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

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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.

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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.

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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).

Table 1
Mineral weathering ability of the bacterial strains used in this study.

Strain	Reference	Origin	Fe*	pH**	GA***	TCP	CAS
Burkholderia							
PML1(4)	Uroz et al., 2007	Forest soil[a]	1.86	3.33	0.2	+	+
PML1(12)	Uroz et al., 2007	Forest soil[a]	1.66	3.37	*n.d.*	+	+
PN3(3)	Uroz et al., 2007	Forest soil	0	6.25	0	–	–
Collimonas							
France							
C2PN2(1)	Lab collection	Forest soil	0.94	3.65	*n.d.*	+	+
PML3(2)	Uroz et al., 2007	Forest soil[a]	1.26	3.50	*n.d.*	+	+
PML3(4)	Uroz et al., 2007	Forest soil[a]	0.95	3.43	*n.d.*	+	–
PML3(7)	Uroz et al., 2007	Forest soil[a]	1.56	3.48	*n.d.*	+	+
PML3(8)	Uroz et al., 2007	Forest soil[a]	1.52	3.46	*n.d.*	+	+
PMB2(3)	Uroz et al., 2007	Forest soil[a]	0.75	3.57	1.47	+	+
PMB3(1)	Uroz et al., 2007	Forest soil[a]	2.88	3.09	1.58	+	+
EPMY1(18)	Lab collection	Forest soil[a]	1.72	3.39	*n.d.*	+	+
EPMY1(19)	Lab collection	Forest soil[a]	1.38	3.15	*n.d.*	+	+
BPN7(2)	Lab collection	Forest soil	1.65	3.96	*n.d.*	+	+
BPN7(3)	Lab collection	Forest soil	0.80	3.82	*n.d.*	+	+
BPML7(1)	Lab collection	Forest soil[a]	1.54	3.44	*n.d.*	+	+
BPML7(4)	Lab collection	Forest soil[a]	1.47	3.9	*n.d.*	+	+
Netherlands							
Ter 6	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.70	3.5	*n.d.*	+	+
Ter 10	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.71	3.43	1.62	+	+
Ter 14	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.19	3.52	1.42	+	+
Ter 90	De Boer et al., 1998; 2004	Coastal dune grassland[b]	1.40	3.46	0.74	+	+
Ter91	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.35	3.35	*n.d.*	+	+
Ter 118	De Boer et al., 1998, 2004	Coastal dune grassland[b]	0.85	3.65	0.67	+	+
Ter 146	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.76	3.47	*n.d.*	+	+
Ter 165	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.21	3.49	*n.d.*	+	+
Ter166	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.28	3.40	1.49	+	+
Ter 228	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.12	3.57	*n.d.*	+	+
Ter 252	De Boer et al., 1998, 2004	Coastal dune grassland[b]	0.98	3.48	*n.d.*	+	+
Ter 282	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.54	3.45	1.24	+	+
Ter 300	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.07	3.43	1.49	+	+
Ter 330	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.49	3.46	*n.d.*	+	+
Ter 331	De Boer et al., 1998, 2004	Coastal dune grassland[b]	1.76	3.48	1.42	+	+
R35512	Höppener-Ogawa et al., 2007	River dune grassland[b]	1.30	3.36	*n.d.*	+	+
R35513	Höppener-Ogawa et al., 2007	Heathland[b]	1.56	3.36	*n.d.*	+	+
R35514	Höppener-Ogawa et al., 2007	Heathland[b]	1.37	3.41	0.92	+	–
R35515	Höppener-Ogawa et al., 2007	Heathland[b]	1.51	3.36	1.80	+	+
Finland							
RAJ3R1	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	1.15	3.7	2	+	+
M2TIA	Männistö and Häggblom, 2006	Mire pond sediment	1.26	3.62	2	+	+
K2X3	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	1.24	3.39	1.98	+	+
RA1BR1	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	1.13	3.44	1.81	+	+
M1J3	Männistö and Häggblom, 2006	Arctic tundra soil[c]	0.77	3.49	1.75	+	+
K2E1	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	1.17	3.45	1.86	+	+
M1R1	Männistö and Häggblom, 2006	Arctic tundra soil[c]	1.27	3.76	1.74	+	+
M1T7	Männistö and Häggblom, 2006	Arctic tundra soil[c]	0.71	3.59	1.78	+	+
S5T5	Männistö and Häggblom, 2006	Arctic tundra soil[c]	0.6	3.6	1.94	+	+
R5TN1	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	1.35	3.43	2.75	+	+
M1T1	Männistö and Häggblom, 2006	Arctic tundra soil[c]	0.87	3.6	*n.d.*	+	+
K1E3	Männistö and Häggblom, 2006	boreal oligotrophic forest soil[c]	0.79	3.55	*n.d.*	+	+
S1E3	Männistö and Häggblom, 2006	Arctic tundra soil[c]	1.25	3.47	n.d.	+	+
Herbaspirillum							
seropedicae DSM6445T	Baldani et al., 1996	Rice roots	0	6.25	0	–	+
rubrisubalbicans ATCC 19308T	Baldani et al., 1996	*Saccharum officinalis*	0.02	5.01	0	–	+
frisingense DSM 13128T	Kirchhof et al., 2001	*Miscanthus sacchariflrus*	0	6.21	0	–	–
Janthinobacterium							
agaricidamnosum DSM9628	Lincoln et al., 1999	Rotting *Agaricus bisporus*	0.18	4.57	0.01	–	+
lividum P63	De Boer et al., 1998	soil	0.05	5.16	0.04	–	–

