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Minireview

The bacterial genus *Collimonas*: mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments

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Summary

This minireview provides a synopsis of past and present research on the biology and ecology of members of the bacterial genus *Collimonas*. From the distribution, abundance and functional behaviours of these so-called collimonads emerges a general picture of bacterial adaptation to low-nutrient soil environments. Among these adaptations is the ability to extract nutrients from living fungi (mycophagy) and from rocks and minerals (weathering). This unique combination of properties will be discussed in the context of other interactions that collimonads have with their biotic and abiotic surroundings, such as the ability to inhibit fungal growth (fungistasis), protect plant roots from fungal disease (biocontrol), and degrade natural polymers and synthetic pollutants (biodegradation). Future research on *Collimonas* is expected to take advantage of the genomic tools and resources that are becoming available to uncover and describe the genes and gene functions that distinguish this group of bacteria and are the basis for its phenotypes. Potential applications of collimonads include the control of unwanted fungi, for example in agriculture, their use as biological indicators of soil quality and fertility, and as a source of bioactive compounds.

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Introduction

The discovery of bacteria that are now referred to as collimonads predates by a decade the taxonomic description of the genus *Collimonas* (de Boer *et al*., 2004). In September of 1994, as part of a larger study on the natural suppression of fungal root pathogens of Marram grass (*Ammophila arenaria*) (de Boer *et al*., 1998a), the most abundant (based on colony morphology) chitinolytic bacteria that were isolated from coastal dune soils of the Dutch Wadden island Terschelling were initially classified as *Pseudomonas* species based on whole-cell fatty acid methyl ester profiles (de Boer *et al*., 1998b). In a follow-up study (de Boer *et al*., 2001), 16S ribosomal RNA gene sequencing revealed that these so-called Ter strains actually belonged to the β-subclass of *Proteobacteria*, which in 2004 led to the description of *Collimonas* 'genus novus', named for cells ('*monas*') from the hill ('*collis*') or dune, in reference to the original site of discovery.

Collimonas taxonomy

The genus *Collimonas* (de Boer *et al*., 2004) belongs to the order *Burkholderiales*, family *Oxalobacteraceae* (Fig. 1), where its closest relatives are *Herbaspirillum* and *Janthinobacterium* species. To date, the *Collimonas* genus encompasses three formally recognized species, i.e. *Collimonas fungivorans* (de Boer *et al*., 2004), *arenae* and *pratensis* (Höppener-Ogawa *et al*., 2008). The type strains Ter6, Ter10 and Ter91, respectively, all originate from the Terschelling sampling site (de Boer *et al*., 1998b). Based on 16S rRNA gene sequencing, DNA–DNA hybridizations, BOX-PCR genomic fingerprints, whole-cell protein electrophoresis and BIOLOG substrate utilization profiles (de Boer *et al*., 1998b; 2001; 2004; Höppener-Ogawa *et al*., 2008), four clusters can be distinguished within the *Collimonas* genus: A and D include *C. arenae* and *C. pratensis* strains, respectively, while clusters B and C comprise *C. fungivorans* strains. Höppener-Ogawa and colleagues (2008) reported seven isolates from Dutch soils that were classified as *Collimonas* based on their 16S rRNA gene

