Genomic analysis of bacterial mycophagy

Mela, F.

Genomic Analysis of Bacterial Mycophagy

Francesca Mela

The study described in this thesis was performed at the institute of Biology of Leiden University; the practical work was performed at the Netherland Institute of Ecology, NIOO-KNAW.
The financial support of this study was provided by the BSIK program Ecogenomics.

Cover picture: *Aspergillus niger* and *Collimonas fungivorans* on water yeast agar plate.

Printed by Gildeprint Drukkerijen, Enschede

ISBN: 978-90-816775-1-6

Genomic Analysis of Bacterial Mycophagy

Proefschrift
ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 22 februari 2011
te klokke 15:00 uur

door
Francesca Mela

geboren in 1978, Imperia, Italy

Promotiecommissie

Promotor	**Prof. Dr. J.A. van Veen**
	Leiden University
Co-promotor	**Dr. W. de Boer**
	Netherlands Institute of Ecology, Heteren
	Dr. J. H. J. Leveau
	University of California, Davis, USA
Overige leden	**Prof. Dr. J. D. van Elsas**
	University of Groningen
	Prof. Dr. C. J. ten Cate
	Leiden University
	Dr. A. F. J. Ram
	Leiden University
	Dr. K. Vrieling
	Leden university

Contents

Chapter 1

General introduction

In many terrestrial environments bacteria and fungi share habitats e.g. organic soil layers, surfaces of plant leaves and crop residues. In these habitats fungi and bacteria have a wide range of interactions, which span from synergism to antagonism. It has been recognized that knowledge of bacterial-fungal interactions is essential for a better understanding of terrestrial ecosystems (1). In addition, knowledge on bacterial-fungal interactions is the base of novel applications in agriculture, food industry and human health. Various bacterial-fungal interactions have been studied extensively and results have been applied widely for agricultural purposes. The antagonism of rhizosphere bacteria against plant-pathogenic fungi has been exploited for the biocontrol of soil-borne plant diseases (2-3), while mycorrhizal helper bacteria have received attention for their ability to promote mycorrhizal symbiosis (4). Understanding the reciprocal influence of bacteria and fungi will also aid in the study of infectious diseases. It has been demonstrated that mixed bacterial-fungal infections have properties which differ from single species infections (5-7) and that several mycotoxins are not produced by fungi but originate from endosymbiotic bacteria hosted into the fungal cytoplasm (8-10). Bacterial-fungal interactions may also result in the production of bioactive compounds, like antibiotics and lytic enzymes, which can be identified and exploited for human therapeutic or agricultural purposes.

Bacterial-fungal interactions have significant impact on growth dynamics of both fungi and bacteria (7). In natural environments the mutual influence of bacteria and fungi plays a role in shaping the structure and ecological properties of the microbial community and is at the origin of fungal-selected bacterial communities and bacterial-selected fungal communities (11-15).

In terrestrial ecosystems the two groups do, to a certain extent, occupy different niches: fungi outcompete bacteria in degradation of recalcitrant

organic matter, while bacteria play a major role in decomposition of simple substrates like root exudates (1). Nevertheless, this niche specialization is not complete, but it is the outcome of a dynamic equilibrium resulting from an ongoing confrontation of fungi and bacteria for both simple and recalcitrant substrates. Confrontation between the two groups might be the driver of evolutionary selective pressure. For example in the rhizosphere bacteria dominate the decomposition of root exudates, but it is likely that fungi play a significant role in this process as well (16-18). Thus, the presence of fungal competitors in the rhizosphere must exert a selective pressure on bacteria to compete for nutrients. This pressure may be at the origin of a higher incidence of antifungal traits in bacteria isolated from fungal-rich soils (19). Bacteria are present in the decay community of lignin substrates (14, 20-21), despite the fact that they play a minor role in decomposition of lignin, which is dominated by lignocellulolytic Basidiomycota and Ascomycota. These bacterial species may be competitors of fungi which scavenge simple substrates released by fungal degradation, or they might be mutualistic species, cooperating in the degradation of these recalcitrant substrates (1, 20, 22). Besides confronting for the degradation of organic substances, fungi and bacteria can represent a nutrient source for one another. Certain fungi are able to lyse and consume bacteria (23-25), and certain bacteria are able to consume fungal exudates and also grow on dead and living fungal hyphae (1, 26-28). This thesis describes the results of a research project which addressed the genetic determinants underlying bacterial mycophagy, a trophic interaction in which bacteria obtain nutrients from living fungal hyphae.

Bacterial mycophagy

Mycophagy can be defined in a broad sense as a trophic interaction, in which an organism exploits a fungus to obtain nutrients. The concept of bacterial mycophagy has been extensively reviewed by Leveau and Preston (29). Bacterial mycophagy is defined as the demonstrable and quantifiable effect of bacterial phenotypic behaviour that makes nutrients available from living fungal hyphae and allows the conversion of living fungal biomass

into bacterial biomass (29). Mycophagous bacteria have an active role in obtaining nutrients from the fungus, and do not simply consume fungal nutrients which have become available for reasons independent from the bacterial action. Bacterial mycophagy was demonstrated for the first time for bacteria of the genus *Collimonas,* based on their ability to grow at the expense of living fungal hyphae in a soil-like microcosm (28, 30). *Collimonas* bacteria inoculated in purified sand without addition of nutrients were shown to increase up to 100-fold as a consequence of the invasion of the microcosm by fungal hyphae growing from an agar plug placed on top of the sand. The growth of *Collimonas* was not observed in the absence of fungal hyphae and other bacterial species, including chitinolytic bacteria (*Cytophaga*-like bacteria and strenotrophomonads) and consumers of fungal exudates *(Burkholderia* and *Pseudomonas* species), did not show a growth response when tested in the same setting. Microscopic observations revealed that *Collimonas* bacteria adhered to the hyphae, which were often swollen or collapsed. The result was taken as evidence that *Collimonas* actively had obtained nutrients from the fungus (30). Later on, Höppener-Ogawa *et al.* (31) demonstrated that mycophagous growth of *Collimonas* is not restricted to artificial laboratory environment, but can also take place in natural soils (31). *C. fungivorans* Ter331 was chosen as a model organism for the study of bacterial mycophagy. Several genomic tools and resources, including the annotated genome sequence of *C. fungivorans* Ter331 and microarrays for expression and comparative genomic studies, have been developed for *Collimonas* research (32).

Collimonas

Collimonas bacteria belong to the family *Oxalobacteraceae* in the order *Burkholderiales* of the β-proteobacteria. Until now there are three recognized species of *Collimonas*: *C. fungivorans*, *C. arenae* and *C. pratensis,* even though the taxonomic status of several *Collimonas* strains needs to be further investigated and might lead to additional *Collimonas* species (33). All three *Collimonas* species possess mycophagous ability; however the morphology and the metabolic characteristics of the three

species are different (33). *Collimonas* bacteria were isolated in the framework of a project searching for a naturally occurring biocontrol agent of fungi pathogenic to marram grass (*Ammophila arenaria*) (34). The main targets of this study were chitinolytic bacteria, capable of degrading chitin, which is a main component of fungal cell wall.*Collimonas* bacteria were isolated as being dominant among the cultivable chitinolytic bacteria in acidic dune soil and the *Collimonas* isolates were initially identified as *Pseudomonas* species based on colony phenotype and whole-cell fatty acid methyl ester (FAME) analysis (34). In 2001 *Collimonas* was reconsidered as constituting a new group of mycophagous β-Proteobacteria (30) and in 2004 this group was recognized as constituting a new genus (28, 30).

A field inventory of the presence of *Collimonas* in 45 soils, carried out using culture-dependent and culture-independent (real-time PCR) methods and covering a wide range of soil physical characteristics, vegetation and management, indicated that bacteria of the genus *Collimonas* are not restricted to dune systems, but are constituents of the soil microbial community in different environments, albeit at a low density (10^4- 10^5 cells g^{-1} dry weight soil) (35). *Collimonas* bacteria are likely to be part of the rare biosphere (32, 36). The occurrence of *Collimonas* bacteria was registered in several soil environments including forests, grasslands, heathlands, tundra, mire and ex-agricultural fields (32), but also coastal areas (37) and water (38), even though the presence of *Collimonas* in water seems to be occasional and resulting from *Collimonas* washing out from soil.

According to Höppener-Ogawa (39), the number of *Collimonas* cells in soil does not correlate with the soil fungal biomass as estimated on the base of ergosterol. This may indicate that mycophagous growth might only be one of the possible survival strategies of *Collimonas* bacteria. The ability of *Collimonas* to oxidize various carbon sources was tested using Biolog GN plates and revealed that *Collimonas* bacteria possess a wide metabolic versatility (33). Mycophagy might be replaced by the consumption of more easily accessible nutrients, when they are available in the environment. In a similar way fungi of the genus *Trichoderma,* which are able to parasitize

other fungi, are also opportunistic avirulent plant symbionts commonly associated with the rhizosphere (40-41).