*Amount of Fe released from the biotite in mg L^{-1}. Mean value of four replicates.
**Mean value of the pH obtained from four replicates.
***GA: Amount of gluconic acid produced in g L^{-1}.
The symbol + indicates the solubilization of phosphorus or the mobilization of iron, and the symbol – indicates no solubilization or mobilization.
n.d.: not determined.
[a] mycorrhizosphere environment.
[b] soil rich in fungi.
[c] soil rich in lichen.

Table 2
Soil characteristics of locations from which the studied collimonads were isolated.

	Site sample	Location	Soil texture and/or bedrock	pH	C (g kg^{-1})	N (g kg^{-1})	C/N	P**(g kg^{-1})	Vegetation
France[a]	Breuil-Chenue (Morvan)	(47°18′N, 4°5′ E)	Sandy-loam*	4	66	3.6	18.3	0.15	Forest (*Quercus sessiliflora*, *Fagus sylvatica* and *Picea abies*)
Finland[b]	Saariselkä located in eastern Lapland.	(68°25′N, 27°25′E)	Lake sediment	n.d.	n.d.	n.d.	n.d.	n.d.	No vegetation
	Kilpisjärvi region	(69°10′N, 20°50′E)	Podzol	5.3	2.9	0.17	25	0.23	Arctic–alpine tundra soils dominated by dwarf shrubs (Empetrum nigrum, Betula nana, Vaccinium spp.)
	Rajajooseppi	(68°28′N, 28°28′E)	Podzol	5	n.d.	n.d.	n.d.	n.d.	Oligotrophic lichen-dominated Scots pine forests
	Kätkäsuvanto	(68°08′N, 23°21′E)		4.9	n.d.	n.d.	n.d.	n.d.	
Netherlands[c]	Island of Terschelling	(53°23′N, 5°16′E)	Sandy	5.3	6.1	0.35	17.4	0.5	Grasses/herbs
	Veluwe	(52°04′N, 5°45′E)	Sandy, podzol	4.1	77.5	2.36	32.8	0.5	Heathland (*Calluna vulgaris*)
	Maas & Waal	(51°10′N, 5°40′E)	Sandy	4.9	9.6	0.81	11.9	0.5	Grasses/herbs

*Developed on the "Pierre qui Vire" granite. The soil is Sandy-loam textured (60% sand and <20% clays).
**Phosphore available.
n.d.: not determined.
[a] Adapted from Calvaruso et al. (2009) and Louis Mareschal (Personal communication).
[b] Adapted from Männistö and Häggblom (2006); Männistö et al. (2007); Eskelinen et al. (2009).
[c] Adapted from Höppener-Ogawa et al. (2007).

