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Comparative genomics of bacteria from the genus *Collimonas*: linking (dis)similarities in gene content to phenotypic variation and conservation

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Summary

Collimonas **is a genus of soil bacteria comprising three recognized species: *C. fungivorans*, *C. pratensis* and *C. arenae*. Collimonads share the ability to degrade chitin (chitinolysis), feed on living fungal hyphae (mycophagy), and dissolve minerals (weathering), but vary in their inhibition of fungi (fungistasis). To better understand this phenotypic variability, we analysed the genomic content of four strains representing three *Collimonas* species (Ter14, Ter6, Ter91 and Ter10) by hybridization to a microarray based on reference strain *C. fungivorans* Ter331. The analysis revealed genes unique to strain Ter331 (e.g. those on the extrachromosomal element pTer331) and genes present in some but not all of the tested strains. Among the latter were several candidates that may contribute to fungistasis, including genes for the production and secretion of antifungals. We hypothesize that differential possession of these genes underlies the specialization of *Collimonas* strains towards different fungal hosts. We identified a set of 136 genes that were common in all tested *Collimonas* strains, but absent from the genomes of three other members of the family *Oxalobacteraceae*. Predicted products of these '*Collimonas* core' genes include lytic, secreted enzymes such as chitinases, peptidases, nucleases and phosphatases with a putative role in mycophagy and weathering.**

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Introduction

The bacterial genus *Collimonas* belongs to the family *Oxalobacteraceae* in the order *Burkholderiales* of the β-*Proteobacteria*. Taxonomic studies of this genus have led to the identification of three species, *C. fungivorans*, *C. arenae* and *C. pratensis* (de Boer *et al.*, 2004; Höppener-Ogawa *et al.*, 2008). Collimonads have been found mostly in soil environments, at relatively low abundances, and their distribution encompasses a wide range of natural and semi-natural environments (Höppener-Ogawa *et al.*, 2007; Leveau *et al.*, 2010).

Collimonas bacteria are known for their ability to grow at the expense of living fungal hyphae. This trophic behaviour is called mycophagy (Leveau and Preston, 2008), and has been demonstrated for *Collimonas* in soil microcosms using *Chaetomium* and *Mucor* species as fungal prey (de Boer *et al.*, 2001). Mycophagous growth of *Collimonas* bacteria is not restricted to the laboratory environment, but also takes place in natural soils (Höppener-Ogawa *et al.*, 2009a,b). All *Collimonas* strains tested so far are mycophagous and share certain other features, e.g. chitinolysis (de Boer *et al.*, 1998) and mineral weathering (Uroz *et al.*, 2009). However, they differ in traits such as colony morphology, the utilization of individual carbon sources, and the ability to inhibit hyphal growth of certain fungi on agar plates (de Boer *et al.*, 2001; Höppener-Ogawa *et al.*, 2008). With the current study, we aimed to uncover the genomic determinants that underlie the variable and shared phenotypes within the *Collimonas* genus (Table 1). To achieve this, we compared reference strain *C. fungivorans* Ter331 with four other *Collimonas* isolates using array-based comparative genomic hybridization (CGH). Three are type strains: *C. fungivorans* Ter6^T, *C. pratensis* Ter91^T and *C. arenae* Ter10^T. The fourth isolate is Ter14, which is another representative of the *C. fungivorans* species. These strains constitute a fair representation of the genus *Collimonas*, based on their phylogenetic relationship to each other and to other *Collimonas* strains (Höppener-Ogawa *et al.*, 2008; Leveau

Table 1. Properties of *Collimonas* isolates featuring in this study.