Different adaptations might aid survival of the *Collimonas* bacteria, depending on the biotic and abiotic environment, ranging from the capacity to efficiently colonize plant roots (42), to the survival in polluted soils (43-45), to obtain nutrients from rocks (46), to hydrolyze chitin (47) and to exhibit antifungal activity. The relationship between the antifungal property of *Collimonas* and its mycophagous ability is not yet clear. *Collimonas* anti-fungal properties were tested against several fungi including plant pathogens like *Fusarium culmorum* (34), *Fusarium oxysporum* (42, 48), *Rhizoctonia solani* (48), *Verticillium dahliae* (37) but also saprophytic fungi like *Mucor hiemalis* and *Chaetomium globosum* (34) and mycorrhizal fungi like *Laccaria bicolor* (49) and *Glomus mosseae* (50). Different susceptibility of fungal species to *Collimonas* isolates were found, as well as a different level of fungal inhibition depending on the *Collimonas* strains used.

The interaction of *Collimonas* with fungi has a minor impact on total fungal biomass turnover, but it had an effect on the species composition of the fungal soil community, possibly because different susceptibility of the various fungal species to *Collimonas* alters the outcome of fungal species competition (51-52). Despite the shift in the architecture of the fungal community, the functional redundancy of the community may ensure the maintenance of the soil functional characteristics, such as the ability to form mycorrhizal symbiosis and the ability to degrade cellulose (51).

The distribution, the abundance and the complex of survival adaptations characterizing this bacterial group delineate *Collimonas* as an oligotrophic bacterium adapted to life in nutrient-poor environments (32).

Tools and resources available for the study of *Collimonas*

This study aimed at increasing the understanding of the mechanisms and the genetic determinants underlying the interaction between *Collimonas* bacteria and fungi. This investigation took advantage of some of the resources available for the study of *Collimonas*. bacteria (32) These available resources include the annotated genome of the model organism *C.*

fungivorans Ter331 (53), a genomic library (54), a plasposon mutant library, which allows the search for fungal-related genes by a loss of function approach (55), and two microarrays designed on the base of the genomic sequence of *C. fungivorans* Ter331: one ORF-base microarray for expression studies and one tiling microarray covering the whole chromosome and plasmid sequence, available for array based comparative genomic studies.

Mechanisms of bacterial-fungal interactions

The strategies that bacteria can use to obtain nutrients from fungi can be classified into three categories: extracellular necrotrophy, extracellular biotrophy and endocellular biotrophy (29). Extracellular necrotrophic bacteria kill the fungal hypha in order to obtain the release of fungal nutrients. The hypha can be killed by breaking the integrity of the cell wall and membrane, by blocking basic metabolic pathways or by inducing programmed cell death. Bacterial induced death of fungal hyphae has been studied extensively in bacterial pathogen of edible mushrooms, which kill the hyphae and grow on their cytoplasmic content (56). Extracellular biotrophic bacteria obtain nutrients from the fungus without killing it, but rather modulating its physiology. Bacteria may affect the permeability of the fungal membrane and the quantity or quality of fungal exudates which results in alteration of fungal morphology and growth (1). Biotrophic modulation of fungal physiology has been described for mycorrhizal helper bacteria (MHB), which promote the formation of the mycorrhizal symbiosis between fungi and plant root (4) and have been shown to be able to alter the morphology and the transcriptome of fungi (49, 57-59). As the main focus of MHB studies are the fungus and its ability to form the mycorrhizal symbiosis, the benefit that the bacterium obtains from the fungus has received little attention. Nevertheless it seems likely that the bacterium obtains nutrients from its association with the fungus (11, 60) and that the interaction among bacterium, fungus and plant is beneficial for all three partners (61-62). In the case of endocellular biotrophy the bacteria use the fungal hypha as a nutrient rich environment in which they multiply. The

first endosymbiotic bacterium identified was "*Candidatus* Glomeribacter gigasporarum", an endosymbiont of the arbuscular mycorrhizal fungus *Gigaspora margarita* (63). Endosymbiotic bacteria have been reported in several species of mycorrhizal (62) and also non-mycorrhizal fungi (10).

Each of these mycophagous interactions involves a set of determinants which can sometimes be common to more than one interaction. For example the production of bioactive compounds such as toxins acting on the fungal membrane transport, could be common to both necrotrophic bacteria and extracellular biotrophic bacteria. The first could use the toxins to kill the hyphae by inhibition of the transport across fungal membrane; the second might use the toxins to modulate fungal release of exudates.

The mechanisms and the determinants characterizing bacterial mycophagy have not yet been elucidated. An increasing body of evidence suggests that bacterial mycophagy is a complex phenotypic trait. Until now attempts to identify mycophagous related genes in *Collimonas* by screening the plasposon mutant library was not successful, as the mycophagous activity of *Collimonas* was not completely suppressed in any mutant (32). It is likely that several genes contribute incrementally to the mycophagous behaviour and that there is no genetic determinant which absence would completely abolish mycophagy. For example mutants impaired in the ability to lyse chitin were not affected in their antifungal properties, or in their biocontrol activity *in vivo* against the fungal pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici* (42, 47). Accordingly, addition of allosamidin, an inhibitor of chitinases, reduced but did not eliminate mycophagous growth of *Collimonas* in sand microcosms experiments (30).

Putative determinants of bacterial mycophagy

A broad range of determinants might be important for mycophagous behaviour. Previous studies about fungal-bacterial interactions form the base for formulating hypotheses. Possible determinants are:

- **Chemotaxis and motility**. It has been shown that fungal-derived substrates can exert an attractive force on bacteria and that bacteria are able to move towards fungal hyphae following a chemical

gradient (64-67). Physical contact with the mycelium might influence the bacterial ability to obtain nutrients from fungi (1, 68). The ability of bacteria to move towards and colonize fungal hyphae plays a role in bacterial inhibition of fungi by siderophores and antibiotics (69).

- **Adhesion.** Many bacteria produce multiple surface polysaccharides and pili that aid in bacterial adhesion to solid surfaces as well as to fungal hyphae (70-72). Several microscopic observations of the interactions between antagonistic bacteria and fungi showed that bacteria adhere to fungal hyphae (42, 73-74). In many cases, the intimate contact between bacteria and fungi appears to trigger specific fungal reactions (75-76).
- **Antifungals.** Antibiotics play a key role in the biological control of plant pathogenic fungi by bacteria (77). Antifungal metabolites are able to weaken fungal structural elements like the fungal wall (56), interfere with fungal self-defence mechanisms and alter the normal physiology of fungal hypha (78-79).
- **Lytic enzymes.** Chitin is the β1,4-linked polymer of N-acetyl-D-glucosamine and is a main structural component of the fungal cell wall. Degradation of fungal cell wall components, including chitin, is expected to be part of mycophagous destabilization of the fungal hypha and consumption of fungal derived substrates (1). Possession of chitinolytic enzymes, chitinases, is essential for mycoparasitism of *Trichoderma* species (40) and is commonly present in bacteria that have antifungal activity (1, 80).
- **Secretion systems.** Secretion systems play a crucial role in the interaction between bacteria and other prokaryotic and eukaryotic cells by delivering toxins and lytic enzymes into the environment or within the cytoplasm of a target cell (81-82). The effector secreted by these systems might have a wide range of roles in bacterial mycophagy, including killing or altering the morphology of the fungal hypha, and interfering with fungal response to bacterial presence (29). There is emerging evidence for an involvement of

secretion systems in fungal-bacterial interactions (61, 68). Enrichment of secretion system-related genes in the bacterial community associated with the mycosphere of *Laccaria proxima* compared to the bulk soil was detected by Warmink and van Elsas (83). The expression of the type III secretion system from *Pseudomonas fluorescens* KD was stimulated by the presence of the oomycete *Pythium ultimum* and decreased the ability of *P. ultimum* to induce damping-off disease in cucumber (84). Inactivation of the type II secretion system rendered avirulent *Burkholderia gladioli* pv. agariciola, causative agent of cavity disease on cultivated mushrooms (85).

- **Growth on fungal derived nutrients and tolerance to fungal produced antibacterial metabolites.** Mycelial exudates support the growth of the bacterial community and influence its species composition (86). The ability to use trehalose, a sugar produced by many fungi, was suggested to play a role in the selection of the bacterial species associated with the mycorrhizosphere (87-88). Organic acids and antibacterial substances contained in the mycelial exudates can selectively affect certain bacterial species which are not able to cope with bioactive compounds and medium acidification (1).
- **Plasmid encoded determinants.** Plasmids are extrachromosomal DNA elements which are able to self replicate and maintain themselves in the host cell. Plasmids often code for host beneficial functions such as catabolic properties and resistance to drugs or heavy metals (89-90). They catalyze genetic exchange among the members of the microbial community helping the dissemination of genes important for survival in a given environment (91-92). The role played by plasmid in bacterial adaptation to fungus determined microhabitats is currently unclear. There are plasmids known to harbour genes involved in bacterial symbiosis (93) and virulence (94-95). Nazir and colleagues suggested that there may be a plasmid-

born asset in bacterial colonization of fungal hyphae via the stimulation of biofilm formation (68).

Fungal responses induced by interaction with bacteria

Just as bacteria rely on several mechanisms to influence fungi, fungi have diverse mechanisms as a response to bacterial action. These mechanisms are just partially known (96-97).