2.6. Genotypic identification of the bacterial strains and phylogenetic analysis

rrs gene amplification was performed on the bacterial strains using the universal primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and 907 R (5′-CCGTCAATTCMTTTGAGTTT-3′). Sequencing reactions were analyzed in the multicapillary BECKMAN CEQ 8000XL automated sequencer system. The sequencing primer used was 518r (5′ATTACCGCGGATGCTGG-3′) (Lane, 1991). The partial 16S rRNA gene sequences from all bacterial strains were aligned with published 16S rRNA gene sequences from α-, β- and γ-proteobacteria using Clustal X (version 1.8) (Thompson et al., 1994). Phylogenetic algorithms and tree design (DNA-DIST, NEIGHBOR, and SEQBOOT) were done using the PHYLIP 3.65 package (version 3.65; J. Felsenstein, University of Washington, Seattle [http://evolution.genetics.Washington.edu/phylip.html]). Bootstrap analysis was based on 1000 replicates.

2.7. Statistical analysis

The effect of the substrate source (glucose, mannitol, trehalose or gluconic acid) on the mineral weathering ability of each bacterial strain was determined by a one-factor analysis of variance (one-factor ANOVA) at the threshold level of $P = 0.05$ and by the Bonferroni–Dunn test. The effect of the ecological origin (acid forest, dune, oligotrophic forest, tundra) on the mineral weathering ability of the collimonads was determined by analysis of variance (one-factor ANOVA) at the threshold level of $P = 0.05$ and by the Fisher test. Both analysis were performed using the Superanova software (Abacus Concepts, Inc., Berkeley, CA).

3. Results

3.1. Mineral weathering ability of members of the genus Collimonas

Fourty five *Collimonas* strains belonging to the *Collimonas* (3×) *fungivorans*, *Collimonas arenae*, *Collimonas pratensis* or unidentified species were analyzed for their ability to weather biotite in the microplate assay developed by Uroz et al. (2007). Five strains belonging to the closely related genera *Herbaspirillum* and *Janthinobacterium* were also tested along with three strains of *Burkholderia* [PN3(3), PML1(4) and PML1(12)] that served as positive and negative controls. After two days of incubation, all collimonads, (clusters E to G in Fig. 1, Table 1), were very efficient in extracting iron from the biotite and acidifying the medium. Their efficiencies ranged from 0.8 to 1.76 mg L^{-1} of Fe released from the biotite and with end-point pH values of ca. 3.5 (Table 1). In contrast, among the *Herbaspirillum* and *Janthinobacterium* strains, *Janthinobacterium agaricidamnosum* DSM9628 was the only one able to weather the biotite, but with a very low efficiency (0.18 mg L^{-1} of Fe). The other strains were null or close to the detection limit level. All the collimonads appeared also capable of hydrolyzing chitin (data not shown).

3.2. Impact of the carbon substrate on the mineral weathering potential of the collimonads

The mineral weathering potentials presented above were measured with glucose as the sole carbon substrate in the microplate assay. As sugar utilization varies among the soil bacteria and could directly or indirectly influence their weathering ability, three other carbon sources that can be present in the fungal microenvironment, were tested on 16 *Collimonas* strains that were randomly chosen from the total collection (Table 3). The impact of these carbon sources was also tested for three of our reference strains of *Burkholderia* [PN3(3), PML1(4) and PML1(12)] described in Uroz et al. (2007). All the bacterial strains tested were able to use these carbon sources as demonstrated by their growth in liquid medium (data not shown). Interestingly, most of the collimonads were able to weather biotite using mannitol or trehalose, but not gluconic acid, as sole carbon source (Table 3). Moreover, no pH acidification was observed in the culture medium amended with gluconic acid whatever the bacterial strain. Weathering potential of collimonads appeared generally higher with glucose than with the other substrates; the exceptions were strains K2E1 which extracted significantly more iron with mannitol than with glucose (ca. 0.9 mg L^{-1} Fe released with glucose and 1.16 mg L^{-1} Fe released with mannitol) and PMB2(3) which extracted slightly more iron with trehalose than with glucose (0. 75 mg L^{-1} Fe released with glucose and 0.80 mg L^{-1} Fe released with trehalose) (Table 3). Interestingly, five strains (S1E3, K2E1, Ter166, Ter91, R35512) showed the same levels of iron extracted from biotite with glucose and mannitol, but not the same levels of acidification (Table 3).

