L^{-1} and $CaCl_2$, 20 mg L^{-1}) devoid of iron, buffered at pH 6.5 and supplemented with glucose (2 g L^{-1}). A selection of bacterial strains was also tested using other carbon sources such as mannitol, trehalose and gluconic acid (2 g L^{-1}). The biotite was obtained from Bancroft (Canada), and is a 2:1 phyllosilicate, which is frequently present in acid soil, which weathers relatively quickly and holds K, Mg and Fe nutrient elements. It is a pure homogeneous mineral and its composition is in g kg^{-1}: SiO_2, 410.1; Al_2O_3, 109; Fe_2O_3, 22.1; FeO, 100.5; MnO, 2.7; MgO, 189; Na_2O, 4.1; K_2O, 94.6; TiO_2, 22.8; F, 44.2 and Zn, 0.8. Its structural formula is (Si_3Al_1) $(Fe^{3+}_{0.12}$ $Fe^{2+}_{0.61}$ $Mg_{2.06}$ $Mn_{0.02}$ $Ti_{0.13})$ and $K_{0.88}$ $Na_{0.06}$ O_{10} $(OH_{0.98}$ $F_{1.02})$. Biotite and culture media were sterilized by autoclaving (20 min at 120 °C).

Each bacterial strain was inoculated in 8 wells of the microplates: 4 were used to estimate weathering ability and 4 to determine the pH, as described below. *Burkholderia* strains [PN3(3)] and [PML1(4)], (Table 1), were used as negative and positive controls, respectively. Another negative control consisted of adding 200 μL of BHm medium only, to the biotite (no bacteria).

After a 48-hr incubation at 25 °C under constant agitation, the MultiScreen microplates were centrifuged and filtrates (0.22 μm) were transferred to a new microplate containing 20 μL of ferrospectral® (Merck, for iron quantification) or bromocresol green (1 g L^{-1}, Sigma, for pH determination). The amount of total iron (Fe^{2+} and Fe^{3+}) released from biotite in the solution and the pH were estimated from $A_{595\ nm}$ measurements on a Bio-Rad model 550 microplate reader, based on calibration curves. The average values of the four replicates for iron quantification and for pH measurements were taken as the weathering potential of each isolate. To investigate which mechanisms could be involved in the bacterial dissolution of the biotite, abiotic assays were performed using serial dilutions of a complexing agent (citric acid, 10^{-3} M) and a strong acid (hydrochloric acid, concentration adjusted to pH 6–2). The synthetic iron chelator Deferoxamine methanesulfonate (DFAM) (Sigma, 75–150 μM) was used as a control (Kalinowski et al., 2000). The data obtained with citric and hydrochloric acids were used to draw two reference curves corresponding to the complexation and acidification reactions that occur during the weathering process.

2.4. In vitro assays for inorganic phosphorus solubilization and siderophore production

The ability of bacterial strains to solubilize tricalcium orthophosphate via the production of acid compounds and to mobilize iron via the production of siderophores was assessed on solid tricalcium phosphate (TCP) and chrome azurol S (CAS) media, respectively, following the protocol of Frey-Klett et al. (2005). Briefly, each bacterial isolate was grown on 10% TSA medium at 25 °C for 48 h. The bacteria were then collected and suspended in sterile water to obtain a suspension with A_{595nm} = 0.7 (ca 10^9 cells mL^{-1}). For each bacterial isolate, 10 μL of inoculum was dropped in the center of three plates. After incubation at 25 °C for 7 days, the clearing of the initially turbid medium indicated phosphate solubilization and iron mobilization on TCP and CAS media, respectively. All the bacterial strains grew on these media and the diameter of the haloes around the bacteria were measured and averaged. According to these values, the bacterial isolates were distributed into two classes based on discoloration response on the CAS or TCP media (0 and +).

2.5. Determination of gluconic acid production

As D-gluconic acid is known to be a weathering agent produced by bacteria (Lin et al., 2006), its production by the bacterial strains was determined in the same culture supernatant as the one used for pH measurement and iron quantification in the weathering microplate assay via an enzymatic bioassay, according to the manufacturer's instructions (kit 10428191035 from r-biopharm/Roche®). Briefly, this assay links two enzymes, Gluconate kinase and 6-Phosphogluconate dehydrogenase respectively, to generate NADPH stoichiometrically with the D-gluconic acid present in the sample. NADPH concentrations were determined from $A_{340\ nm}$ measurements (Krishnaraj and Goldstein, 2001).