- **Hyphal growth and sporulation.** Bacteria are able to stimulate fungal growth and interfere with fungal germination and sporulation (23, 98-99). The enhancement of fungal production of fruiting bodies and hyphal length is generally interpreted as a positive effect of bacteria on fungal physiology, although in some instances these phenomena may represent a stress response (1).
- **Secondary metabolites.** Interaction with bacteria can trigger fungal production of secondary metabolites such as antibiotics (75) or melanin, which encrusts fungal cell wall increasing resistance to several stresses e.g., by cell-wall degrading enzymes (100-101).
- **Resistance to antibiotics.** Fungal response to bacterial antibiotics includes resistance, export via membrane bound pumps or enzymatic degradation of the antibiotics, and also production of molecules able to interfere with antibiotic production in bacteria (97, 102).
- **Altering the environment.** Fungi can alter the physical environment in a way that reduces the fitness of bacteria. For example lowering the pH can create a habitat less conducive to bacterial growth and influence bacterial antibiotic production (77, 97)
- Additional mechanisms known to function in self-defense, like i**nduction of programmed cell death** (76) and **production of reactive oxygen species** (103), might also play an important role in fungal response to bacterial presence (96).

Aim and research questions

The work described in this thesis aimed at exploring the genetic determinants underlying the interaction of the mycophagous bacterium *Collimonas* and fungi, making use of the genomic tools available for *Collimonas* research. The distribution of the putative determinants of mycophagy was investigated by means of comparative genomics.

Research questions

In this thesis the following research questions were addressed:

- Which of the mechanisms putatively involved in *Collimonas* mycophagy are actually activated when *Collimonas* interact with a fungus
- What is the fungal response to the presence of *Collimonas* bacteria
- What is the role played by plasmid pTer331, detected in the genome of the mycophagous bacterium *C. fungivorans* Ter331, in the ecology of this bacterium? Are the genes encoded on plasmid pTer331 involved in mycophagy?
- Are the putative determinants of mycophagy uniformly distributed among *Collimonas* species?

Outline of the thesis

An *in vitro* assay was set up to study the interaction between the model organism *C. fungivorans* Ter331 and the fungus *Aspergillus niger*. The genetic determinants activated in *C. fungivorans* Ter331 as a consequence of its interaction with the fungus were analyzed by microarray analysis. A parallel microarray analysis was carried out in the same study to investigate the change in the expression profile of the fungus as a consequence of the presence of the bacterium. (Chapter 2)

The sequence of plasmid pTer331, isolated from the genome of *C. fungivorans* Ter331, was determined and annotated. The occurrence of the plasmid in the *Collimonas* strains present in our collection was investigated. The role of the plasmid in the mycophagous and antifungal behavior of

Collimonas was evaluated, as well as its role in other phenotypic traits characterizing *Collimonas* bacteria, such as the ability of colonizing efficiently the root system of tomato, mineral weathering and chitinolysis. (Chapter 3)

Microarray based comparative genomic hybridization was used to investigate the conservation of genes of the model organism *C. fungivorans* Ter331 in four other strains, representing the three *Collimonas* species identified to date. A set of highly conserved genes was identified as well as a set of variable genes. The conservation of genes putatively involved in bacterial fungal interaction was examined. (Chapter 4)

A general discussion is presented in Chapter 5

Chapter 2

Listening in on a bacterial/fungal dialog: transcriptional profiling of an antagonistic interaction

F. Mela, K. Fritsche, W. de Boer, J. A. van Veen, L. de Graaff , M. van den Berg, J. H. J. Leveau

Submitted to The ISME Journal

Abstract

Interactions between bacteria and fungi cover a wide range of incentives, mechanisms, and outcomes. The genus *Collimonas* consists of soil bacteria that are well known for their antifungal activity and for mycophagy, i.e. the ability to grow at the expense of living fungi. The aim of the current study was to gain a better understanding of the mechanisms of antagonism of *Collimonas* bacteria towards fungi, the involvement of the mycophagous phenotype, and the role of the fungus as a responsive partner in the interaction. In an *in vitro*, non-contact confrontation assay with the fungus *Aspergillus niger, Collimonas fungivorans* showed accumulation of biomass concomitant with inhibition of hyphal spread. Bacterial and fungal RNA were isolated at two time points during the interaction and analyzed by microarray analysis. *Collimonas* responded to the presence of the fungus by activating genes for the utilization of compounds of fungal origin and the production of a putative antifungal compound. In *A. niger*, transcriptional changes were observed for genes involved in lipid and cell wall metabolism and in cell defense. These changes correlated well with the hyphal deformations that were observed microscopically. Transcriptional profiles revealed signs of distress in both partners: in the bacteria, these included downregulation of ribosomal proteins and upregulation of mobile genetic elements, while the fungus showed endoplasmic reticulum stress and expression of conidia-related genes. Also, both partners in the interaction experienced a shortage of nitrogen due to each other's presence. Overall, our results indicate that the interaction between *Collimonas* and *Aspergillus* is characterized by a complex interplay between trophism, antibiosis, and competition for nutrients.

Introduction

Fungi and bacteria co-exist in many terrestrial environments, where they compete for access to nutrients, or even constitute a nutrient source for each other (1, 104). Bacterial mycophagy (29) is a trophic behavior by which bacteria actively obtain nutrients from living fungal hyphae. For bacteria belonging to the genus *Collimonas* (28), mycophagy was demonstrated in a gnotobiotic sand system, in which bacterial numbers were shown to increase after invasion of common soil fungi like *Mucor hiemalis* and *Chaetomium globosum* (105). Follow-up studies revealed that invasion of field soils by fungal hyphae resulted in a transient but significant increase of indigenous collimonads, suggesting that mycophagy also occurred under natural circumstances (52). The mycophagous phenotype of collimonads was recently explained as an adaptation to life under conditions of limited nutrient availability (32).

The relationship between the mycophagous behavior of collimonads and their demonstrated ability to arrest fungal growth is currently unclear. So far, the two phenomena have been studied in parallel to each other, although it is fair to assume that they share underlying mechanisms (29). The ectomycorrhizal fungus *Laccaria bicolor* (49), arbuscular mycorrhizal fungus *Glomus mosseae* (50) and various soil fungi including *Chaetomium*, *Fusarium*, and *Mucor* species (34) all showed reduced fungal growth when co-inoculated on agar plates with *C. fungivorans* strain Ter331. While the detriment to the fungus was well documented in each of these cases, it was not assessed if and how the bacteria caused it or how they might have benefited from it. Their antagonistic activity likely involves the production of an antibiotic compound, but whether this provided the bacteria an advantage over the fungus in competition for limiting nutrients, or helped them in a mycophagous fashion, e.g. by causing hyphal damage and release of fungal content, is not clear. These scenarios are not mutually exclusive, and both may be explored by mycophagous bacteria under conditions of nutrient limitation in the presence of fungi.

In preliminary confrontations of *C. fungivorans* Ter331 with *Aspergillus niger* on nutrient-poor plates, it was observed that 1) the growth of this

fungus was severely inhibited by the bacterium and 2) the presence of the fungus on the confrontation plates resulted in the accumulation of bacterial biomass. These results suggest simultaneous expression of the mycophagy phenotype (i.e. conversion of fungal biomass into bacterial biomass) and the production of one or more antibiotic compounds. As such, it provides a unique opportunity and starting point for assessing the interrelatedness between mycophagy, antibiosis, and competition in this bacterial/fungal interaction. Best known for its role as a common food spoiler, but also as a ”cell factory” in the fermentation industry, and an opportunistic human pathogen, *A. niger* had its complete genome sequenced recently (106). In addition, an Affymetrix microarray is available for carrying out gene expression studies, and there is a large body of literature on *Aspergillus* mutants and their phenotypes. Similarly, genomic resources are available for *C. fungivorans* strain Ter331 (32), including an annotated genome (32), a plasposon mutant library (55) and a large insert genomic library (54). We exploited the availability of genomic resources for both partners and took a transcriptomic approach and listen in on the dialog that goes on during their interaction on nutrient-poor plates. Typically, such approaches have been very valuable by offering new insight into the complexity of bacterial/fungal interactions (49, 57-58, 107-108). However, in many of these studies only one partner was profiled, treating the other one as a black-box component of the biotic environment. In the approach taken here, we profiled the transcriptomes of both the bacterium and the fungus, which allowed us to interpret any changes in gene expression in one organism in terms of the other’s possible cause or effect. The strategy of dual partner profiling has been applied successfully to the study of prokaryotic-host cross-talk (109-110), providing access to important information on organisms interaction.

Materials and Methods

Strain cultivation and confrontation assay. *Collimonas fungivorans* Ter331 (28) was inoculated from 1/10 TSB agar plates (1 g KH_2PO_4, 1 g NaCl, 3 g Tryptone Soya Broth (Oxoid, Basingstoke, UK), and 20 g agar (J. T. Baker, Phillipsburg, NJ) per liter) into 1/10 liquid TSB medium. After

o/n growth at 25 °C, bacterial cells were harvested by centrifugation, washed once with Wash Solution (0.25 g KH_2PO_4 per liter, pH 6.5), and re-suspended to an optical density of 1 at 600 nm. *Aspergillus niger* strain 400 (CBS 120.49) was grown on PDA medium (19.5 g Potato dextrose agar (Oxoid) and 7.5 g agar (J. T. Baker) per liter) for 4 days at 30 ºC. Spores were harvested by washing with sterile saline solution (111) supplemented with 0.005 % Tween 80, filtered through Miracloth (Calbiochem, Nottingham, UK) to remove mycelial fragments, washed twice and re-suspended in saline solution. For the confrontation assay (Fig. 1), Petri dishes (9 cm diameter) contained 25 ml of WYA (1 g KH_2PO_4 , 0.1 g NaCl, 0.1 g Bacto™ Yeast-Extract (Difco), and 20 g agar (J.T. Baker) per liter) supplemented with 10 µg bromocresol purple per ml.