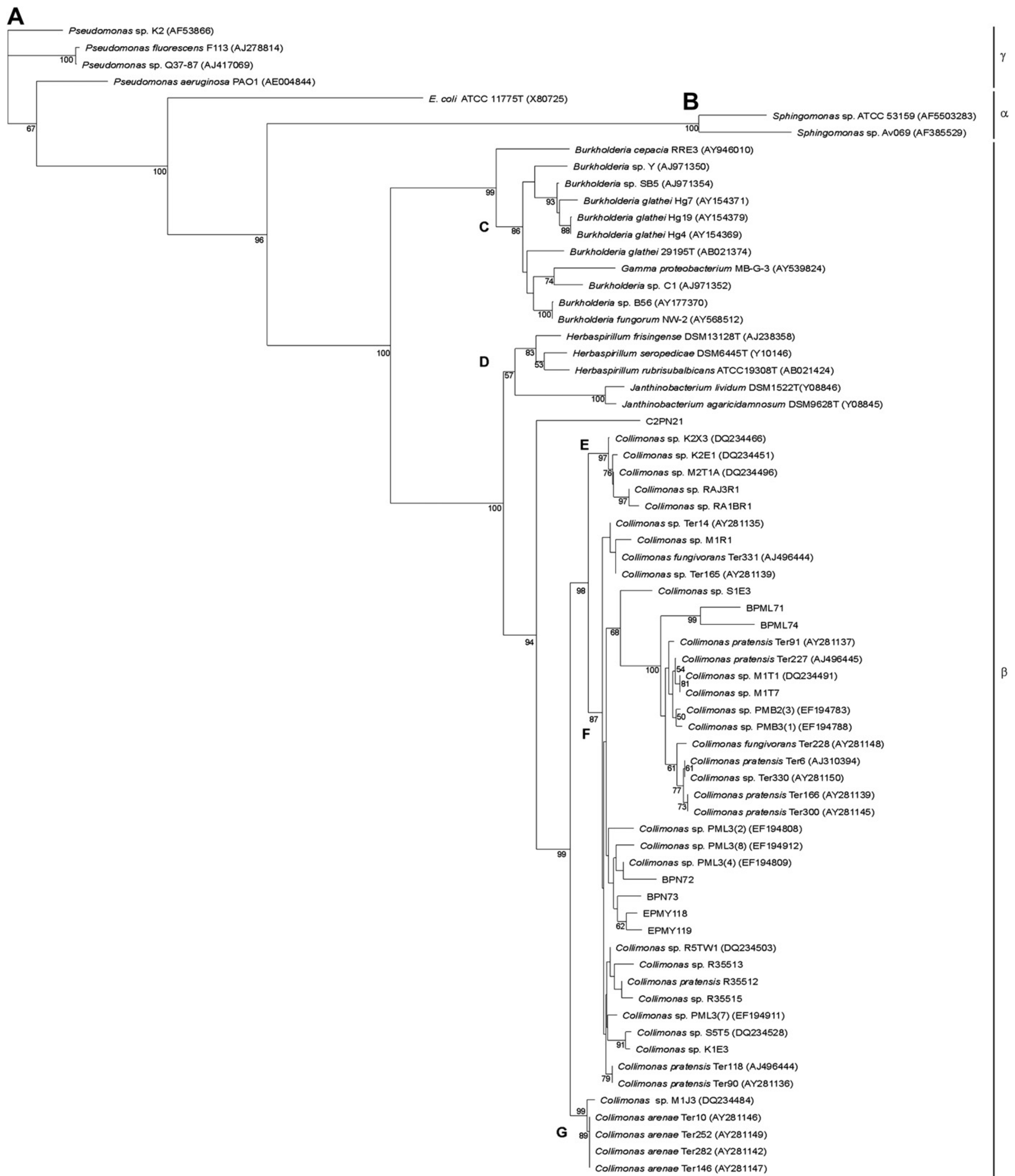

Fig. 1. Neighbor-joining tree showing the phylogenetic relationships of the bacterial strains used in this study and their closest relatives among known species, based on PCR sequencing of a portion of the 16S rRNA gene with primers pA and 907R. A bootstrap analysis was performed with 1000 repetitions, and only values greater than 50 are indicated at the nodes. The bootstrap analysis identified seven clusters: A, B, C, D, E, F and G. The origin of each strain is described in Table 1.