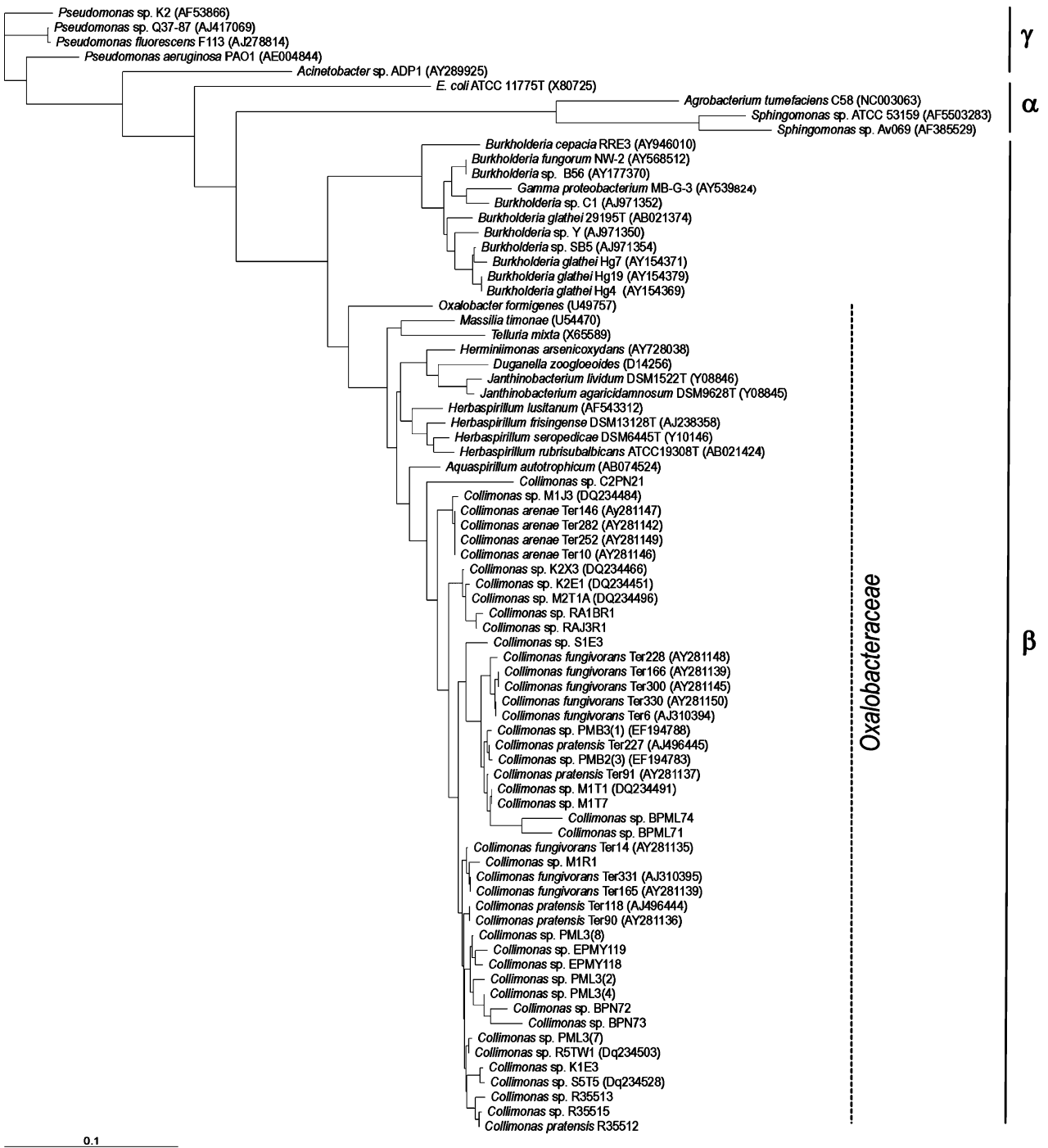


Fig. 1. Neighbour-joining tree showing the placement of *Collimonas* strains in the bacterial tree of life, as members of the family of *Oxalobacteraceae*, and in relation to representatives from the *Beta-*, *Alpha-* and *Gammaproteobacteria*. Tree is based on the CLUSTAL X (version 1.8) alignment of partial DNA sequences from pA-907R-amplified 16S rRNA genes. A bootstrap analysis was performed with 1000 repetitions. For reasons of legibility, bootstrap values are not presented on the figure. Bacterial strains without accession numbers originate from our lab collections or were kindly provided by Dr Minna Männistö.

sequences but did not belong to any of the four clusters. The precise taxonomic status of these still needs to be verified, but it is likely that additional species of *Collimonas* will be identified in the future.

Ecological niche

Since the study of de Boer and colleagues (1998b), numerous other sightings of *Collimonas* isolates have

been reported (Table 1). These include culture-dependent and -independent observations, based on 16S rRNA gene sequence similarity to the original Ter strains (Fig. 2). Sightings occurred at various locations around the world, but the majority involved soil environments. Often these were described explicitly as being vegetated. Soil types, insofar specified, included dune, forest, grassland, heathland, tundra, mire and (ex)agricultural soil. In instances where the soil compartment was explicitly stated, *Collimonas* was found in the bulk soil, the rhizosphere (i.e. the plant root zone), the mycorrhizosphere (i.e. in association with mycorrhizae), the soil layer below lichen mats and/or the mineral layer. Many of the soils or soil compartments that harboured *Collimonas* were characterized by slight acidity, fungal presence, low nutrient availability and limited human disturbance. Individually or in combination, these conditions seem to define the preferred natural niche of *Collimonas* species. Conversely, the presence of *Collimonas* may be indicative of the above characteristics and may thus have quantitative utility as an indicator of soil quality.

So far, only one attempt has been made to estimate the natural abundance of collimonads (Höppener-Ogawa *et al*., 2007). Real-time quantitative PCR using a *Collimonas*-specific primer/probe set on total bacterial DNA isolated from Dutch soils with a wide range of physical and chemical characteristics and management practices revealed abundances up to 10^5 cells per gram of soil. *Collimonas* numbers were significantly higher in forest and grassland soils than in arable soil. In forest soils, the nutrient-poor mineral layer contained more collimonads than the organic layer. In other studies, comparative analyses by real-time PCR (S. Höppener-Ogawa and S. Uroz, unpublished) and 16S rRNA gene pyrosequencing (S. Uroz *et al*., unpublished) of the bulk and rhizosphere soil showed that *Collimonas* bacteria prefer the latter compartment. Abundances of 10^5 or less bacteria per gram soil are low, relatively speaking, and suggest that collimonads are members of the so-called rare biosphere (Sogin *et al*., 2006). This notion is supported by the observation that *Collimonas* 16S rRNA gene sequences are rarely found in metagenomic or clone libraries from soil environments (Höppener-Ogawa *et al*., 2007).

Isolation and identification

Culturable collimonads have been isolated on several types of media (Table 1), but with a clear bias towards those that mimic oligotrophic conditions, such as R2A (Aspray *et al*., 2005) and diluted tryptic soy agar (Axelrood *et al*., 2002), mineral medium supplemented with a single carbon/energy source (Wilson *et al*., 2003), and chitin-yeast extract agar (de Boer *et al*., 1998b; Höppener-Ogawa *et al*., 2007). None of these media is truly *Collimonas*-specific, meaning that they are weak predictors of *Collimonas* identity for the bacterial colonies that they sustain. To verify whether a bacterial isolate belongs to the genus *Collimonas*, amplification and sequencing of its 16 rRNA gene sequence is the most straightforward approach, with the V6 region being the most informative about *Collimonas* status and the V2 region most suitable to identify at the species level (Fig. 2). Verification of a suspected *Collimonas* isolate can also be achieved using restriction fragment length polymorphism analysis of its amplified 16S rRNA gene (Höppener-Ogawa *et al*., 2007). This method is based on the presence of a *Bst*BI restriction site (5′-TTCGAA-3′) that is unique to *Collimonas* around position 1000 of the 16S rRNA gene. Other methods available for *Collimonas* identification are BIOLOG and FAME analysis, for which *Collimonas*-specific reference data are available (de Boer *et al*., 2004; Höppener-Ogawa *et al*., 2008), fluorescent *in situ* hybridization using a *Collimonas*-specific fluorescently labelled probe (de Boer *et al*., 2004; Leveau *et al*., 2004), and PCR with *Collimonas*-specific primers (Höppener-Ogawa *et al*., 2007).

Phenotypes and functions

The availability of culturable representatives from the *Collimonas* genus, which include the Terschelling strains as well as isolates from other locations (Table 1), has allowed a functional characterization of this group of *Oxalobacteraceae*. Many of the observed properties can be interpreted as adaptations to life in nutrient-poor soils, including chitinolysis, antifungal activity, mycophagy, plant root colonization and weathering activity. They are discussed in greater detail below, to provide an overview of the types of interactions that *Collimonas* bacteria have with their biotic and abiotic environment.