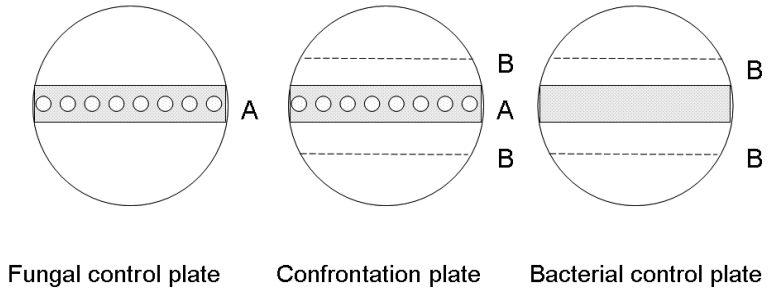

Figure 1. Schematic representation of the *Collimonas*/*Aspergillus* confrontation assay. The central panel represents a plate that was inoculated, as described in Materials and Methods, with 8 drops of fungal spore suspension (A) on top of a rectangular polycarbonate membrane, and with two lines of bacterial inoculum (broken line B) on either side of the membrane. On the control plates, only one partner was inoculated, either *A. niger* (left panel) or *C. fungivorans* (right panel).

In the center of the plate, an autoclaved 2-cm wide strip of Nuclepore Track-Etch polycarbonate membrane (Whatman, s'-Hertogenbosch, The Netherlands, catalog number 113506, 0.2-µm pore size) was placed, on top of which eight 2.5-µl droplets each containing 10^4 *A. niger* spores were equidistantly deposited. The polycarbonate membrane separated the mycelium from the agar medium, thus preventing the fungus from growing

into the agar and allowing easy and complete removal of hyphal material for RNA extraction (see below). Next, eight 2.5-µl droplets of bacterial suspension were placed at a distance of 2.5 cm on either side of the membrane and streaked into a single line parallel to the edge of the membrane. Plates were sealed with Parafilm and incubated at 20 °C. Control plates were inoculated as described above, but with only *A. niger* or *C. fungivorans* on the plate.

RNA isolation. RNA was isolated at two time points during the interaction between the bacterium and the fungus, i.e. 5 days after inoculation (time point T1), at which time the production of bacterial slime and slowdown of fungal growth were first apparent, and one day after T1 (T2). For fungal RNA isolation, approximately 60 mg mycelium was retrieved and pooled from the combined membranes of 7 confrontation plates. After grinding in a mortar with liquid nitrogen, total RNA was extracted using Trizol Reagent (Invitrogen, Breda, The Netherlands) following instructions of the manufacturer. Each RNA isolation was performed in duplicate for both treatments (i.e. presence/absence of *C. fungivorans*) and both time points (i.e. T1/T2), resulting in 8 fungal RNA samples. For bacterial RNA isolation, biomass was collected by retrieving and pooling cells from the same seven plates from which fungal RNA was isolated. After resuspension in pre-cooled (4ºC) Wash Solution and centrifugation at 3000 g at 4 ºC, RNA was extracted from the bacterial cell pellet using Trizol Reagent according to the manufacturer's instructions. Each bacterial RNA isolation was done in duplicate for both treatments (presence/absence *A. niger*) and both time points (T1/T2), resulting in 8 bacterial RNA samples. RNA quantity and quality were checked using a NanoDrop (Isogen Life Science, IJsselstein, The Netherlands) and Experion (Bio-Rad, Veenendaal, The Netherlands), respectively.

Microarray analysis. Synthesis, labelling and hybridization of fungal cDNA to *A. niger* GeneChips were performed according to instructions of the manufacturer (112). The dsmM_ANIGERa_coll511030F GeneChips are based on the genome sequence of *A. niger* CBS513.88 (106) and contain 14,554 probe sets representing all annotated ORFs and genetic elements of

A. niger. Chip information is available in NCBI's Gene Expression Omnibus (accession number GPL6758). *C. fungivorans* Ter331 cDNA synthesis, array hybridization and scanning were performed by NimbleGen (Roche NimbleGen Systems, Iceland). The cDNA was synthesized from 20 µg total RNA and hybridized on a custom made expression array produced by NimbleGen. Each of the 4,480 annotated chromosomal (32) and 43 plasmid-located (Chapter 3) genes was represented by 5 replicates of sixteen 60-mers. The array also contained 32,483 control probes used by Nimblegen to quantify background signal and non-specific hybridization.

Data analysis. *C. fungivorans* microarray data were normalized by NimbleGen using quantile normalization (113) and the Robust Multichip Average (RMA) algorithm (114-115). *A. niger* microarray data were normalized using the Bioconductor package "affy" (116). Both data sets were analysed using the ArrayStar version 2.1.0 (DNAStar, Madison, Wisconsin, USA). To identify genes with significant changes in expression levels, the following criteria were applied: (i) the *p*-value for a Moderated t-Test (117) with FDR multiple testing correction should be equal to or less than 0.05, and (ii) an absolute fold change in transcript level should be equal to or greater than 2.

Validation of microarray data. Validation of microarray data was performed by real-time PCR analysis of a subset of differentially expressed genes. For *Collimonas*, we confirmed the expression of 10 genes that were up-regulated based on microarray data and one with unaltered expression. *Aspergillus* microarray results were validated by qPCR analysis on 3 differentially expressed genes and 1 unchanged gene. The same RNA used for cDNA synthesis in the microarray experiment was used for reverse transcriptase PCR analysis. Primer pairs (Tables 1 and 2) were designed using the software Primer express 3.0 (Applied Biosystems, Warrington, UK). A total of 250 ng of RNA was treated with DNase using a TURBO-DNA-free kit (Applied Biosystems, The Netherlands) and converted into cDNA using the RevertAid H minus First strand cDNA synthesis kit (Fermentas, Germany). To confirm the absence of genomic DNA contamination, a reaction without the reverse transcriptase was performed

for each sample. Each cDNA sample was diluted 5x in sterile H_2O and 5 µl of the diluted samples were used as template in a 25-µl qPCR reaction containing 100 nM of each primer and 1x ABsolute QPCR SYBR green PCR master mix (ABgene, UK). Reactions were carried out in a Corbett Rotor-Gene 3000 instrument (Westburg, Leusden, The Netherlands), with the following cycling conditions: 95°C for 15 min followed by 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. The relative standard curve method was applied to estimate gene copy numbers (118). Standard curves for each gene were prepared using genomic DNA.

Table 1. List of the primers used in this study and the corresponding gene in the *C. fungivorans* Ter331 genome.

Gene name	primers		primer sequences	
Cf_2790	Cf_2790_f	5'	AGGCTGCGGCTGGAATG	3'
	Cf_2790_r	5'	GCAGCAGGAACGTCAGGATATC	3'
Cf_1140	Cf_1140_f	5'	CCAGGCGCTGGTACGCTAT	3'
	Cf_1140_r	5'	CTGAGCGGGTTGTTGTTGGT	3'
Cf_1141	Cf_1141_f	5'	ATCGAGGCAGCGTTTTCAGTT	3'
	Cf_1141_r	5'	GCCAATCGCGGAATCGT	3'
Cf_223	Cf_223_f	5'	GCGATAGAGGGTGGGAAAAAG	3'
	Cf_223_r	5'	CGAAACAAGCGCCGTAAGTAA	3'
Cf_2072	Cf_2072_f	5'	CCTTGCTGGCATGGTTTGTATT	3'
	Cf_2072_r	5'	GGCCTAGCGCTTTCAGTTCAG	3'
Cf_2080	Cf_2080_f	5'	TCGGCAAGGATGAATGGATAGA	3'
	Cf_2080_r	5'	ACCGCTTCGAACTTGGTCTTG	3'
Cf_1004	Cf_1004_f	5'	AGATGCAAGGACCGGAAGAAGT	3'
	Cf_1004_r	5'	AGGAACGGGATAAAGATGGTGAA	3'
Cf_986	Cf_986_f	5'	TATATGGGGCGCAACCAGAGT	3'
	Cf_986_r	5'	GAAGTTGAAGCGCTCCAGGAA	3'
Cf_1007	Cf_1007_f	5'	GGCCGGAGCTGCCTATTATT	3'
	Cf_1007_r	5'	GGATCGAGGGCGACAAAGAT	3'
pTer_ 17	pTer_17 VirD4_f	5'	GCAAACCGGTCAAGCAACA	3'
	pTer_17 VirD4_r	5'	ATCTTCCCCGACATCGTTCAG	3'
Cf_1225	Cf_1225_f	5'	GGCATCGGTGCTGATTCCTA	3'
	Cf_1225_r	5'	ACTGACTTGTCCGGCATGGT	3'

Table 2. List of the primers used in this study and the corresponding gene in the *A. niger* genome.