Table 3
Impact of the carbon source on the mineral weathering ability.

	Fe**				pH***			
	Glucose*	Mannitol	Trehalose	Gluconate	Glucose	Mannitol	Trehalose	Gluconate
Collimonas								
PML3(2)	1.26[a]	0.29[b]	0.26[b]	0[c]	3.50[a]	4.31[b]	4.43[c]	6.22[d]
PML3(4)	0.96[a]	0.02[b]	0[c]	0[c]	3.43[a]	4.86[b]	6.22[c]	6.22[c]
PML3(7)	1.56[a]	0.27[b]	0[c]	0[c]	3.48[a]	4.54[b]	6.22[c]	6.22[c]
PML3(8)	1.52[a]	0.30[b]	0.84[c]	0[d]	3.46[a]	4.69[b]	4.77[c]	6.22[d]
PMB2(3)	0.75[a]	0.31[b]	0.80[c]	0[d]	3.57[a]	4.18[b]	4.79[c]	6.22[d]
PMB3(1)	2.88[a]	0.32[b]	0.15[c]	0[d]	3.09[a]	4.42[b]	5.05[c]	6.22[d]
S1E3	1.25[a]	1.18[a]	0.30[b]	0[c]	3.47[a]	4.75[b]	4.49[c]	6.22[d]
K2E1	0.90[a]	1.16[b]	0[c]	0[c]	3.53[a]	4.72[b]	6.22[c]	6.22[c]
K2X3	1.24[a]	1.11[a]	0.28[b]	0[c]	3.41[a]	4.93[b]	4.43[c]	6.22[d]
Ter330	1.49[a]	0.90[b]	0.22[c]	0[d]	3.46[a]	4.83[b]	4.43[c]	6.22[d]
Ter166	1.28[a]	1.16[a]	0.23[b]	0[c]	3.41[a]	4.86[b]	4.44[c]	6.22[d]
Ter165	1.21[a]	1.16[b]	0.54[c]	0[d]	3.49[a]	4.86[b]	3.97[c]	6.22[d]
Ter91	1.35[a]	1.26[a]	0.58[b]	0[c]	3.35[a]	4.82[b]	4.20[c]	6.22[d]
R35513	1.56[a]	1.06[b]	0.02[c]	0[c]	3.36[a]	4.77[b]	6.22[c]	6.22[c]
R35512	1.30[a]	1.26[a]	0.31[b]	0[c]	3.36[a]	4.83[b]	5.04[c]	6.22[d]
R35515	1.51[a]	1.09[b]	0.21[c]	0[d]	3.36[a]	4.77[b]	4.45[c]	6.22[d]
Burkholderia								
PML1(4)	1.86[a]	0.24[b]	0[c]	0[c]	3.33[a]	4.20[b]	6.23[c]	6.22[c]
PML1(12)	1.66[a]	0.11[b]	0[c]	0[c]	3.37[a]	4.83[b]	6.23[c]	6.22[c]
PN3(3)	0[a]	0[a]	0[a]	0[a]	6.23[a]	6.23[a]	6.23[a]	6.22[a]

*Each carbon source was tested at 2 g L^{-1}.
**Amount of Fe released from the biotite in mg L^{-1}. Mean value of four replicates.
***The pH is a mean value of four replicates.
For each bacterial strain (in line) different letters indicate that the amount of Fe released or the pH measured are significantly different according to a one-factor (substrate) ANOVA ($P = 0.05$) and the Bonferroni–Dunn test.

Concerning the other substrates, the collimonads were generally more efficient for mineral weathering with mannitol than trehalose, apart from the strains PML3(8) and PMB2(3) which were more efficient with trehalose (ca. 0.8 mg L^{-1} Fe released with trehalose and 0.3 mg L^{-1} Fe released with mannitol). Each *Collimonas* strain showed significantly more acidification with glucose than with mannitol or trehalose (Table 3).