	Ter331	Ter14	Ter6	Ter91	Ter10	Reference
Species						
fungivorans	•	•	•			de Boer *et al*. (2004)
pratensis				•		Höppener-Ogawa *et al*. (2008)
arenae					•	Höppener-Ogawa *et al*. (2008)
Plasmid pTer331	+	–	–	–	–	Mela *et al*. (2008)
Mycophagy						
Chaetomium globosum	+	+	+	+	+	de Boer *et al*. (2001)
Fusarium culmorum	+	+	+	+	+	de Boer *et al*. (2001)
Mucor hiemalis	+	+	+	+	+	de Boer *et al*. (2001)
Antifungal activity						
Chaetomium globosum	+	–	–	nd	+	de Boer *et al*. (1998)
Fusarium culmorum	+	–	+	nd	–	de Boer *et al*. (1998)
Fusarium oxysporum	–	–	–	nd	–	de Boer *et al*. (1998)
Idriella bolleyi	+	+	+	nd	–	de Boer *et al*. (1998)
Mucor hiemalis	+	+	+	nd	+	de Boer *et al*. (1998)
Phoma exigua	+	+	+	nd	+	de Boer *et al*. (1998)
Ulocladium sp.	+	+	+	nd	+	de Boer *et al*. (1998)
Aspergillus niger	+	+	–	–	–	Mela *et al*. (2011)
Colony type[a]	I	I	I	III	II	de Boer *et al*. (2004)
Swimming motility	+	+	+	+/–	+	de Boer *et al*. (2004)
Assimilation of D-trehalose	+	+	+	+	–	de Boer *et al*. (2004)
Chitinolytic activity	+	+	+	+	+	de Boer *et al*. (2004)
Mineral weathering	+	+	+	+	+	Uroz *et al*. (2009)

a. Colony types have been described as follows (de Boer *et al*., 2004): I, flat, glossy, turbid, whitish colonies with a diameter of 3–7 mm and a layered structure; II, flat colonies with a diameter of 3–7 mm, a yellowish central part and a translucent, granular-structured periphery; III, small, glossy, whitish colonies with a diameter of 1–3 mm.

et al., 2010). We discuss the implications of our findings for the niche specialization of collimonads within the *Oxalobacteraceae*.

Results and discussion

Microarray design and hybridization

Based on the genome sequence of *C. fungivorans* Ter331, i.e. its chromosome (GenBank accession number CP002745) and plasmid pTer331 (GenBank accession number EU315244), a custom *Collimonas* microarray (Roche NimbleGen Systems, Iceland) was designed to assess which of the Ter331 genes were present in the genomes of other *Collimonas* strains (Table S1). *Collimonas* CGH array hybridization and scanning were performed by NimbleGen. Total genomic DNA that was extracted from each test strain (Ter6, Ter14, Ter10 or Ter91) using a QIAGEN Genomic-tip kit (QIAGEN, Venlo, the Netherlands) from bacterial cultures that were grown overnight at 25°C in King's B (KB) medium (King *et al*., 1954) was fluorescently labelled with Cy3 and co-hybridized on the microarray with Cy5-labelled genomic DNA from reference strain Ter331. Each array experiment was performed in a dye-swap replicate, in which dye assignment was reversed in the second hybridization. To evaluate the hybridization efficiency of the microarray and to detect probes that might yield false negatives, we also hybridized in duplicate genomic DNA isolated from strain *C. fungivorans* Ter331 to the microarray. A gene was considered present in a test strain if its hybridization value was equal or greater than a predetermined threshold (Fig. S1, Table S1). Microarray data for a selection of genes were validated using PCR (Table S2; true positive rate = 100%, false positive rate = 19%). Microarray design and hybridization results were deposited in the EMBL-EBI ArrayExpress Archive as experiment E-MTAB-349.

Genes that are differently shared between Collimonas *strains*

Analysis of the CGH data (Fig. 1, Table S1) indicated that 2343 (54.7%) out of the 4283 genes analysed were conserved in all *Collimonas* strains. A total of 156 genes (3.6%) were unique to Ter331, while 1784 (41.7%) scored absent in one or more of the *Collimonas* test strains. The percentage of *C. fungivorans* Ter331 genes detected in the test strains ranged from 64.2% in *C. arenae* Ter10, 68.2% in *C. pratensis* Ter91, to 82.8% and 95.1% in the two *C. fungivorans* strains Ter6 and Ter14 respectively (Fig. 2A). These numbers were in good agreement with the taxonomic placement of these isolates in relation to Ter331, based on 16S rRNA gene sequence comparison (Fig. 2B).

Figure 3 shows by use of colour-coding the absence/presence of Ter331 genes in the four *Collimonas* test