Gene name	primers	primer sequences
An01g14550	An01g14550_f	CTTTGGGATACTCTGAGGTGGATTT
	An01g14550_r	TGCGGCGGTGGTTGAG
An01g06750	An01g06750_f	TCTGGGTCCGGTTGATGGT
	An01g06750_r	GAGTACACTTTCCAGCTTCCACAAA
An16g01400	An16g01400_f	GGGAGTCAGCCGAAACAGTCT
	An16g01400_r	GAGTTGCCGTGCTGGTTTGT
An11g11300*	An11g11300_f	TGAACAAGCTCCTGGGTCAT
	An11g11300_r	CTTTCCACTCTTGGGGGTCT

* These two primers were previously published (Douwe van der Veen, 2009. Transcriptional profiling of *Aspergillus niger*, Ph.D. thesis, Chapter 2, Wageningen University).

Results and Discussion

Visible responses of *C. fungivorans* and *A. niger* to each other's presence. The co-inoculation of *C. fungivorans* Ter331 clearly inhibited the mycelial extension of *A. niger* (Fig. 2A and C). At the microscopic level, we observed deformation and increased branching of the fungal hyphae (Fig. 2B). During mycelial development, the agar became acidic, as indicated by the change in color of the pH indicator bromocresol purple (not shown). This acidic halo expanded gradually and ahead of the fungal front. Control plates confirmed that acidification was independent of the presence of bacteria. On the confrontation plates, the arrival of the fungal-induced acidification wave coincided with the accumulation of biomass in the form of slime (Fig. 2A and D). In the later stages of the interaction, fungal mycelium turned a darker color, likely as a result of a change in the process of conidiation (see the discussion below).

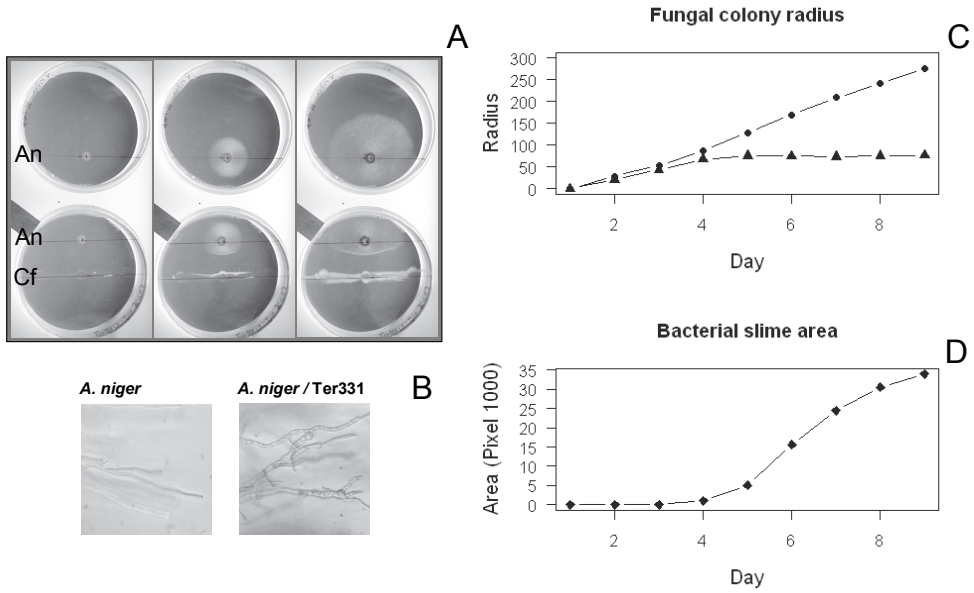

Figure 2. (A) Visualization of the *Collimonas/Aspergillus* confrontation 2, 4, and 7 days after inoculation. The setup shown here is different from the one described in Figure 1: it has only one spot of *A. niger* (An) spores and a single line of *C. fungivorans* inoculum (Cf), and it does not feature the polycarbonate membrane. Inclusion of the latter was necessary for complete recovery of fungal mycelium from the agar surface for RNA extraction, but it did not change the response of either microorganism to the presence of the other. (B) Microscopic view of *A. niger* hyphae on the plate without (left) or with (right) *Collimonas*. (C) Increase in the radius of the fungal colony (measured from the point of inoculation towards the center of the plate) in the absence (circles) or presence (triangles) of *Collimonas*. (D) Production of slime by *Collimonas* in response to the presence of *A. niger*. Slime production was quantified by digital image analysis and expressed as the number of pixels in the area covering the plate in slime.

Transcriptional responses of the organisms to each other's presence. Figure 3 shows the numbers of bacterial and fungal genes that were differentially regulated at two time points (T1 and T2) during the

confrontation. At the transcriptional level, the effect of the fungus on the bacterium appeared much greater than that of the bacterium on the fungus.

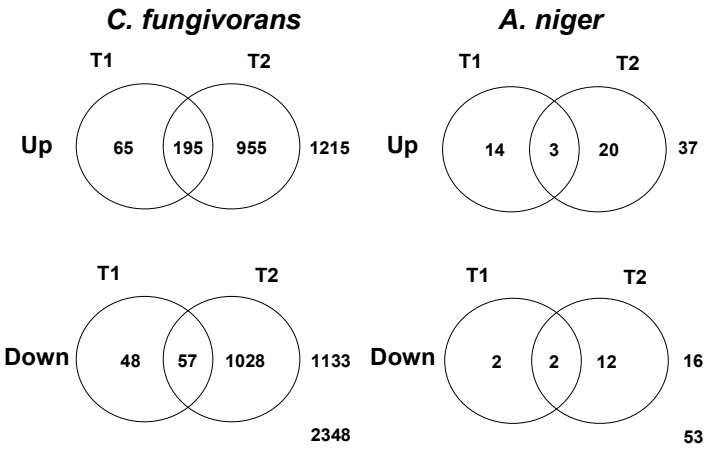

Figure 3. Venn diagrams showing the number of genes differentially expressed in *Collimonas* and *Aspergillus* in the absence or presence of each other at two different time points (T1 and T2). Values represent the number of genes that were ≥2-fold induced or repressed at the 95% confidence level. Intersects show the numbers of genes that were up- or down-regulated at both time points.

Only up to 0.4% of the fungal genome (53 genes) showed altered expression. In contrast, the presence of *Aspergillus* evoked considerable changes in the transcriptome of *Collimonas*. At T1, i.e. at the onset of slime production by the bacteria, 8 % (365) of the analyzed genes were differentially expressed at the 95% confidence level. Most of these (71 %) were up-regulated (Figure 3) and appeared in clusters on the genome (Figure 4). At T2, which was one day later, as many as 49 % of the analysed genes showed altered expression. Microarray data were validated by quantitative PCR measurements on a subset of regulated genes (Figure S1).

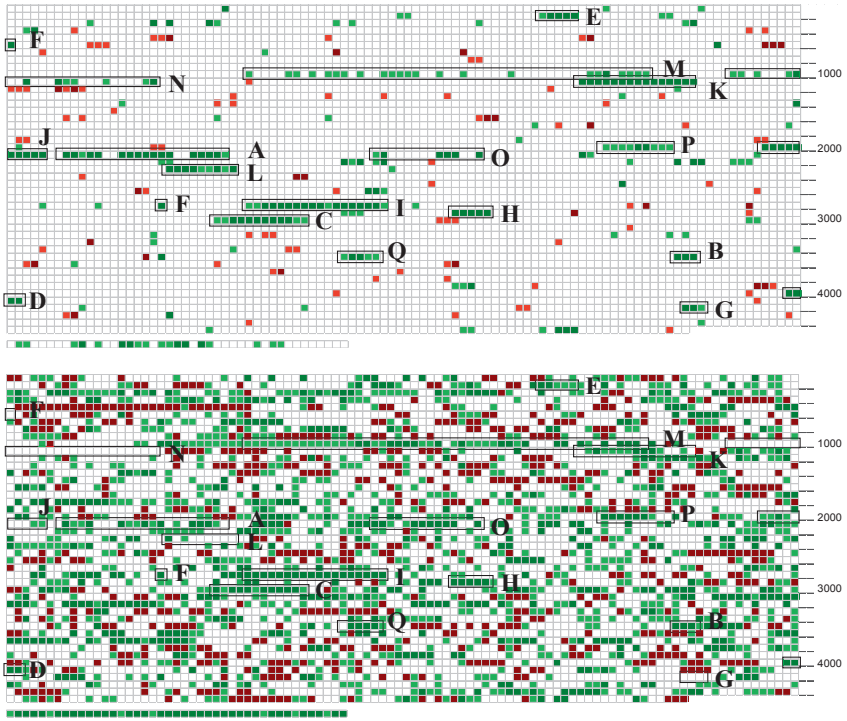

Figure 4. Representation of differentially expressed genes in *C. fungivorans* Ter331 in confrontation with *A. niger* at time points T1 (upper panel) and T2 (lower panel). Each gene is represented by a square in the order as it appears on the genome. The color of a square indicates whether the gene was ≥2-fold induced (green) or repressed (red), at the 99% (dark green or dark red) or 95% (light green or light red) confidence level. Boxed are gene clusters A-Q that are referred to in the text. The bottom row represents genes present on plasmid pTer331.