3.3. Mineral weathering mechanisms

To determine the potential mechanism of biotite weathering by the collimonads, serial dilutions of citric acid and hydrochloric acid were tested as well as different concentrations of the synthetic iron chelator Deferoxamine methanesulfonate. The results obtained using the microplate assay with glucose as sole carbon source demonstrated that the data points for all tested collimonads fell along the reference curve for acidification (Fig. 2), suggesting that they use mainly an acidification process to solubilize biotite. To elaborate further on the mineral weathering mechanism, we tested the *Collimonas, Herbaspirillum* and *Janthinobacterium* strains for their ability to solubilize phosphate and to mobilize iron on TCP and CAS media, respectively. All the *Collimonas* strains were able to solubilize inorganic phosphorus with glucose as sole carbon source, whereas the *Herbaspirillum* and *Janthinobacterium* strains were not (Table 1). On the contrary, most of the *Collimonas*, *Herbaspirillum* and *Janthinobacterium* strains were capable of producing siderophores and/or complexing molecules, except two strains of *Collimonas* and one strain of *Herbaspirillum* and *Janthinobacterium* (Table 1). These two strains of *Collimonas* (PML3(4) and R35514; Table 1) were nevertheless as efficient in weathering biotite in the microplate assay as the other collimonads.

As gluconic acid is among the very few bacterial metabolites shown to be involved in phosphorus solubilization (Kim et al., 1997; Rodriguez et al., 2001) and mineral weathering (Wu et al., 2007), we tested 12 randomly picked *Collimonas* strains from our collection and all the *Herbaspirillum* and *Janthinobacterium* strains for gluconic acid production. After two-days incubation in the microplate assay with glucose as sole carbon source, production of gluconic acid (0.66–1.8 g L^{-1}) was shown for the collimonads but not for the *Herbaspirillum* and *Janthinobacterium* (Table 1).

3.4. Relationship between the ecological origin of the collimonads and related genera and their mineral weathering ability

All the strains of *Collimonas* that we tested came from non-fertilized soil environments (temperate forest, coastal dune, river dune, heathland, tundra and oligotrophic forest) characterized by

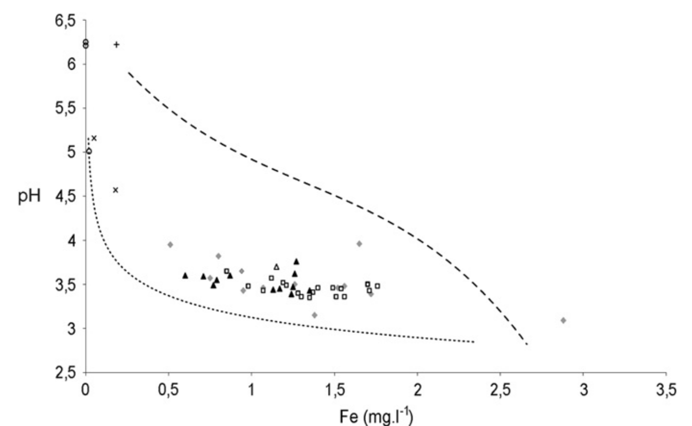

Fig. 2. Relationship between the ecological origin of the bacterial strains and their mineral weathering ability. Each symbol represents a collimonads strain from one of the three origins, from The Netherlands (coastal dune grassland, river dune grassland, heathland; open squares), France (acid forest soil; grey diamonds) and Finland (boreal oligotrophic forest soil, Mire pond sediment, Artic tundra soil; solid triangles). The two curves indicate the mineral weathering effect of a complexing agent (citric acid)(upper line) and a strong acid (hydrochloric acid) (lower line). The synthetic siderophore DFAM (deferroxamine) is presented by a plus sign. The *Herbaspirillum* and *Janthinobacterium* strains are indicated by an open circle and a cross, respectively.