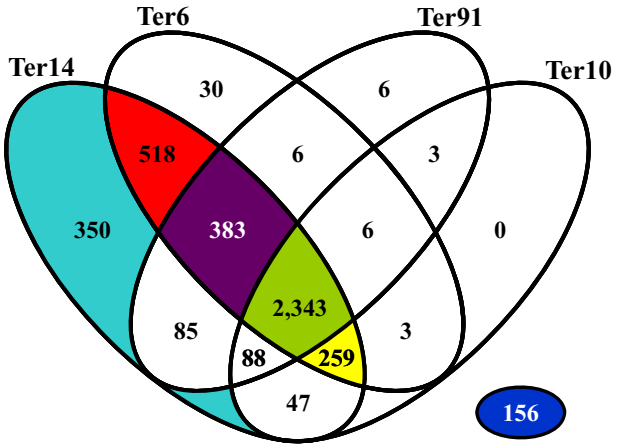

Fig. 1. Venn diagram showing the number of *C. fungivorans* Ter331 genes present in each of the four test strains (*C. fungivorans* Ter6, *C. arenae* Ter10, *C. fungivorans* Ter14 and *C. pratensis* Ter91) as determined by CGH analysis. In total, 156 genes were found to be unique to reference strain Ter331. Colour coding is the same as in Fig. 3: blue: not found in any of the other strains; cyan: shared only with Ter14; red: shared with Ter6 and Ter14; purple: shared with Ter6, Ter14 and Ter91; yellow: shared with Ter6, Ter10 and Ter14; green: shared with Ter6, Ter10, Ter14 and Ter91.

strains. Many genes that were not shared by all collimonads were found to group in clusters of one colour. These clusters are likely to have been lost or gained by individual strains of *Collimonas* in the course of intrageneric divergence. Of the genes that were unique to Ter331, the majority belonged to the mobile genetic pool, such as those encoded by plasmid pTer331 (Mela *et al*., 2008) and by putative prophages. For example, CF_1041–1075 showed substantial similarity to bacteriophage φCTX, a temperate phage originally identified in *Pseudomonas aeruginosa* (Nakayama *et al*., 1999), whereas Cf_2197–2205 and Cf_3425–3453 shared genes with *Xanthomonas* phage Cf1c (Kuo *et al*., 1991) and bacteriophage φKO2 from *Klebsiella oxytoca* (Casjens *et al*., 2004) respectively. These observations suggest that collimonads are vulnerable to phage infection and amenable to the role of DNA recipient during horizontal gene transfer.

Of the 383 genes that were detected in all strains except *C. arenae* Ter10, Cf_228 encodes a periplasmic trehalase, an enzyme that catalyses the hydrolysis of trehalose into two molecules of glucose. Consistent with this finding is the fact that isolate Ter10 is not able to grow at the expense of trehalose (Table 1). Many fungi accumulate trehalose as a reserve compound and stress protectant (Arguelles, 2000), and the ability to use trehalose as a growth substrate and chemoattractant has been demonstrated for bacteria that interact intimately with fungi (Deveau *et al*., 2010). Several of the other genes that were absent only in *C. arenae* Ter10 fell within clusters that encode bacterial secretion systems: Cf_2276–2288 encodes a type II secretion system (T2SS) (Cianciotto, 2005),