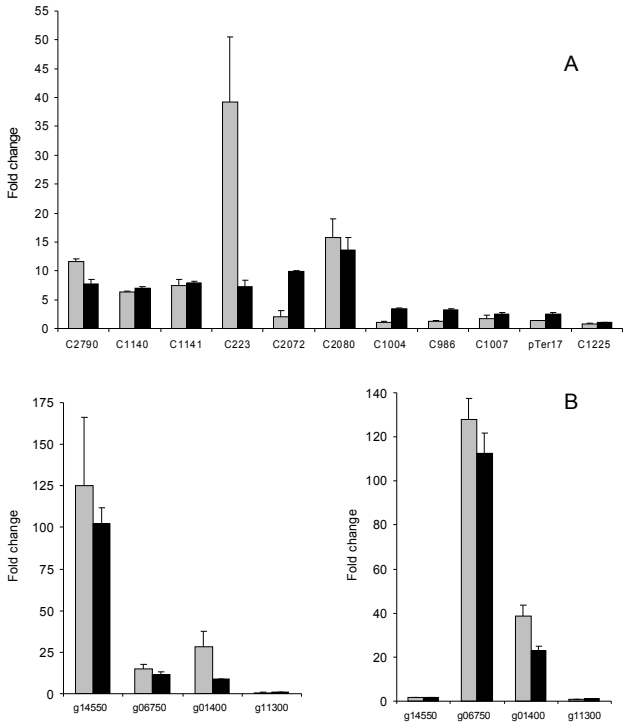

Figure S1. Validation of micro-array expression data by real-time PCR analysis. Shown is the fold-change and standard error in gene expression as determined by PCR (grey) or microarray (black). (A) Comparison of 10 *C. fungivorans* genes at T1. (B) Comparison of 3 *A. niger* genes, from T1 (left) and T2 (right).

Genes differentially expressed in *C. fungivorans*. As mentioned, the majority of differentially expressed *Collimonas* genes at T1 were upregulated (Figure 4, Appendix Table A1). A detailed description of these genes is given below.

Carbon and energy metabolism. Cluster A features two genes that code for an oxalate/formate antiporter (Cf_2072 and Cf_2075) and two for a formyl-CoA transferase (Cf_2080 and Cf_2082). The same cluster harbors two genes (Cf_2063 and Cf_2079) with coding homology to several thiamine pyrophosphate-requiring enzymes, including oxalyl-CoA decarboxylases. In the anaerobic bacterium *Oxalobacter formigenes*, which belongs the the same family as *Collimonas*, the net activity of these activities (i.e. oxalate in, formate out) generates a proton gradient from which the bacterium derives energy (119-120). Downstream of Cf_2063 are four genes (Cf_2064-2067) that code for the α-, β-, γ-, and δ-subunits of an NAD-dependent formate dehydrogenase (121). This enzyme catalyzes the

conversion of formate to CO_2 with concomitant production of $NADH_2$, allowing aerobic bacteria such as *Cupriavidus oxalaticus* (122) to use oxalate as an energy source. Cluster B encodes several enzymes of the glycerate pathway (123). This pathway includes tartronic semialdehyde reductase (Cf_3540) and glyoxylate carboligase (Cf_3542) and is used by bacteria to assimilate C_2 compounds, including oxalate (124). Together, these data suggest that in confrontation with *A. niger*, *C. fungivorans* Ter331 is utilizing oxalate as a source of carbon and/or energy. Indeed, oxalate is known to be one of the main acids produced by *A. niger* (106) the presence of oxalic acid on plate was confirmed experimentally (data not shown). We tested the ability of *Collimonas* to grow on oxalate as sole source of carbon and energy in both semi-solid and liquid media. Surprisingly, oxalate did not sustain bacterial growth under the applied conditions. Yet, we cannot rule out that oxalate adds positively to *Collimonas* energy budget and might be used as an energy source even though cellular growth requires additional C sources (125-126).

Nitrogen metabolism. Several *Collimonas* genes that were upregulated in response to the presence of *A. niger* code for activities that mobilize ammonia. For example, in cluster C, gene Cf_2985 expresses an *fmdA*-like formamidase and Cf_2986 an *amiE*-like acylamide amidohydrolase. FmdA and AmiE release NH_3 from formamide (127) and aliphatic amides (128), respectively. Cluster D contains three genes (Cf_4055-4057) with coding homology to nitrate transporter NasFED (129), while cluster E (Cf_224-227) contains *nasCBA*-like genes (129) that are involved in the reduction of nitrate to nitrite to NH_3. An additional nitrate transporter is encoded by upregulated gene Cf_223 (also in cluster E). Also upregulated was Cf_556 (cluster F), coding the large chain of glutamate synthase. By itself (i.e. in the absence of the small chain, which is encoded by Cf_557 and which is not induced in the presence of the fungus), this enzyme exhibits glutaminase activity (130), converting glutamine to glutamate and NH_3.

We hypothesize that the mobilization of ammonia is a mechanism that protects *Collimonas* against the acidification of the medium by the fungus. Spontaneous protonation of ammonia to ammonium (NH_4^+) leads to

alkalinization of the cytoplasm, which is known to help bacteria to counter proton influx in low pH environments (131). Consistent with this hypothesis is the upregulation of genes Cf_4242-4243 (cluster G), coding for a *glnK-amtB* gene pair (132). The *amtB* gene codes for a NH_3 channel that recruits external NH_4^+, strips it of its proton, and facilitates movement of NH_3 to the cytoplasm. Inside, ammonia is protonated again to NH_4^+, thereby raising the internal pH (133).

The increased expression of NH_3-mobilizing genes could also point at a bacterial reaction to nitrogen deficiency. It is possible that the fungal production of organic acids led to a perceived increase in C/N ratio and to the induction of bacterial pathways for nitrogen scavenging. This would be consistent with the elevated expression of Cf_2775, which codes for a putative permease for the alternative nitrogen source allantoin. Also upregulated were genes Cf_2912-2916 (in cluster H) and Cf_2987-2991 (in cluster C), both of which code for an UrtABCDE-type transporter of urea (134). However, expression of the urease *ureABC* genes (Cf_2918-2920), which would release NH_3 from urea, was not elevated.

Slime production. Clusters I (Cf_2786-2803) and J (Cf_2051-2060) both contain genes involved in exopolysaccharide production. Cluster J comprises 10 upregulated genes, several of which resemble genes coding for the synthesis of colanic acid. In *E. coli*, this polyanionic heteropolysaccharide confers a strong negative charge to the cell surface and has been shown to offer protection against acidic conditions (135). Several genes in cluster I resemble *gum* homologs for xanthan production in *Xanthomonas campestris* (136). Exposure of *X. campestris* to acid stress has previously been shown to increase xanthan synthesis (137). Similarly, the production of slime by *Collimonas* might constitute a protective mechanism against low environmental pH. We tested experimentally whether exposure of *Collimonas* to an acidic environment triggers the production of slime and found that the slime is not produced unless a suitable C source is available. Citric acid, which is a suitable substrate for *Collimonas* growth, induced slime formation, but oxalic acid and HCl did not induce slime production (results not shown). A possible motivation for slime production is the

dissipation of excess carbon available inside the cell. This has been observed for *Xanthomonas* in the presence of organic acids and absence of growth (138). *Collimonas* might use a similar strategy to dispose of excess C derived from the oxidation of fungal organic acids when the growth rate is limited by the diversion of ATP from anabolic processes to acid stress responses. Producing exopolysaccharides might prove beneficial to the cell for an additional reason. Exopolysaccharides generate microscale gradients around the cell influencing nutrients concentration, pH and resistance to antimicrobials (139-141) and protect the cell against several kinds of stress enhancing bacterial survival in unfavourable environments.

Secondary metabolites and bacterial secretion. Cluster K (Cf_1128-1142) encompasses 15 upregulated genes with unclear function. Most likely, they code for the synthesis of a secondary metabolite, given the resemblance of some of these genes to polyketide synthases and nonribosomal peptide synthetases. It is tempting to speculate (also see the discussion below) that these genes code for the synthesis of a compound with antifungal activity and that this compound is responsible for the inhibition of fungal growth and deformation of fungal hyphae (Fig. 2). Cluster L (Cf_2276-2284) codes genes belonging to a type II secretion system, a pilus-like structure specialized in transporting toxins and hydrolytic enzymes in the extracellular space (142-143). The upregulation of this gene cluster offers further support to the idea that the presence of the fungus elicits the production of antimicrobial compounds in *Collimonas.*

Motility. Cluster M (Cf_986-1036) contains 23 upregulated genes related to flagellum synthesis and chemotaxis. We don't know whether this means that *Collimonas* is trying to get away from the fungus or move towards it. Our confrontation plates contained 2% agar, which typically does not allow flagella-driven motility.

Horizontal gene pool. Many of the genes located on plasmid pTer331 (Chapter 3) showed increased expression in response to the fungus. The same was true for genes in clusters N (Cf_1047-1074) and O (Cf_2102-2115), both of which carry genes belonging to putative prophages. The

activation of mobile genetic elements is a common feature of bacterial response to stress (144).

General stress response and ribosomal proteins. In total, only 57 genes were significantly downregulated at both T1 and T2 (Fig. 3). Closer examination of these revealed a disproportionate number of genes coding for ribosomal proteins. Ribosomal proteins are essential for *de novo* synthesis of proteins (145-146). Their down-regulation has been linked to various stress experiences (147-150) and a decrease of cellular growth (151). Genes C_3502 and C_2039, in cluster P and Q respectively, encode two Csb proteins (152-153), which are activated during the general stress response. The general stress response is triggered by energy or environmental stress and aims at protecting DNA, proteins and membranes from damage (154). Consistent with this, genes C_2035 and C_2036, in cluster P, encode two ATP-dependent DNA ligase, involved in repairing DNA double-strand breaks (155).