a low content of inorganic nutrients. Most of the collimonads were associated with an environment abundantly colonized by fungi (mycorrhizal and non mycorrhizal fungi or lichens)(Table 1). All the collimonads, independent of their ecological origin, showed a mineral weathering potential ranging from 0.6 to 2.88 mg L^{-1} of Fe released (Fig. 2). Interestingly, the mineral weathering efficacies of the collimonads originated from tundra appeared significantly lower than the ones of dune and acid forest, according to a one-factor ANOVA (ecological origin, $p = 0.048$) and the Fisher test performed on the iron concentrations in the microplate culture filtrates. Among the collimonads related genera, *J. agaricidamnosum* strain DSM9628, which was the only one able to somewhat weather biotite (0.18 mg L^{-1} of Fe released), was isolated from the mushroom *Agaricus bisporus* (Lincoln et al., 1999). The other *Janthinobacterium* and *Herbaspirillum* strains originated in contrast from fertile soils and cultivated plants (Baldani et al., 1996; Kirchhof et al., 2001).

4. Discussion

4.1. *Collimonas genus: a reservoir of efficient mineral weathering bacteria*

Here, we tested the hypothesis that *Collimonas* bacteria, which are best known as mycophagous (fungal-eating) bacteria, possess good mineral weathering abilities. This is the first time that a wide range of *Collimonas* strains was tested for this trait. The 45 *Collimonas* strains tested in this study belong to the *C. fungivorans*, *C. arenae*, *C. pratensis* or unidentified species. The mineral weathering potential measured here was in the same range of that of six collimonads previously described in Uroz et al. (2007) and presented here as reference strains. Notably, strain PMB3(1) remained the most efficient one (Table 1). In contrast, all the strains belonging to the closely related *Herbaspirillum* and *Janthinobacterium* genera were inefficient in weathering biotite, suggesting a specific functional evolution of the collimonads. Moreover, the mineral weathering efficacy of the collimonads measured in this study (mean value: 1.10 mg $L^{-1} \pm 0.04$ Fe released) appeared higher than the mean values measured for *Burkholderia*, *Sphingomonas* or *Pseudomonas* strains by the same microplate bioassay (Uroz et al., 2007). All together, these results expose the *Collimonas* genus as a reservoir of efficient mineral weathering bacteria. Interestingly all the collimonads were isolated from environments with relatively low contents of inorganic nutrient (such as forest soils, tundra soils, heathland and coastal dunes) and fungal rich environments. We hypothesize that the presence of collimonads in environments where inorganic nutrients are limiting factors compared to agricultural soils could result from the high mineral weathering efficacy and the chitinolytic ability of this bacterial genus. Indeed, all the known and newly isolated strains were able to hydrolyze chitin. The significantly lower mineral weathering efficacy measured for collimonads originated from the tundra compared to those originated from the acid forests and dunes, suggests that the mineral weathering efficacy of the bacteria could be impacted by the biogeochemical conditions of their environment. However, the analysis of our data by principal component analysis did not allow us to discriminate different ecotypes among the *Collimonas* strains (data not shown).

4.2. *Effect of the carbon source on the weathering efficacy of the collimonads*

Because collimonads are preferentially associated with the fungal environment (De Boer et al., 2005), we compared different potentially fungal-derived carbon substrates (gluconic acid, mannitol and trehalose) to glucose with respect to their impact on the mineral weathering efficacy of the collimonads. Indeed, it has been demonstrated that the carbon source directly impacts the mineral weathering ability of bacteria (Hameeda et al., 2006; Nautiyal et al., 2000; Uroz et al., 2007). It is also well established that the carbon sources contained in the root- or mycorrhizal root-exudates impact the phylogenetic structure and the functions of the soil bacterial communities (Frey-Klett et al., 2005; Haichar et al., 2008). In this study, the choice of the carbon sources (glucose, gluconic acid, mannitol and trehalose) was based on two reasonings. First, glucose is among the most abundant carbohydrates in soil. Concentrations ranging from milligram to gram per kg of soil were reported in arable and forest soils respectively (Jolivet et al., 2006; Medeiros et al., 2006). Gluconic acid is a by-product of glucose metabolism produced by a direct oxidation of glucose via a membrane-bound dehydrogenase (Geiger and Görisch, 1986; Goldstein, 1995), and suspected to be involved in phosphorus solubilization. Second, mannitol and trehalose are two polyols commonly found in fungi (Martin et al., 1984). Their concentrations range from microgram to milligram per kg of rhizosphere soil (Medeiros et al., 2006). Our result clearly highlighted the relationship between bacterial metabolism and mineral weathering ability (Hameeda et al., 2006; Lin et al., 2006; Nautiyal et al., 2000; Welch and Ullman, 1999). A similar result was previously reported when comparing the mineral weathering potential of *Burkholderia* strains in the presence of glucose or mannitol in the same microplate assay. Most of the *Burkhloderia* strains appeared significantly less efficient with mannitol than glucose (Uroz et al., 2007). Here, we demonstrated for the first time that some collimonads were able to weather biotite with the same efficacy using different carbon substrates (Table 2). Their ability to weather minerals using fungal polyols (mannitol or trehalose) instead of glucose could be an adaptation of the collimonads to their ecological niche permitting them to adjust their activity in relation to the available carbohydrates.