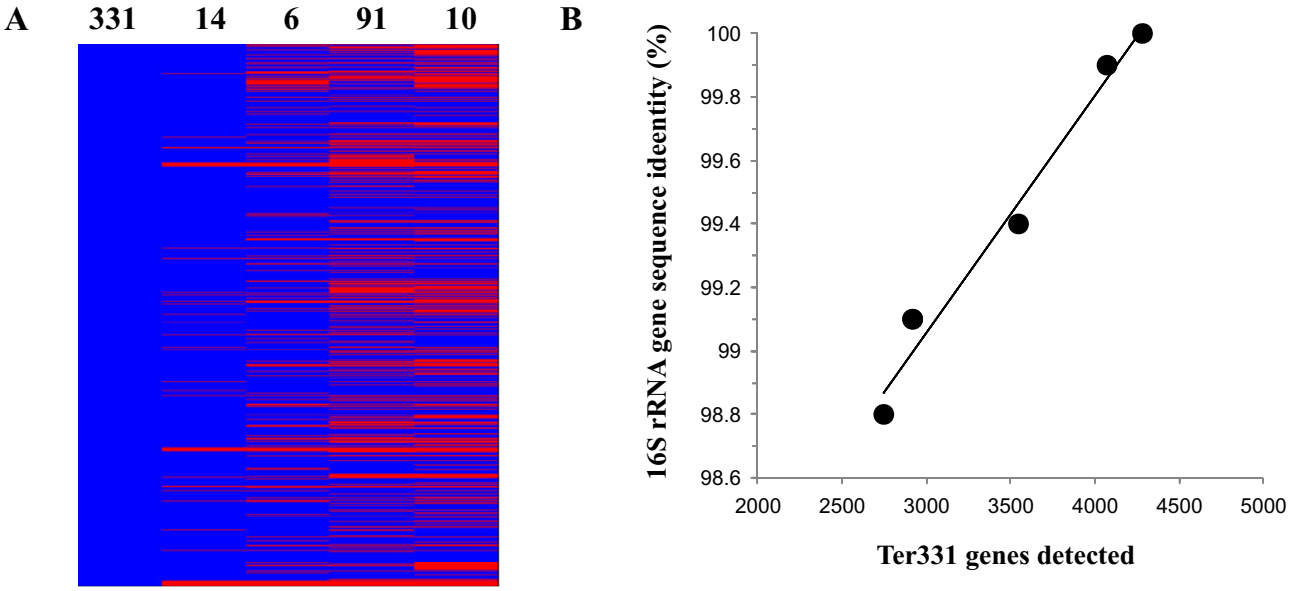

Fig. 2. A. Heat map showing the presence/absence of *C. fungivorans* Ter331 genes in other *Collimonas* strains. The presence and absence of individual genes, represented as lines in vertical order of their appearance on the *C. fungivorans* Ter331 genome is indicated as blue and red respectively. The genes located on plasmid pTer331 (unique to Ter331) are at the bottom of the figure.
B. Scatter plot showing for each of the five *Collimonas* test strains (Ter10, Ter91, Ter6, Ter14 and Ter331) the relationship between the number of Ter331 genes detected (*x*-axis) and the percentage 16S rRNA gene sequence identity with Ter331 (*y*-axis). Values on the *y*-axis were obtained by aligning the 16S rRNA gene sequence of Ter331 (GenBank accession number AJ310395) to those of Ter6 (AJ310394), Ter10 (AY281146), Ter14 (AY281135) and Ter91 (AY281137) in the MegAlign module of Lasergene (DNASTAR, Madison, WI).