At T2, almost 50% of the *Collimonas* analysed genes showed an altered transcription level. In contrast with what we observed at T1, the differentially expressed genes were distributed all over the genome.

Genes differentially expressed in *A. niger*. As mentioned, very few fungal genes were differentially expressed in response to the presence of *Collimonas* (Fig. 3, Appendix Table A2 and A3). However, their putative functions collectively suggest the fungal experience of (nutrient) stress and the need for rearrangement of cell wall and cell membrane.

Nitrogen metabolism. The differential expression of several *Aspergillus* genes suggests that in confrontation with *Collimonas*, the fungus experienced a shortage of nitrogen. One of the downregulated genes, An01g11380, codes for a homolog of NmrA, a protein that is involved in controlling nitrogen metabolite repression in various fungi (156). In *Aspergillus nidulans*, a *nmrA* deletion partially de-repressed the utilization of non-preferred nitrogen sources (157). Consistent with this, gene An02g00560, which codes for a uric acid-xanthine transporter and is normally repressed by NmrA (156, 158) was upregulated at T2. Three other genes that were induced at T1, i.e. An07g00370, An07g08770 and

An13g03910 (also induced at T2), show coding homology to proteins involved in the transport and metabolism of the alternative nitrogen source allantoin/allantoate. Three of the ten most highly upregulated genes in the fungus at T1 (i.e. An01g14550, An18g01740 and An10g00730) are coding for nitrilases, suggesting that nitriles may also serve as sources of nitrogen for the fungus.

Stress response. Three genes that were down-regulated at T2, i.e. An06g00900, An06g00930 and An06g00940, are located near each other on the genome. Of these, An06g00940 was annotated as NUCB1, a conserved regulatory protein (159). One of its proposed functions is to suppress the Endoplasmic Reticulum (ER) stress response, which is activated under conditions that lead to malfunctioning of the ER and unfolding/misfolding of proteins (160). Down-regulation of this repressor suggests that such stress conditions are met in confrontation with *Collimonas*. Up-regulated at T2, gene An01g10790 is a *con-10* homolog, typically expressed during conidial differentiation (161-162). It might be another indication that exposure to *Collimonas* induces stress in the fungus.

Cell membrane. Several of the differentially expressed genes in *A. niger* are linked to the metabolism and integrity of the fungal cell membrane. Two genes, both upregulated at T2, are involved in regulating cell membrane fluidity. The first, An12g09940, resembles the *sdeA* gene of *A. nidulans* (163), which encodes a Δ9-stearic acid desaturase for the production of unsaturated fatty acids. The second, An03g00580, codes for a putative CypX-like cytochrome P-450 (164). The latter is a key enzyme in the biosynthesis of ergosterol, which is required for membrane permeability and fluidity. Upregulation of An12g09940 and An03g00580 suggests an effort by the fungus to regulate membrane fluidity, which in *Candida albicans* and *Aspergillus fumigatus* was suggested to confer resistance to amphotericin B (165-166). This polyene antifungal agent binds to ergosterol to form pores that cause leakage of hyphal content and eventually death of the fungus (167). Based on this observation, it seems worth considering the possibility that the product of *Collimonas* gene cluster K is an antifungal molecule able to induce hyphal leakage with a mode of action similar to polyene

antibiotics. An16g05910 and An16g05920 are adjacent genes, both down-regulated in our experiment. While An16g05920 codes for a putative membrane receptor of a yet unidentified signal (168), An16g05910 is homologous to gene *ahbB1* of *A. nidulans*, which is believed to be involved in cell membrane synthesis (164). Its deletion in *A. nidulans* led to an increased branched phenotype (169) which closely resembled the hyphal deformations that we observed for *A. niger* in confrontation with *Collimonas* (Fig. 2B).

Cell wall. The function of An12g10200, up-regulated at T1, is unknown, but it was shown to be induced upon exposure of *A. niger* to caspofungin (170), an antibiotic that inhibits the synthesis of the fungal cell wall component β-1,3-glucan. Another cell-wall related gene was down-regulated at T2, i.e. An08g09420, coding for galactomannoprotein (106), a structural component of the *Aspergillus* cell wall (171). Also downregulated was An09g06400, a homolog of the *A. nidulans chiA* gene. Its product functions as a chitinase involved in cell wall remodelling and/or maturation (172). During branching of *A. niger* hyphae, gene An09g06400 was downregulated (173). Differential regulation of these genes suggests that the presence of *Collimonas* promotes changes in fungal cell wall dynamics, some of which are possibly linked to the changes in hyphal morphology (Fig. 2B).

Secondary metabolites. An08g08000 and An08g08010 are two adjacent genes, up-regulated at T2. The first is a membrane transporter, while the second is homologous to a gene involved in the synthesis of mitomycin in *Streptomyces lavendulae* (174) Two other genes presumably involved in fungal secondary metabolism, An12g02660 and An08g03810, were up-regulated at T2. The interaction with bacteria can trigger the biosynthesis of fungal metabolites which would not otherwise be produced (75); it remains to be investigated whether expression of these genes constitutes a counter offense against *Collimonas*.

A working model for the *Collimonas*/*Aspergillus* interaction. Figure 5 presents a schematic summary of the confrontation between *C. fungivorans* and *A. niger*, as deduced from the transcriptional profiles of both organisms. It is clear that the interaction is quite complex, and resembles a back-and-forth dialog between the two partners.

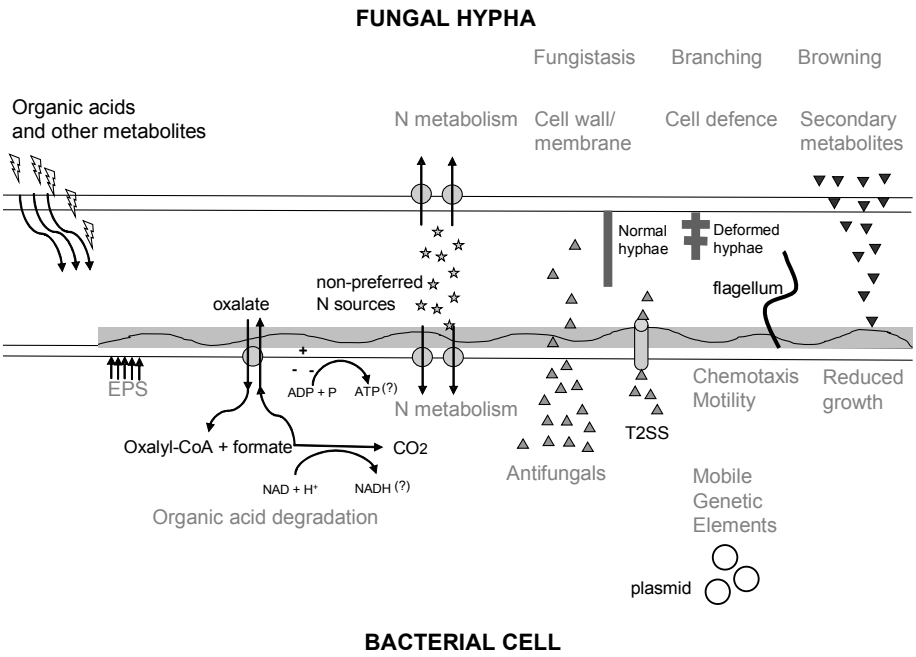

Figure 5. Schematic overview of the *Collimonas*/*Aspergillus* dialog, as interpreted from transcriptional profiling of both partners. Details are discussed in the text.

Central to the model is the production of organic acids by the fungus, including oxalate and citrate. The upregulation of *Collimonas* genes involved in the uptake and metabolism of oxalate, together with the production of slime, can be taken as indirect evidence for the conversion of fungal biomass into bacterial biomass. One might argue that *Collimonas* did not play an active role in the release of organic acids, since *A. niger* caused acidification of the agar also in the absence of bacteria. Under this assumption -i.e. bacteria as opportunistic consumers of fungal exudates-, the strict definition of mycophagy was not fully met, i.e. it did not involve an

active bacterial investment in causing the release of hyphal content (29). However, we cannot exclude that *Collimonas* was in fact capable of changing the quantity and/or quality of organic acids that were secreted by *A. niger*. This possibility will be tested in future studies, which will also take into consideration our finding that *Collimonas* induced severe deformation of *A. niger* hyphae (Fig. 2B). Are these hyphae leakier than normal mycelium? Hyphal leakage is often associated with membrane damage (175), and the changes we observed in the expression of several *A. niger* genes indeed suggest that membrane integrity was compromised in this fungus during its confrontation with *Collimonas*.