Chitin hydrolysis

Hydrolysis of chitin, more specifically halo formation on colloidal chitin agar plates, was used as a defining property of the original Ter strains (de Boer *et al*., 1998b). Such activity has also been demonstrated for *Collimonas* strains isolated by other groups in other locations (Männistö and Häggblom, 2006; S. Uroz *et al*., submitted). Chitin-hydrolysing activity has been described for other members of the *Oxalobacteraceae* as well, including *Janthinobacterium* and *Telluria* species, but not for the closest relatives of *Collimonas*, i.e. *Herbaspirillum* species. The activity of Ter strains towards particulate rather than colloidal chitin was found to be relatively low, at least compared with chitinolytic filamentous fungi and actinomycetes (de Boer *et al*., 1999). Thus, collimonads probably have a limited role in the recycling of solid

Table 1. Culture-dependent and -independent sightings of *Collimonas* reported in the literature and GenBank.[a]

ID[b]	Environment	Region, country	Detection method	Reference
22 Ter isolates	Soil (pH 4.9–6.1) from the upper 10 cm within the tussocks of Marram grass (*Ammophila arenaria*); low in calcium carbonate, slightly acidic	Inner dune sites on the Wadden island of Terschelling, the Netherlands	Isolated on chitin agar plates as halo-forming colonies	de Boer *et al.* (1998b; 2001; 2004)
2 isolates and 4 uncultured	Mineral soil from mature forest, dominated by lodgepole pine (*Pinus contorta* Dougl.) and rhizosphere soil of lodgepole pine seedlings	British Columbia Ministry of Forests Long-Term Soil Productivity installation sites, Canada	General medium (not specified, probably 0.5× TSA); clone library	Axelrood *et al.* (2002); Chow *et al.* (2002)
Isolate Hg1	Hillside soil, with pine tree vegetation, 3 m from naphthalene-contaminated seep	South Glens Falls, upstate New York, USA	Isolated as naphthalene-degrading bacterium	Wilson *et al.* (2003)
Isolates A8 and A23	Phytosphere of the moss *Aulacomnium palustre*, growing in mire behind dunes at Baltic Sea coast	Natural reserve 'Ribnitzer Grosses Moor', near Rostock, Germany	Isolated on R2A medium, with antifungal activity against *Verticillium dahliae*	Opelt and Berg (2004)
Isolates F11 and F14	Flow chamber biofilm from 2,4-D-spiked brown forest soil (pH 5.0), with no previous exposure to 2,4-D	Location of forest not specified, but in the UK	Isolated from flow cell effluent on mannitol yeast extract and R2A plates	Aspray *et al.* (2005)
G sequences (ab011716-28,37/38,44)	Not specified	Not specified, but probably in Japan	Not specified, but culture-independent	Shikano and Mitsui (unpublished)
Isolate RE1 (dq365897)	Not specified	Jeju, Southern Korea	Isolated as pigment-producing colony	Yoon *et al.* (unpublished)
33 isolates	Forest soil, stream water, tundra soil, mire pond sediment	Finnish Lapland	Isolated on different agar media, 33 isolates identified several with violet pigment; 11 were tested and scored positive for chitinase activity	Männistö and Häggblom (2006)
Isolate GCM11	Ikaite tufa columns from a depth of approximately 10 m	Ikka Fjord, South West Greenland	Not specified, but probably isolated on 1/10 R2A, pH 10, at 5°C	Schmidt *et al.* (2006)
Drobligo_Isolate34 3 (am710196)	Organically managed farm soil	Droevendaal, the Netherlands	Isolated using oligotrophic-specific conditions (A. Speksnijder, pers. comm.)	Senechkin *et al.* (unpublished)
6 wged isolates (ef408849,50,54, 58,59,67)	Unspecified, possibly from ginseng roots	Unspecified (probably China)	Unspecified, most likely as isolates	Qiu and Song (unpublished)
4 PML and 2 PMB isolates	Mycorrhizosphere and symbiotic mantle of oak (*Quercus petraea*)-*Scleroderma citrinum* ectomycorrhizae	Experimental forest site, Breuil, France	Isolated on 0.1× TSB agar, with demonstrated capacity for weathering and chitin hydrolysis	Uroz *et al.* (2007)
26 isolates	Dune grassland (pH 4.7–5.3), unfertilized grassland (pH 4.9–5.8), ex-agricultural land (pH 5.1–5.6), heathland (pH 4.1)	Various locations in the Netherlands	Isolated on chitin/yeast agar as halo-forming colonies; some were violet pigmented	Höppener-Ogawa *et al.* (2008)
MB1S1_H07 (ef664464)	Forest soil	GASP KBS-LTER sampling site, Michigan, USA	Unspecified, but culture-independent	Jangid *et al.* (unpublished)
KN-1	Soil (suppressive to phytopathogenic fungi?)	Unspecified, but presumably in Europe	Probably isolated on King's B medium, possibly antagonistic against *Rhizoctonia solani* AG3 and/or *Fusarium oxysporum* f.sp. *lini*	Adesina *et al.* (2007)
Toolik_Jun2005_ Intertussock_64	Moist acidic tundra soil site, intertussock microsites dominated by mosses with small shrubs	Arctic Toolik Lake Long-Term Ecological Research	Culture-independent	Wallenstein *et al.* (2007)
4 isolates	Bulk soil, grass-clover crop	Organic farm with arable rotation in Garderen, Gelderland, the Netherlands	Isolated on R2A plates	Postma *et al.* (2008)
CB7 (fm206304)	Rhizosphere soil of Monterey pine (*Pinus radiata*)	Rotorua, New Zealand	Band in PCR-DGGE	Lottmann *et al.* (unpublished)
LM19	Decomposing beech wood from forest soil	The Netherlands	Band in PCR-DGGE	Folman *et al.* (2008)

a. This table does not include sightings of *Collimonas* for which no 16S rRNA gene sequence was deposited and *Collimonas* taxonomy could not be verified. These include isolates BS290 (Warmink and van Elsas, 2008), band 3 (Mahmood *et al.*, 2005) and V63C (Allaire, 2005).
b. Accession numbers for unpublished *Collimonas* sequences are given in parentheses.

chitinous materials derived from arthropod or fungal remains in soil. Instead, it was hypothesized that the chitinolytic activity of collimonads helps these bacteria to obtain nutrients from living fungal hyphae ('bacterial mycophagy', see below) or to defend bacterial colonies from being predated by fungi (de Boer *et al*., 1998b; 2001).

The chitinolytic system of *C. fungivorans* strain Ter331 has recently been analysed by a loss-of-function approach using plasposon technology (Leveau *et al*., 2006) and described in detail (Fritsche *et al*., 2008). It consists of several genes organized in two loci on the chromosome and coding for an inducible cascade of events that involves extracellular hydrolysis of chitin into chitooligosaccharides by a single chitinase, transport of these chitooligosaccharides across the outer membrane by a dedicated uptake system, breakdown to chitin dimers, uptake across the inner membrane, and conversion to monomers that are channelled into the central metabolism of the cell. The observation that *Collimonas* has apparently only one extracellular chitinase and possesses an uptake mechanism for chitooligosaccharides has been explained as a competitive adaptation which avoids the release of di- and monomers of chitin which might be used by other microorganisms or might alert them to the availability of (pre-)digested chitin (Fritsche *et al*., 2008). Chitinolytic activity by collimonads is repressed in the presence of tryptic soy broth or glucose (de Boer *et al*., 1998b), suggesting that the utilization of chitin is secondary to other, more palatable nutrients.