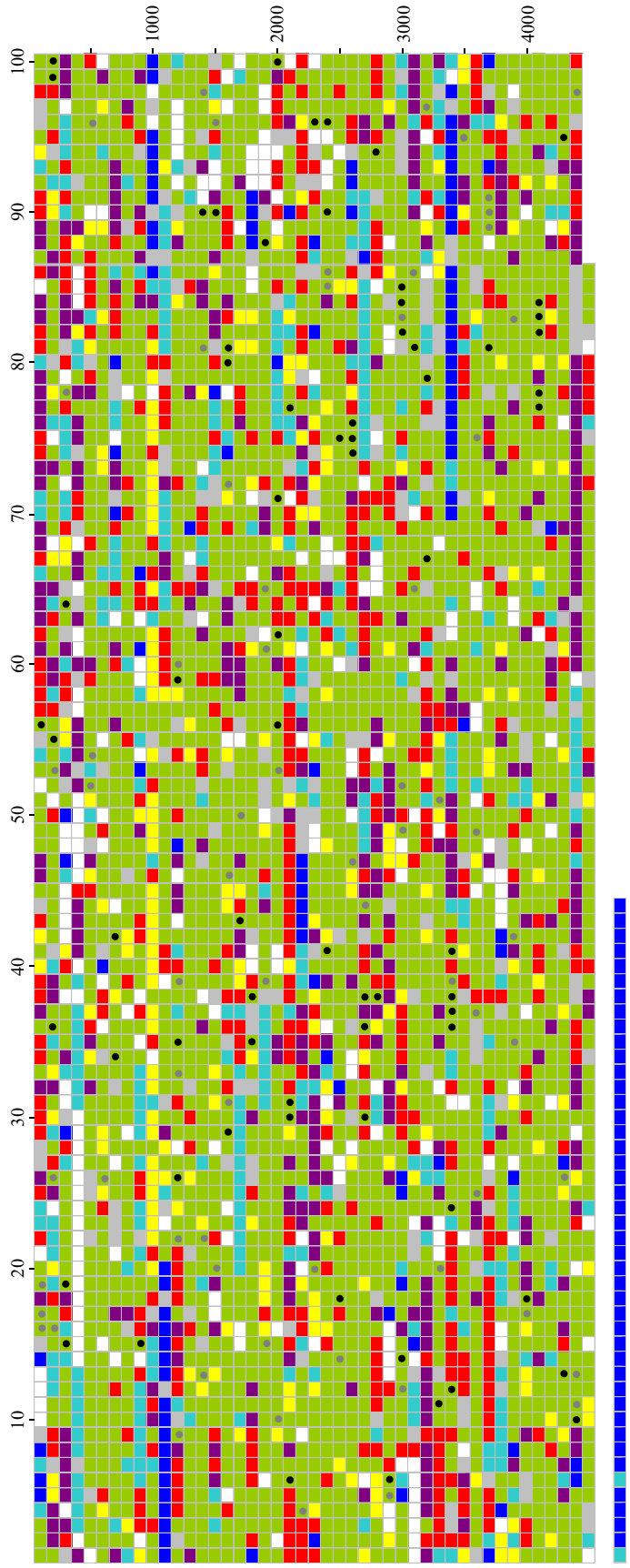

Fig. 3. Variable distribution of *C. fungivorans* Ter331 genes among *Collimonas* isolates. Each gene is represented by a square in the order as it appears on the genome of Ter331. There are 100 genes per row. The relationship between gene identifier Cf_ and its position in this matrix is given in Table S1. The colour of a square corresponds to the colours used in Fig. 1. For example, genes labelled in red were detected in Ter 14 and Ter6, but not Ter91 or Ter10. White squares indicate genes falling in the non-coloured parts of the Venn diagram in Fig. 1, while grey squares represent genes that were excluded from CGH analysis, because they were represented by less than 13 probes on the array. The bottom row represents plasmid pTer331. Genes with dots represent '*Collimonas* core' genes (Table 2): black dots are genes with a predicted function, grey dots are genes annotated as 'hypothetical protein'. *Collimonas* 'core' genes were identified among the genes that were found in all test strains by scoring them as present in or absent from the genomes of three other *Oxalobacteraceae* family members: *Herbaspirillum seropedicae*, *Janthinobacterium* sp. Marseille (*Minibacterium massiliensis*) and *Herminiimonas arsenicoxydans* using the Sequence-Based Comparison function in RAST (Aziz *et al.*, 2008): genes with a bi- or unidirectional sequence identity match lower than 20% were scored as absent.

Cf_4382–4403 and Cf_4415–4435 both encode a type III secretion system (T3SS) (Buttner and He, 2009; Mukaihara and Tamura, 2009) and Cf_116–144 encodes a type VI secretion system (T6SS) (Cascales, 2008; Filloux *et al*., 2008). Secretion systems deliver toxins and proteins into the environment or a target cell and play a crucial role in the interaction between bacteria and other prokaryotic and eukaryotic cells (Beeckman and Vanrompay, 2010). There is an increasing body of evidence suggesting that secretion systems play a role in the interaction between bacteria and fungi (Rezzonico *et al*., 2005; Chowdhury and Heinemann, 2006; Warmink and van Elsas, 2008; Nazir *et al*., 2010). It also has been hypothesized that possession of different secretion systems influences host specificity (Fauvart and Michiels, 2008).

We found 259 of the Ter331 genes to be conserved in *C. fungivorans* Ter6 and Ter14 and in *C. arenae* Ter10, but not in *C. pratensis* Ter91. Several of these genes fell into cluster Cf_975–1036, which covers more than 56 kb and codes for chemotaxis-related genes and the flagellar apparatus (Terashima *et al*., 2008). Consistent with this finding is the reduced motility of Ter91 on low-strength agar, compared with other collimonads (Table 1).

Genes shared between strains of C. fungivorans

In total, 518 genes were conserved in all *C. fungivorans* strains (Ter6, 14 and 331) but undetected in the strains of the other two species (*C. arenae* Ter10 and *C. pratensis* Ter91). Many of these presumed *fungivorans*-specific genes fell into cluster Cf_2087–2127, encoding a putative prophage, cluster Cf_3651–3687, encoding a T1SS (Beeckman and Vanrompay, 2010) and cluster Cf_2240–2245. The latter encompasses genes that code for the production of syringomycin and syringopeptin, two non-ribosomal peptides with antibacterial and antifungal activity (Raaijmakers *et al*., 2006).

Several of the other genes specific to the species *fungivorans* showed coding similarity to enzymes that function in cell wall and membrane biogenesis. Changes in the bacterial cell envelope are often related to variation in colony morphology (Long *et al*., 1998; Hasman *et al*., 2000), and our findings are consistent with the classification of *Collimonas* colony types (Table 1). Genes in cluster Cf_2052–2060 resemble genes coding for the synthesis of the exopolysaccharide colanic acid in *Escherichia coli* and are likely to play a role in the *C. fungivorans* morphology type. Exopolysaccharides aid bacterial adhesion to solid surfaces, including fungal hyphae (Broek and Vanderleyden, 1995; Bianciotto *et al*., 2001). Adhesion to fungal hyphae has been demonstrated for *C. fungivorans* Ter331 (de Boer *et al*., 2001), and has been explained as a beneficial albeit not essential contributing factor to the mycophagous phenotype (Leveau and Preston, 2008).