4.3. *Mineral weathering mechanism potentially used by collimonads*

Under aerobic conditions, microorganisms are known to influence the dissolution of soil minerals by two main mechanisms: acidification and complexation (Banfield et al., 1999). Apart from these two mechanisms, the release of protons accompanying respiration and/or ammonium assimilation were also related to phosphorus solubilization (Illmer and Schinmer, 1995). Acidification and complexation mechanisms are linked to the production of geochemically reactive by–products of bacterial metabolism such as organic acids, protons and siderophores. In this study, we combined four bioassays to try to elucidate by which mechanisms collimonads weather minerals. Comparing the iron and pH data measured in the microplate assay using glucose as sole carbon source, with the two acidification and complexation reference curves mostly suggests an acidification mechanism. This hypothesis is consistent with the efficient phosphorus solubilization by the collimonads on the TCP medium. Indeed, the ability to solubilize inorganic phosphorus from the TCP medium is strongly linked to the production of acidic molecules (Goldstein, 1995; Kim et al., 1997, 2005). These results are in accordance with the high concentrations of gluconic acid measured in the culture filtrates of the collimonads, which may thus explain at least partly the mineral weathering efficacy of the collimonads. This is the first report of the production of gluconic acid by collimonads. Beside this acidification mechanism, collimonads are also able to produce chelating compounds as deduced from the results with the CAS medium. The ability to mobilize iron from CAS medium is known to be linked to the production of hydroxamate- and catechol-type siderophores

(Liermann et al., 2000; Schwyn and Neilands, 1987). Further work will be necessary to identify the siderophores produced by collimonads. Interestingly, the nature of the carbon substrate impacts the mineral weathering mechanisms involved. The higher pH values obtained with mannitol or trehalose compared to glucose suggest that collimonads are able to use an alternative mechanism to weather minerals. All together, our results suggest that collimonads can adjust their mineral weathering mechanisms depending on the carbon sources present in their environment. We hypothesize that the soil collimonads, which have access to glucose, weather minerals mainly through an acidification process whereas those which use alternative carbohydrates may implement alternative weathering mechanisms. More research will be needed to elucidate these alternative mechanisms.

4.4. Concluding remarks

This study demonstrates that bacteria belonging to the *Collimonas* genus harbour potential to weather minerals. The *Collimonas* genus, hitherto known for its chitinolytic ability (De Boer et al., 2004; 2005), could have evolved differently from the two closely related genera *Herbaspirillum* and *Janthinobacterium*, by developing nutrient-mobilizing mechanisms especially adapted to its ecological niche in nutrient-poor soils. We hypothesize that in forest mineral horizons, collimonads live in the vicinity of ectomycorrhizal fungi, providing inorganic nutrients to their fungal associate, both participating in the tree nutrition. This mutualism could be based on enhanced inorganic nutrient uptake by mycorrhizal fungi via associated collimonads which, in turn, grow and perform the mineral weathering in exchange for fungal carbohydrates. Such carbohydrates e.g. storage sugars such as trehalose or mannitol would be released either voluntarily by the fungus or involuntarily through mechanisms of bacterial mycophagy.

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