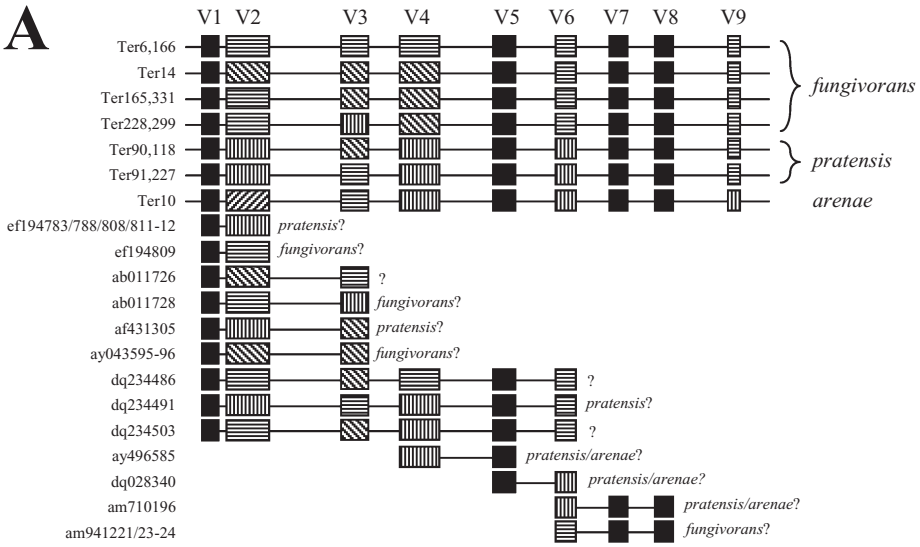
Antifungal and biocontrol activity

Antifungal activity has been reported for several *Collimonas* isolates (Table 1). The original Ter strains showed *in vitro* antagonism on water-agar plates against several plant-pathogenic and saprotrophic dune soil fungi (de Boer *et al*., 1998b). These included *Chaetomium globosum*, *Fusarium culmorum*, *Fusarium oxysporum*, *Idriella (Microdochium) bolleyi*, *Mucor hiemalis*, *Phoma exigua* and *Ulocladium* sp., which were introduced to the water-agar plates as potato-dextrose agar plugs. The study revealed clear differences in susceptibility of individual fungi to different Ter strains, while individual Ter strains differed in the type of fungi that they inhibited as well as in the extent of inhibition. For example, *F. oxysporum* was not affected by any of the *Collimonas* strains Ter6, 10, 14, 146, 227, 228, 330 and 331, while *M. hiemalis* and *P. exigua* were affected at least somewhat by all strains except Ter330. Other groups have reported antifungal activity of *Collimonas* isolates against the fungi *Cylindrocladium floridanum* (Allaire, 2005), *Rhizoctonia solani* AG3, and *F. oxysporum* f.sp. *lini* (Adesina *et al*., 2007) and *Verticillium dahliae* (Opelt and Berg, 2004). These fungi represent pathogens of economically important plants and crops, such as spruce, potato, flax and lettuce, respectively, suggesting the potential for broad application of *Collimonas* as a biocontrol agent.

For *C. fungivorans* strain Ter331, antifungal activity has been demonstrated against several additional fungi. One is the ectomycorrhizal fungus *Laccaria bicolor* S238N (Deveau *et al*., 2007). The *in vitro* confrontation bioassay involved a modified Pachlewski agar plate with 5% glucose and featured a central plug of *L. bicolor* surrounded by four drops of *Collimonas* suspension. Compared with the control, the pre-contact presence of *Collimonas* slowed down growth of the fungus macroscopically and increased branching density of the fungal hyphae microscopically. These effects were accompanied by an enhanced expression in *L. bicolor* of a tectonin II-like gene, which was suggested (Deveau *et al*., 2007) to play a role in fungal recognition of and/or interaction with bacteria, based on observations that in the slime mold *Physarum polycephalum*, tectonins seem to be involved in the aggregation of bacteria during the phagocytosis process. The confrontation with *Collimonas* also altered the expression of several fungal genes coding for elements of the Spt–Ada–Gcn5–acetyltransferase (SAGA) complex, which is involved in nucleosome remodelling to make promoter sequences accessible to DNA-binding transcriptional regulators. Follow-up experiments will be needed to establish whether this observation hints at the ability of *C. fungivorans* Ter331 to modulate fungal gene expression, or whether it represents a more general stress response of the fungus to the presence of *C. fungivorans* Ter331.

Collimonas fungivorans Ter331 also was found to inhibit hyphal growth of germinating spores of the arbuscular mycorrhizal fungus *Glomus mossae* BEG12 (Pivato *et al*., 2009). However, no effect of *Collimonas* was observed *in vivo* on the frequency and intensity of arbuscular mycorrhizal colonization, or on the frequency of arbuscules in *Medicago truncatula* roots inoculated with *G. mossae* BEG12 (Pivato *et al*., 2009). In a study by Kamilova and colleagues (2007), *C. fungivorans* Ter331 significantly suppressed disease symptoms caused by the plant-pathogenic fungus *F. oxysporum* f.sp. *radicis-lycopersici* (*Forl*) on tomato plants under *in vivo* greenhouse conditions in potting soil. The degree of disease control was very similar to that obtained with previously identified biocontrol agents *Pseudomonas fluorescens* WCS365 and *Pseudomonas chlororaphis* PCL1391 (Kamilova *et al*., 2007). *In vitro*, however, strain Ter331 was not inhibitory to the growth of the fungus on solid potato dextrose, King's B or water agar. The use of a GFP-labelled derivative of *C. fungivorans* Ter331 demonstrated its ability to colonize *Forl* hyphae *in vitro*, but only on agar lacking a carbon source, not on Armstrong agar,