Out of all Ter331 genes, 350 were shared exclusively with *C. fungivorans* Ter14, the strain most closely related to *C. fungivorans* Ter331 based on 16S rRNA gene sequence similarity. This group of genes includes cluster Cf_2729–2745 encoding a T1SS (Beeckman and Vanrompay, 2010), as well as cluster Cf_1127–1146. The latter codes for the production of a putative antifungal compound, which is induced in Ter331 during confrontation with *Aspergillus niger* (Mela *et al*., 2011).

Genes shared by all Collimonas *strains and absent from other* Oxalobacteraceae

To identify '*Collimonas* core' genes, i.e. genes that define the genus *Collimonas*, we asked the question which of the 2343 genes that were shared by all tested *Collimonas* isolates were absent from three closely related *Oxalobacteraceae* strains for which a genome sequence is available. These include *Herbaspirillum seropedicae* SmR1, an endophytic plant-growth promoting bacterium (GenBank accession number CP002039), *Janthinobacterium* sp. Marseille (*Minibacterium massiliensis*) which was isolated from groundwater (Audic *et al*., 2007; GenBank accession number CP000269) and *Herminiimonas arsenicoxydans*, a metabolizer of arsenic (Muller *et al*., 2007; GenBank accession number CU207211). By this approach, we identified 136 '*Collimonas* core' genes (Table 2, Table S1; also marked by dots in Fig. 3). About half of these were annotated as coding for hypothetical proteins, while the rest could be assigned to one of 12 functional categories (Table 2). Many of these genes encode putative lytic enzymes such as chitinases, peptidases, nucleases, lipases and phosphatases. Also, many of the predicted enzymes were found to feature a signal peptide at the N-terminus, suggesting that they are transported outside of the cell (Table 2). What follows is a brief description of some *Collimonas* core genes and a preliminary assessment of their putative role in niche specialization by collimonads.

Genes Cf_3037 and Cf_3039 are part of the *chi* locus A of *C. fungivorans* Ter331 (Fritsche *et al*., 2008) and code for the previously characterized proteins ChiI and ChiII respectively. Our identification of these genes as *Collimonas* core is in excellent agreement with the fact that chitinolysis is a defining characteristic of the strains used in this study (Table 1), as well as other strains of *Collimonas* (Leveau *et al*., 2010). ChiI is an extracellular chitinase responsible for the formation of cleared haloes on colloidal chitin plates, while ChiII is a periplasmic chitinase that converts chitooligosaccharides into chitobiose. Another core gene is Cf_1790, the predicted product of which possesses a chitin-binding domain similar to that of ChiI. Conservation of this gene among the tested colli-

Table 2. *Collimonas* core genes[a] and their predicted functions.