A



B

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                FISH probe                  BstBI
TTACCTACCCTTGACATGTACAGAATCCCGAAGAGATTTGGGAGTGTTCGAAAGAAAGCTGTAAC  Collimonas fungivorans Ter6 (AJ310394)
.....................................................A.......  Collimonas arenae Ter10 (AY281146)
.....................................................A.......  Collimonas pratensis Ter91 (AY281137)
.....................G......T..........A......C........G.A.C.....  Herbaspirillum lusitanum P6-12 (AF543312)
.....................G......T..........A......C........G.A.C.....  Herbaspirillum hiltneri N3 (DQ150563)
.................G.TG......T..........A......C........G.A.CA.C..  Herbaspirillum chlorophenolicum CPW301 (AB094401)
.................G.TG.......................C........G...CA.C..  Herbaspirillum frisingense GSF30 (AJ238358)
.................G.TG.......................C........G.A.CA.C..  Herbaspirillum huttiense ATTC14670 (AB021366)
.................G.TG.......................C........G.A.CA.C..  Herbaspirillum putei 7-2 (AB109890)
.................GT.G......T.................C........G.A.C.GCG.  Herbaspirillum rubrisubalbicans ATCC19308 (AB021424)
.................GT.G......T.................C........G.A.C.GCG.  Herbaspirillum seropedicae DSM6445 (Y10146)
.....................G.......TG.......A.......C........G.A.C.....  Herminiimonas fonticola S-94 (AY676462)
.....................G......TTG......CA.......C........G.A.C.....  Herminiimonas aquatilis CCUG36956 (AM085762)
.................G.AG......T.G......C........CC......GG.A.CT.C..  Naxibacter alkalitolerans YIM 31775 (AY679161)
.................GCTG.......CG.......G.......C........G.A.CAGT..  Janthinobacterium lividum DSM 1522T (Y08846)
.................G..G.......................C........G...C.NT..  Janthinobacterium agaricidamnosum W1r3T (Y08845)
.................GCAGA.....TCG.......GA......C........G.ATCTGC..  Duganella zoogloeoides IAM12670 (D14256)
.................GCAG.N......G..............C........G.A.CTGC..  Duganella violaceinigra YIM 31327 (AY376163)
.................CAG...GG...G......C...TT...CC......G..C.CTG...  Massilia timonae (U54470)
.....................G........G..............C........G.A.C.....  Massilia dura 16 (AY965998)
.................CAG......T.G......CC.......CC......GG.A.CTG...  Massilia albidiflava 45 (AY965999)
.....................G...G..ACG......CGA..T...C........G.A.C.....  Massilia plicata 76 (AY966000)
.................CAG........G......CC.......CC......GG.A.CTG...  Massilia lutea 101 (AY966001)
.................CAG...C.T.CG.......G.A.G...CC......GG...CTG...  Telluria mixta ACM17 (X65589)
...........T......CAGA.....................C........G...CTG...  Oxalobacter formigenes OXB (U49757)
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C

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                                                                                                  Japan Finland France Canada
Ter6,166,165,331,228,299 TCGAAAGACTAGCTAATACCGCATACGATCTACGGATGAAAGTGGGGG-CTCGCAAGA-CCTCATGCTCA   1     1      1
Ter14                    .....................................................A-........-.........   1     3             1
Ter90,91,118,227         ........T.....................................A-........-.........   3     5      5      1
Ter10                    ........T.....................................-........-.........         1             2
seq1                     ........T.....................................-....T....-.........   7
seq2                     ........T..................................C.....A-........-....G.....   4                    1
seq3                     ........T.....................................A.C..T...GG.........         3
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which contains 2% glucose. Colonization of *Forl* hyphae by *Collimonas* was also observed *in vitro* on agar containing tomato root exudates, but not *in vivo* on tomato root surfaces that showed reduced disease symptoms. Clearly, expression of antifungal activity by *Collimonas* is context-dependent and more work is needed to define the environmental factors, including nutrient availability, that contribute to this phenotype.

Mycophagy

Bacterial mycophagy is defined as the demonstrable and quantifiable effect of bacterial phenotypic behaviours that make available nutrients from living fungi and allow the conversion of living fungal biomass into bacterial biomass (Leveau and Preston, 2008). The first evidence that collimonads can grow at the expense of fungal hyphae was

Fig. 2. Detailed phylogeny of the collimonads.
A. Schematic showing the location of the nine variable regions (V1–V9) in the 16S rRNA genes of representative Ter strains and a subset of other *Collimonas* species or sequences (named by GenBank accession numbers). For most of the latter, no full-length sequences are available. This panel shows a per-region comparison, where identical shading represents 100% sequence identity. Note that for most, but not all, of the non-Ter sequences, a species name can be tentatively assigned, based on Ter-based combinations of variable regions. Some sequences, however, contain combinations of V sequences that are not found within the Ter group (e.g. ab011726, dq234486 and dq234503); these possibly represent new *Collimonas* species or subspecies.
B. Comparison of V6 sequences of *Collimonas* type strains with those of all other recognized type strains of the family *Oxalobacteraceae*. This V6 region is highly predictive of the genus *Collimonas*, and encompasses the previously developed fluorescent *in situ* hybridization (FISH) probe sequence and *Bst*BI restriction site unique to *Collimonas*.
C. Part of the V2 region revealing single nucleotide differences between different species of *Collimonas*, including *fungivorans* (Ter6, 166, 165, 331, 228, 299, 14), *pratensis* (Ter90, 91, 118, 227), *arenae* (Ter10), and three unidentified species of *Collimonas* (seq1, seq2 and seq3). To the right of the alignment is shown how many of these sequences were found in four independent surveys of bacterial diversity at different geographical locations, i.e. Japan (Shikano and Mitsui, unpublished), Finland (Männistö and Häggblom, 2006), France (Uroz *et al*., 2007) and Canada (Axelrood *et al*., 2002; Chow *et al*., 2002). This analysis reveals that most sequences occur in more than one location and that each location sustains at least two different sequences.

reported by de Boer and colleagues (2001). A mixture of Ter strains was inoculated in purified sand without added nutrients. Common dune soil fungi, i.e. *M. hiemalis*, *C. globosum* and *F. culmorum*, were allowed to invade the sand from an agar plug that was placed on top of a microscope slide or steel disk resting on the sand. In an earlier study, these fungi were found to be inhibited by collimonads on water agar (de Boer *et al*., 1998b). In the purified sand set-up, numbers of collimonads increased up to 100-fold (10^5–10^7 cfu g^{-1} soil) during invasion of the sand by fungal hyphae. No such growth response was observed in the absence of fungi, or with two other types of chitinolytic soil bacteria (*Cytophaga*-like bacteria and stenotrophomonads), or with non-chitinolytic dune soil bacteria (mixture of pseudomonads and *Burkholderia* strains). The latter results were taken as evidence that collimonads had an active role in obtaining nutrients from the fungi. Microscopic observations revealed that clusters of *Collimonas* cells were often present on the tips of fungal hyphae and that these tips appeared swollen or collapsed. Based on this, it was suggested that only young hyphae are sensitive towards attack by collimonads. No evidence for invasion of the hyphae by collimonads was found.

Growth of collimonads was more supported on the zygomycete *M. hiemalis* than on the ascomycete *C. globosum* (de Boer *et al*., 2001). One possible explanation for this may be that zygomycetes lack septa, which would give more access to cytoplasmic nutrients, if opening hyphal tips were to be a major mycophagous mechanism. Addition of the chitinase inhibitor allosamidin to the purified sand set-up reduced but did not abolish the ability of *Collimonas* to grow on *C. globosum* or *F. culmorum* (de Boer *et al*., 2001), suggesting that chitinase activity is probably involved but by itself not sufficient to explain the bacterial proliferation. *Collimonas* growth on *Mucor* was unaffected by allosamidin, which can be understood in light of the fact that *Mucor* features chitosan, not chitin, as a major component of the cell wall (de Boer *et al*., 2001).

In a purified sand experiment with another zygomycete, i.e. *Absidia* sp., it was shown (Höppener-Ogawa *et al*., 2009a) that growth of *Collimonas* in response to the fungus had no significant effect on fungal numbers (as measured by real-time PCR) or biomass (expressed as ergosterol). Calculations showed that the estimated increase in *Collimonas* biomass was three orders of magnitude lower than the total biomass of *Absidia* present in the assay. Thus, no measurable effect of collimonads on fungal abundance would be expected under these circumstances. This apparent lack of a quantitative effect of *Collimonas* on fungal abundance was also found in a follow-up study by Höppener-Ogawa and colleagues (2009b), in which addition of *Collimonas* to an ex-arable soil with low levels of indigenous collimonads did not alter total fungal biomass, or have a measurable effect on fungal activity, such as cellulose degradation or arbuscular mycorrhization. However, a clear qualitative effect of the addition was observed, in that the composition of the soil fungal communities changed dramatically, as determined by denaturing gradient gel electrophoresis. The explanation offered for this observation was that differences in sensitivity of fungal species to the presence of mycophagous or antifungal collimonads change the competitive relationships between functionally equivalent fungal species.

Recently, Höppener-Ogawa and colleagues (2009b) showed evidence that mycophagous growth of collimonads is not restricted to the artificial conditions of purified sand but also occurs in natural soils. Using an *Absidia* sp. as bait fungus, it was found, through real-time PCR using a *Collimonas*-specific primer/probe set, that indigenous collimonads increased in numbers in the soil zone that was invaded by the fungal hyphae. No growth responses were observed for other soil bacteria tested in the same soils, i.e. *Pseudomonas* and *Burkholderia*. Hence, it appears that the stimulation of growth of *Collimonas* bacteria by fungal hyphae in these soils was specific and did not benefit other bacteria present. The latter suggests that *Collimonas* employs mycophagous mecha-

nisms that keep fungal-derived nutrients unavailable to other bacteria.

The studies described above are the only ones that actually show by experimental design the occurrence of fungal-induced growth by collimonads. Several other observational studies point indirectly to the possibility of mycophagous growth of collimonads, e.g. the apparent prevalent presence of collimonads in fungal-rich environments such as mycorrhizospheres (Uroz *et al*., 2007). However, in their study on the abundance of collimonads in soils with different fungal content, Höppener-Ogawa and colleagues (2007) observed no significant relationship between the number of collimonads and ergosterol concentration as an estimate of fungal abundance. Possible explanations are that mycophagy is not an essential growth strategy for collimonads under natural conditions, collimonads exist that are not mycophagous, or *Collimonas* strains feed preferentially on specific taxonomic or functional fungal groups (Höppener-Ogawa *et al*., 2007).

The relationship between antifungal activities of collimonads and their mycophagous growth is not yet clear. Enumeration of collimonads (and other bacteria) is usually not included in standard *in vitro* antagonism tests on agar media and therefore the fungal-dependent contribution to bacterial growth would be unknown (de Boer *et al*., 1998b; Deveau *et al*., 2007; Pivato *et al*., 2009). An additional complication is the presence of nutrients in the agar which would mask the growth of collimonads on fungal substrates. In other words, it is difficult to uncouple growth on fungus from antifungal activity. Future research will need to deal with quantifying the individual and added effects of the *Collimonas* antifungal arsenal compared with the efficiency of these bacteria to sequester fungal nutrients and convert those into more bacteria with that same antifungal arsenal.

Bacterial mycophagy is not a property unique to the *Collimonas* genus. Leveau and Preston (2008) identified a number of demonstrated or suspected examples of mycophagous bacteria in the literature. One of these is *Janthinobacterium agaricidamnosum*, a soft rot pathogen of mushroom (*Agaricus bisporus*) and a member of the *Oxalobacteraceae* with close relationship to *Collimonas*. When *C. fungivorans* Ter331 was tested in a mushroom assay, no symptoms typical of *J. agaricidamnosum* infection were observed (Fritsche *et al*., unpublished). Additional experiments are needed, especially if *Collimonas* is going to be considered as a biocontrol agent for the management of fungal plant diseases (Kamilova *et al*., 2007), to further define the specificity of these mycophagous bacteria and to confirm their harmlessness towards fungi with economical or ecological value such as commercially important mushrooms or mycorrhizal associations with agricultural crops.

Pigmentation, antibiotic activity and resistance

Several authors have reported the isolation of purple-coloured collimonads (Männistö and Häggblom, 2006; Höppener-Ogawa *et al*., 2007). In the lab, the violet pigmentation is often irreversibly lost. Männistö and Häggblom (2006) linked this to cultivation at high temperatures (> 25°C), indicating that the pigment may confer an advantage in cold habitats. They also reported that the violet pigment of their *Collimonas* isolates had a spectrum similar to violacein which is known for its broad-spectrum antibiotic, antiprotozoal, antiviral and cytotoxic properties (Duran and Menck, 2001) and was identified as a protection mechanism in *Janthinobacterium lividum* and *Chromobacterium violaceum* against bacteriovorous nanoflagellates (Duran and Menck, 2001). No reports of antibacterial activity have been published for *Collimonas* isolates. Strain Ter331 was tested for hydrogen cyanide production but scored negative (Kamilova *et al*., 2007). Several of the Ter strains have been tested for their susceptibility to antibiotics (W. de Boer, unpublished). Most were resistant to ampicillin, chloramphenicol and kanamycin, but not tetracycline.