	Chitinases/glucanases
Cf_1790 (1735)	Chitin binding protein[b]
Cf_3037 (2982)	Chitinase ChiI[b]
Cf_3039 (2984)	Chitinase ChiII[b]
Cf_3040 (2985)	Hydrolase[b]
Cf_2793 (2738)	Glucanase[b]
	Peptidases
Cf_254 (199)	Peptidase S9, prolyl oligopeptidase
Cf_255 (200)	Peptidase S9, prolyl oligopeptidase[b]
Cf_319 (264)	Peptidase S9, prolyl oligopeptidase[b]
Cf_1214 (1159)	Family S54 peptidase, transmembrane
Cf_1698 (1643)	Peptidase S9/S15
Cf_2473 (2418)	Peptidase S46[b]
Cf_3234 (3179)	Peptidase S9, prolyl oligopeptidase[b]
Cf_3736 (3681)	Peptidase M13[b]
Cf_4268 (4213)	Pyroglutamyl peptidase C15
	Amino acid catabolism
Cf_2061 (2006)	NAD-glutamate dehydrogenase
Cf_2085 (2030)	Glycine cleavage system P protein
Cf_2086 (2031)	Glycine cleavage system T protein
Cf_2445 (2390)	*N*-formylglutamate amidohydrolase
Cf_2451 (2396)	Urocanase
Cf_3266 (3211)	Lysine 6-dehydrogenase
Cf_3391 (3336)	Kynurenine formamidase
Cf_3392 (3337)	Kynureninase
Cf_3393 (3338)	Tryptophan 2,3-dioxygenase
	Regulation
Cf_210 (155)	Signal transduction response regulator
Cf_270 (215)	Transcriptional regulator, HTH-type
Cf_1944 (1889)	Transcriptional regulator, TetR-like
Cf_2011 (1956)	Transcriptional regulator, HTH-type
Cf_2351 (2296)	Transcriptional regulator, ArsR-like
Cf_4350 (4295)	Isocitrate dehydrogenase phosphatase
	Transferases
Cf_697 (642)	Thiopurine S-methyltransferase
Cf_870 (815)	L-seryl-tRNA selenium transferase
Cf_1190 (1135)	Acyl-CoA *N*-acyltransferase
Cf_1445 (1390)	4′-Phosphopantetheinyl transferase
Cf_1545 (1490)	Acyl-CoA *N*-acyltransferase
Cf_1584 (1529)	Acyl-CoA *N*-acyltransferase
Cf_2693 (2638)	Methyltransferase
Cf_2849 (2794)	Acyl-CoA *N*-acyltransferase
Cf_2861 (2806)	Leucine carboxyl methyltransferase
	Nucleases/nucleoside hydrolases
Cf_111 (56)	HNH endonuclease family protein
Cf_274 (219)	Nucleoside hydrolase[b]
Cf_2969 (2914)	Nucleoside hydrolase[b]
Cf_3396 (3341)	Extracellular endonuclease[b]
	Lipases/esterases
Cf_191 (136)	Lipolytic protein, GDSL family[b]
Cf_2026 (1971)	Secreted lipase[b]
Cf_3379 (3324)	Esterase, SGNH hydrolase-type
	Phosphatases
Cf_689 (634)	Phosphonoacetate hydrolase
Cf_2530 (2475)	Metallo-dependent phosphatase
Cf_2685 (2630)	Phosphatase, HAD superfamily
Cf_3973 (3918)	Alkaline phosphatase[b]
	Sugar uptake/conversion
Cf_1793 (1738)	Quinoprotein glucose dehydrogenase[b]
Cf_2629 (2574)	Sugar-binding periplasmic protein[b]
Cf_2630 (2575)	6-Phosphofructokinase
Cf_2631 (2576)	Fructose-bisphosphate aldolase class I
Cf_3222 (3167)	Xylose isomerase
	Cell wall/fimbriae
Cf_1635 (1580)	Fimbrial protein
Cf_1636 (1581)	Fimbrial assembly chaperone
Cf_2055 (2000)	Polysaccharide pyruvyl transferase
Cf_4365 (4310)	UDP-*N*-acetylglucosamine transferase
	Secretion
Cf_2396 (2341)	VirJ component of T4SS[b]
Cf_4132 (4077)	General secretion pathway protein N
Cf_4133 (4078)	General secretion pathway protein M
Cf_4136 (4081)	General secretion pathway protein J
Cf_4137 (4082)	General secretion pathway protein I
Cf_4138 (4083)	General secretion pathway protein H
	Other
Cf_1181 (1126)	Sulfide quinone-reductase
Cf_2017 (1962)	Low temperature requirement A protein
Cf_2132 (2077)	Rhodanese-type protein
Cf_2691 (2636)	OsmC-like protein
Cf_3136 (3081)	Thioredoxin[b]
Cf_3367 (3312)	Activator of heat shock protein

a. Defined as genes that are shared between *Collimonas* strains Ter331, Ter14, Ter6, Ter91 and Ter10, but are absent from the genomes of *Herbaspirillum seropedicae, Janthinobacterium* sp. Marseille (*Minibacterium massiliensis*) and *Herminiimonas arsenicoxydans*. The number in parentheses indicates for each gene its position in Fig. 3. Not included in this table are 66 *Collimonas* core genes for which the annotation was 'hypothetical protein'. These genes included Cf_71, 72, 74, 171, 181, 208, 333, 507, 509, 551, 581, 1164, 1177, 1188, 1194, 1215, 1368, 1377,1436,1453, 1475, 1551, 1586, 1601, 1627, 1705, 1870, 1894, 1916, 1920, 1965, 2008, 2159, 2275, 2440, 2441, 2469, 2602, 2699, 2860, 2967, 3004, 3007, 3038, 3120, 3141, 3252, 3275, 3306, 3394, 3550, 3580, 3592, 3604, 3630, 3744, 3745, 3746, 3890, 3897, 3938, 3972, 4281, 4366, 4368 and 4453.

b. These gene products featured a signal peptide at their N-terminus, as predicted by SignalP (Petersen *et al.*, 2011).

monads together with other genes involved in chitin breakdown suggests it has a role in the chitinolytic system of *Collimonas*.