Quorum sensing

There is convincing evidence that shows quorum sensing is an inherent property of collimonads. Analyses by thin-layer chromatography and reporter strains (Liu *et al*., 2004) revealed remarkable similarity of the acyl-HSL patterns produced by *Collimonas* strains Ter6, 14, 165 and 331 to those described for *P. fluorescens* 2-79 (Shaw *et al*., 1997; Khan *et al*., 2007). Consistent with these experimental data, a set of *luxI* and *luxR* homologues with homology to the *phzI* and *phzR* genes of *P. fluorescens* 2-79 was identified on the genome sequence of *C. fungivorans* strain Ter331 (see below). Interestingly, *P. fluorescens* 2-79 is best known for its antifungal and biocontrol activity, in particular against the causative agent of take-all disease of wheat, i.e. *Gaeumannomyces graminis* var. *tritici* (Thomashow and Weller, 1988). Two other *Collimonas* strains, Ter10 and 72, showed somewhat different acyl-HSL patterns, suggesting intrageneric variation in the ability to produce quorum-sensing molecules.

Weathering capacity

A recently discovered characteristic of collimonads is their weathering capacity, i.e. the ability to decompose rocks and minerals. Using a microplate assay, Uroz and colleagues (2007) demonstrated that *Collimonas* isolates from a forest soil were among the most efficient bacteria to release iron from biotite, a widespread mineral in soil. Another *in vitro* assay showed the ability of the bacteria to

dissolve inorganic phosphorus (S. Uroz *et al*., submitted). Their efficacy as biological weathering agents appeared strongly linked to their ability to acidify the culture medium by conversion of glucose into gluconic acid (S. Uroz *et al*., submitted). The mineral weathering phenotype was confirmed for strains Ter6 and Ter331 (Uroz *et al*., 2007). Iron mobilization also occurred on chromoazurol S medium, which evokes the prediction that collimonads produce chelating compounds such as hydroxamate- and catechol-type siderophores (Schwyn and Neilands, 1987; Liermann *et al*., 2000). Strains of *Herbaspirillum* and *Janthinobacterium* were also tested but were much less efficient in weathering biotite or solubilizing phosphorous than the collimonads (S. Uroz *et al*., unpublished).

It remains to be tested whether there exists a link between the weathering properties of collimonads and their mycophagous behaviour. *Collimonas* and other weather-capable bacteria were enriched near the mycorrhizae of *Scleroderma citrinum* on roots of oak, beech and spruce. One role of mycophagy in this context is that it might provide collimonads with a mechanism to compete with fungi for available minerals. However, *S. citrinum* is not efficient in mineral weathering. In an alternative scenario, the mycophagous behaviour of collimonads represents an ability to exploit mycorrhiza as a provider of organic nutrients, obtained from the plant, in exchange for inorganic nutrients released by the bacteria from the soil minerals. In this last hypothesis, all actors (plant, bacteria and fungus) engage in and benefit from a tripartite, give-and-take interaction.

Interactions with plants, mobility in soil and degradation of xenobiotics

One of the preferred habitats of *Collimonas* appears to be the soil surrounding plant roots. This suggestion is based on the frequent isolation of collimonads from rhizosphere soils (Table 1). It is in line with the observation that *C. fungivorans* Ter331 is a competitive colonizer of tomato roots (Kamilova *et al*., 2007). Collimonads also are rapid colonizers of soil in general: it was shown by cultivation-independent methods that collimonads were among the first to colonize a soil freshly sterilized with gamma irradiation (Wertz *et al*., 2007). The mechanisms for this rapid colonization are unknown, but an intriguing hypothesis is that *Collimonas* bacteria can travel in soil by association with exploring fungal hyphae. This may involve chemotaxis towards the target fungus (Warmink and van Elsas, 2008), and attachment through the production of extracellular polysaccharides or components of the chitinolytic system. Spread of bacteria along fungal hyphae has also been demonstrated for polycyclic aromatic hydrocarbon-degrading bacteria (Kohlmeier *et al*., 2005). In this context, it is worth noting that collimonads were featured in studies on the degradation of xenobiotic compounds or on the amendment of such compounds to soil with no prior history of exposure to those xenobiotics. The presence of *Collimonas* sequences or isolates has been reported in mostly pristine soils amended with the pollutants 2,4-dichlorophenoxyacetic acid (Aspray *et al*., 2005), pentachlorophenol (Mahmood *et al*., 2005) or naphthalene (Wilson *et al*., 2003). In the latter study, naphthalene-mineralizing activity was demonstrated for the *Collimonas* isolate that was tested.

Genomic resources

Recently, the complete genome sequence of one of the original Terschelling isolates, *C. fungivorans* Ter331, was determined and annotated (J.H.J. Leveau *et al*., in preparation). The genome consists of one chromosome of 5.3 Mbp and an extrachromosomal element of 40.5 kb. This plasmid pTer331 (Mela *et al*., 2008) is a new member of the pIPO2/pSB102 family of environmental plasmids. The plasmid is stably maintained in its host and is self-transferable, but has no role in the *Collimonas* mycophagy or antifungal phenotype (Mela *et al*., 2008). Annotation of the Ter331 genome, which harbours about 4500 open reading frames (ORFs), has revealed several candidates for mycophagy-related genes. These include genes that code for lytic enzymes, antibiotics, chemotaxis, attachment, quorum sensing, resistance to fungal-derived antibiotics, and utilization of mannitol and trehalose, two compatible solutes that accumulate to high concentrations in several fungal species (Dijksterhuis and de Vries, 2006). Among the most promising mycophagy- and antifungal-related genes are those that code for non-ribosomal peptide synthetases as well as a gene cluster for a type III secretion system. The latter discovery nurtures the hypothesis that *Collimonas* bacteria, in a contact-dependent manner, deliver effector molecules into the fungus, thereby altering the physiology of the fungus or lowering its defence against a mycophagous assault. In a recent study (Warmink and van Elsas, 2008), it was shown that type III secretion system genes were enriched in the mycosphere of *L. bicolor* compared with the bulk soil, indeed suggesting a potential role of these types of genes in bacterial–fungal interactions. The discovery of genes for non-ribosomal peptide synthetases, together with the ability to produce enzymes with industrial application, such as chitinases, may prompt interest in *Collimonas* as a potentially rich source of bioactive compounds.

Attempts to identify mycophagy-related genes in *Collimonas* by a loss-of-function approach and map them onto the genome sequence have so far had limited success. Screening of several thousands of plasposon mutants of strain Ter331 (Leveau *et al*., 2006) for their ability to increase biomass in an *in vitro* confrontation with a

Trichoderma species did not yield mutants that were completely blocked in this ability (J.H.J. Leveau *et al*., unpublished). This suggests that mycophagy is not based on a single allele or under the control of one master regulator. Instead, it is more likely that the phenotype is the sum of many gene functions, each of which incrementally contributes to growth on fungal hyphae. A mutant of *C. fungivorans* Ter331 in which the chitinase responsible for halo formation on chitin agar plates was inactivated was affected neither in antifungal activity nor in mycophagy, suggesting that its role in these phenotypes is marginal at most (Fritsche *et al*., 2008).

One preliminary observation from the genome mining effort is the relatively high proportion of genes that seem to have been acquired by *C. fungivorans* through horizontal gene transfer. It is tempting to speculate on the mechanisms that are involved in this acquisition. One is plasmid-mediated horizontal gene transfer. Through its (retro)mobilizing activity, i.e. ability to move and recruit plasmids from other bacteria, plasmid pTer331 of *C. fungivorans* Ter331 is a likely catalyst of the dissemination of the mobile gene pool ('mobilome') within a bacterial community (Mela *et al*., 2008). Another interesting hypothesis is the role of the chitin uptake system as was demonstrated for *Vibrio cholerae* (Meibom *et al*., 2005). This bacterium can acquire new genetic material by natural transformation during growth on chitin. Transformation competence was found to require a type IV pilus assembly complex, the coding information for which is also present on the *Collimonas* genome.

To date, the genomes of two representatives from the family *Oxalobacteraceae* have been sequenced. These include *Herminiimonas arsenicoxydans* (Muller *et al*., 2007) and *Janthinobacterium* sp. Marseille (*Minibacterium massiliensis*) (Audic *et al*., 2007). The former is known for its metalloresistance, the latter for its survival in water. A comparison of these genomes with that of *C. fungivorans* Ter331 will shed a first light on the functional diversity within the *Oxalobacteraceae* family and provide evidence from a genomic perspective for niche specialization in the genus *Collimonas*. Even more insightful will be the comparison with the in-progress genome sequence of *Herbaspirillum seropedicae* (http://www.genomesonline.org), which represents one of the closest relatives to *C. fungivorans* Ter331. The availability of a genome sequence will drive the research on *Collimonas* also towards the exploitation of microarray technology to reveal the genes and gene clusters that are specifically expressed during its interaction with different components of its biotic and abiotic environment. Among other things, this will provide greater insight into the context-dependent expression of the mycophagous and antifungal phenotype of collimonads.

Conclusion

From this minireview, it becomes clear that the study of collimonads is touching upon and influencing research areas that deal with interactions between fungi and bacteria, the impacts of rare taxonomic groups and bacterial adaptations to oligotrophic environments. Collimonads also represent a valuable model and resource for the discovery of new bioprocesses and novel molecules/enzymes, due to their ability to hydrolyse polymers,

Table 2. Tools and resources available for *Collimonas* research.

Resource	Detail	Reference
Type strains	*Collimonas fungivorans* Ter6 (DSM 17622, LMG 21973, NCCB 100033)	de Boer *et al*. (2004)
	Collimonas arenae Ter10 (DSM 21398, LMG 23964, R-22719)	Höppener-Ogawa *et al*. (2008)
	Collimonas pratensis Ter91 (DSM 21399, LMG 23965, R-22721)	Höppener-Ogawa *et al*. (2008)
Genome	*Collimonas fungivorans* Ter331 (LMG 21588, NCCB 100024)	de Boer *et al*. (2004)
	(1) circular chromosome, approximately 5.3 Mbp	J.H.J. Leveau *et al*. (in preparation)
	(2) plasmid pTer331, 40 457 bp	Mela *et al*. (2008)
Genomic library	3200 *Escherichia coli* clones with pCC1FOS carrying genomic DNA fragments from *C. fungivorans* Ter331 with average size of 33.8 $\pm$ 3 kb	Leveau *et al*. (2004)
Mutant library	2016 random mutants of *C. fungivorans* Ter331 generated with plasposon p*Tn*Mod–KmO*lacZ*	Leveau *et al*. (2006); Fritsche *et al*. (2008)
FISH protocol	Probe CTE998-1015 (5′-CTCTTCGGGATTCTGTAC-3′) for specific detection of *Collimonas* 16S rRNA by fluorescence *in situ* hybridization	de Boer *et al*. (2004); Leveau *et al*. (2004)
Real-time PCR	Based on 16S rRNA gene detection, primer/probe combination (Eddy3for, Eddy3rev, Sophie)	Höppener-Ogawa *et al*. (2007)
PCR-RFLP	Based on unique *Bst*BI-digestion site (5′-TTCGAA-3′) on amplified 16S rRNA gene sequence from collimonads	Höppener-Ogawa *et al*. (2007)
Transcriptomics	Nimblegen platform, 4529 predicted ORFs, 16 probes per ORF, strain Ter331	Mela *et al*. (in preparation)
CGH	Nimblegen platform, tiling array, strain Ter331	Mela *et al*. (in preparation)
GFP-labelled strain	*C. fungivorans* Ter331	Kamilova *et al*. (2007)
Educational tool	The Mycomuncher DNA Puzzle	Leveau (2007)

weather minerals and decompose fungal matter. They have great applicable potential as control agents of unwanted fungi or as biological indicators of soil quality/fertility. To aid in realizing this potential, numerous tools and resources are available for *Collimonas* research (Table 2), which will help in framing and answering important questions, for example regarding the specificity and timing of the interactions of *Collimonas* with its biotic and abiotic environment. The rapid development in next-generation sequencing technologies will undoubtedly reveal new *Collimonas* species in other environments and will unveil the degree of diversity, both phylogenetic and functional, within the genus. Culturable collimonads will continue to be useful additions to the *Collimonas* toolbox/database by providing the raw material to test new hypotheses, whereas comparative genomics approaches will feed our growing appreciation for the ecology and evolution of collimonads and its relatives in the family *Oxalobacteraceae*.

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