The *Collimonas* core list (Table 2, Table S1) features nine peptidases, five of which are predicted to be extracellular. The list includes several (cytosolic) enzymes involved in the catabolism of amino acids, including tryptophan (Cf_3391, 3392, 3393), lysine (Cf_3266), histidine (Cf_2445, 2451), glycine (Cf_2085, 2086) and glutamate (Cf_2061). We also identified two endonucleases and two nucleoside hydrolases as *Collimonas* core gene products. One of the former is annotated as an extracellular endonuclease with non-specific activity towards DNA and RNA, while nucleoside hydrolases are involved in the salvage of purines and pyrimidines formed during the degradation of DNA and RNA. The conservation of *Collimonas* core genes with putative activity towards peptides, DNA/RNA and their respective building blocks suggests a

lifestyle for collimonads that is specialized in feeding off the biopolymers of live or dead organisms. Biotrophy and necrotrophy both have been presented as strategies for bacteria that seek to convert fungal biomass into bacterial biomass (Leveau and Preston, 2008). The possession of a complete chitinolytic system lends support to the idea (de Boer *et al*., 2001) that collimonads have the means to access the content of living fungi that have chitin as a major structural component in their hyphae. Fully compatible with this idea is the hypothesis that collimonads play a role as recyclers of dead fungal and other biomass. This may be one of the niches that *Collimonas* has filled as a common colonizer of oligotrophic environments.

We identified a *virJ* homologue in all tested collimonads (Table 2). In *Agrobacterium tumefaciens*, VirJ has been demonstrated to form complexes with proteins in the periplasm before interacting with components of the T4SS (Pantoja *et al*., 2002). As pointed out (Gauthier *et al*., 2003), this might allow periplasmic proteins to be translocated across the outer membrane via the T4SS but be exported to the periplasm by another pathway. However, only strain Ter331 has a full complement of T4SS genes, located on plasmid pTer331 (Mela *et al*., 2008), so why the other Ter strains also have retained a copy of VirJ remains unclear under this hypothesis.

The conserved possession of a predicted quinoprotein glucose dehydrogenase (Cf_3973) is interesting in light of the ability of *Collimonas*, but not *Herbaspirillum* and *Janthinobacterium*, to form haloes on tricalcium phosphate plates (Uroz *et al*., 2009). This enzyme catalyses the periplasmic oxidation of glucose to gluconic acid and has been identified as an important contributor to the mineral phosphate-solubilizing phenotype in *Pseudomonas* and *Pantoea* species (Babu-Khan *et al*., 1995). A gene of interest in the category 'Phosphatases' is Cf_689, the product of which resembles phosphonoacetate hydrolases. These enzymes catalyse the conversion of phosphonoacetate into acetate and phosphate, and their C-P bond breaking activity has been implicated in phosphate cycling (Quinn *et al*., 2007).

Conclusion

The results of our CGH analysis suggest that within the family *Oxalobacteraceae*, collimonads have adopted a lifestyle that is based on the possession of genes coding for the production of extracellular and periplasmic enzymes with lytic and solubilizing activities. Many of the genetic determinants shared among the tested collimonads could be placed in the framework of two defining properties of members of the *Collimonas* genus, i.e. weathering and bio- or necrotrophic mycophagy. We revealed several correlations between the presence/absence of certain genes and the intrageneric variation in *Collimonas* phenotypes, including the utilization of the fungal storage compound trehalose, motility, the production of exopolysaccharide, and the secretion of proteins and/or toxins. Our findings are the basis of future work which will focus on the *Collimonas* core genes, to confirm by experimentation their functional contribution to the survival of collimonads in their natural habitats.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Receiver operating characteristic (ROC) curve indicating different presence score thresholds used to separate true positive from false positive calls. The points on the curve represent true positive and false positive rates at various thresholds, including the chosen threshold of –0.9, which offers a 100% true positive rate with a 5% false positive rate. Further details are provided in Table S1.

Table S1. Distribution of Ter331 genes among Collimonas isolates Ter6, Ter10, Ter14 and Ter91.

Table S2. Confirmation of CGH-called presence/absence of 12 selected genes by PCR analysis.

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