Our discovery of *Collimonas* gene cluster K, coding for a putative metabolite with antifungal activity, aligns well with the leakage hypothesis. Mycoparasitic fungi such as *Gliocladium virens* (176) induce cytoplasmic leakage of proteins, amino acids, and carbohydrates from their host through the production of low-molecular-weight metabolites. The fact that the genes in cluster K were upregulated in the presence of the fungus under noncontact conditions suggests that the fungal compound(s) that activate these genes are reaching *Collimonas* by diffusion. In this context, it is interesting to note that in a confrontation between *Amanita muscaria* and *Streptomyces* strain AcH 505, oxalate-induced acidification of the medium by the fungal partner *A. muscaria* stimulated production of a secondary metabolite by the bacterium (177). In this study, it was suggested that fungal organic acids serve as information signals for other microorganisms. Along similar lines, several of the genes that were found to be upregulated in *Pseudomonas putida* 06909 during colonization of the fungus-like *Phytophthora parasitica* were also found to be induced *in vitro* by organic acids (178). Some of these served as growth substrates for strain 06909. In our *Collimonas*/*Aspergillus* model, compounds such as citric and oxalic acid may thus represent a signal indicating the presence of (more) fungal food and stimulating the expression of genes that take advantage of this presence.

It is interesting that *Collimonas* genes for chemotactic motility (cluster M) were upregulated. This suggests an expectation on the part of the bacteria to find a substrate to adhere to somewhere along a gradient. Based on the

available data, it is tempting to speculate that that substrate is the fungal cell wall and that the gradient is provided by the fungal acids. Bacterial attraction to fungally produced organic acids has been reported previously. For example, *Pseudomonas fluorescens* is chemoattracted to fusaric acid produced by *Fusarium oxysporum* f. sp. *radicis lycopersici* (*Forl*) (64). Of particular interest is oxalate, given the upregulation of *Collimonas* genes involved in the transport and metabolism of this compound. Secretion of oxalate is a common trait among fungi (179), but also plants (180). It is worth noting that several collimonads known to date were isolated from the ectomycorrhizae of pine trees (32), which produce oxalate as a weathering agent to release phosphate and other micronutrients from the soil environment (181). *Collimonas* was also detected on the hyphae of *Resinicium bicolour* (182), a fungus that is known to accumulate oxalate crystals on its surface. These observations make it likely that oxalic acid plays a major role in the attraction of *Collimonas* by fungi.

Both partners in the *Collimonas*/*Aspergillus* confrontation experienced nitrogen deficiency in each other's presence. This indicates competition for a limited resource, which adds an additional level of complexity to the interaction. It remains to be determined how this competition contributed to the response of both organisms to each other's presence. It is possible that the aggressive sequestration of NH_3 by *Collimonas*, as suggested by its transcriptional profile, exacerbated the nitrogen shortage experienced by *A. niger*. Other types of stresses were noted as well, e.g. acid stress in the bacteria and ER stress in *A. niger*. How 'natural' these responses are is uncertain. Obviously, the arena in which we confronted the bacterium and fungus lacked the complexities and dynamics of real-life environments. However, the transcriptional profiles clearly offered insight into the functional hardwiring of both microorganisms that allows them to survive in their natural habitats. The *Collimonas* data showed concurrent expression of genes involved in mycophagy, nutrient deficiency, low pH stress and antibiosis, which fits well with its description in the literature as a soil bacterium with antifungal activity and that prefers acidic, nutrient-poor environments (32).

Chapter 3

Comparative genomics of the pIPO2/pSB102 family of environmental plasmids: sequence, evolution, and ecology of pTer331 isolated from *Collimonas fungivorans* Ter331

Francesca Mela, Kathrin Fritsche, Hidde Boersma, Jan D. van Elsas, Daniela Bartels, Folker Meyer, Wietse de Boer, Johannes A. van Veen, and Johan H. J. Leveau

Published in FEMS Microbiology Ecology (2008) 66: 45–62

Abstract

Here, we report on the isolation and characterization of plasmid pTer331 from the bacterium *Collimonas fungivorans* Ter331. It represents a new member of the pIPO2/pSB102 family of environmental plasmids. The 40,457-bp sequence of pTer331 codes for 44 putative ORFs. Based on sequence similarity, most of these represent genes involved in replication, partitioning and transfer of the plasmid. We confirmed that pTer331 is stably maintained in its native host. By deletion analysis, we identified a mini-replicon capable of replicating autonomously in *Escherichia coli* and *Pseudomonas putida*. Furthermore, plasmid pTer331 was shown to be able to mobilize and retromobilize IncQ plasmid pSM1890 at typical rates of 10^{-4} and 10^{-8}, respectively. The high degree of DNA sequence identity (91%) between pTer331 and pIPO2 was exploited to hypothesize on the forces that underlie the divergent evolution of these two plasmids. Such forces likely include the functional conservation of coding sequences, the deletion of DNA fragments flanked by short direct repeats, and sequence preservation of long direct repeats. In addition, we experimentally established that pTer331 has no obvious contribution in several of the phenotypes that are characteristic of its host *C. fungivorans* Ter331, including the ability to efficiently colonize plant roots. Based on our findings, we hypothesize that cryptic plasmids such as pTer331 and pIPO2 might not confer an individual advantage to bacteria, but, due to their broad-host-range and ability to retromobilize, benefit bacterial populations by accelerating the intracommunal dissemination of the mobile gene pool.

Introduction

Plasmids are extrachromosomal self-replicating DNA elements. Two rather extreme but not mutually exclusive views on plasmids exist regarding their relation to bacterial hosts. One stresses the benefits of plasmids to bacteria. Heavy metal resistance genes, antibiotic resistance genes, or genes coding for degradative pathways are typically located on and mobilized by plasmids (89, 183). By contributing to the genetic plasticity of bacteria, plasmids assume the status of symbionts which enhance the ability of bacteria to adapt to a changing environment. Another type of advantage that some plasmids confer involves a process known as retrotransfer or retromobilization (91-92). This involves the acquisition of plasmid-encoded genes from other bacteria. Retromobilization-active plasmids can be thought of as a kind of "gene-fishing devices" for their host, as they effectively increase the accessibility of the host to the mobile gene pool available in a given environment.

The other view on plasmids is one that represents plasmids as selfish DNA (184-186), which essentially groups them together with bacteriophages and transposons in the same superfamily of parasitic sequences. This classification is based on the understanding that the presence of plasmids in a bacterial population is mainly due to their efficiency in spread and not to the reproductive success of the individuals carrying the plasmid (187-188). Examples of selfish DNA are cryptic plasmids: they are stably maintained in the host population but do not confer any evident or demonstrable advantage to their hosts.

The increasing availability of completely sequenced plasmids (http://www.ncbi.nlm.nih.gov/genomes/static/o.html, http://www.ebi.ac.uk/ genomes/ plasmid.html) offers new excitement to the study of plasmids by allowing novel answers to questions regarding their biological role, coding potential, and contribution to host fitness. Furthermore, it opens the way for comparative genomics approaches to elucidate the mechanisms of plasmid evolution, i.e. the forces that drive plasmid divergence and diversity. The general consensus is that plasmids are subject to two parallel evolutionary processes: micro- (189-190) and macro-evolution (191-192). The former

includes the accumulation of nucleotide substitutions and insertion/deletions (indels), which may be neutral or selected for or against, depending on their effect on plasmid functioning and/or on their compatibility with host biology (e.g. codon usage). Macroevolution describes the acquisition of whole operons and the creation of "mosaic" plasmids, which typically involves the activity of other mobile elements such as transposons and insertion elements.

All sequenced plasmids fall into one of two groups: one for which the native host is known because that is where the plasmid was originally isolated from or identified in, and one for which no native host is (yet) known. Typically, this latter group of 'orphan plasmids' features elements that have been captured by and maintained in a surrogate bacterial host through one of several available methods, including bi-and tri-parental mating and transposon-aided capture of plasmids (for an overview see (193-195)). These methods have played an important role in broadening our knowledge on plasmid diversity as they opened the way for the exogenous isolation of plasmids from bacterial hosts that typically resist cultivation in the laboratory. However, it should be recognized that knowledge and availability of a plasmid's natural host(s) is always desirable, as it allows for experimental testing of hypotheses on the plasmid and associated functions in its natural background.

The recently recognized pIPO2/pSB102 family of environmental broad-host-range plasmids consists both of plasmids with known hosts and orphan plasmids. Proposed members of this family include pIPO2 (196), pSB102 (197), and pXF51 (198). The former two (39,815 and 55,578 bp, respectively) were isolated exogenously, while pXF51 (51,158 bp) was identified as an extrachromosomal element in the genome of *Xylella fastidiosa* strain 9a5c (198). All three plasmids originated from bacterial communities associated with the plant environment (phytosphere): pIPO2 and pSB102 were isolated from the rhizosphere of wheat and alfalfa, respectively, while *X. fastidiosa* is a plant pathogen colonizing the xylem of citrus plants (198). Their complete nucleotide sequences are similar in gene content and synteny, and in all three cases the majority of the coding

potential seems dedicated to 'selfish' traits including plasmid replication, maintenance and transfer. Only pSB102 harbors a set of genes with a demonstrable advantage to its host by conferring resistance to mercury. However, like pIPO2 and pXF51, it remains cryptic as to if and how it contributes to its host's fitness in the phytosphere. A likely fourth member of the pIPO2/pSB102 family is pES1 (199). It was isolated exogenously from a hydrocarbon-polluted soil and partially (10.2 kb) sequenced as a mini-Tn*5*-Km1 derivative named pMOL98 (199) to reveal high similarity to the predicted replication regions of pIPO2 and pSB102. Lastly, it has been suggested that pFBAOT6 (200) also represents a member of the pIPO2/pSB102 family. With a size of 84,748 bp, this IncU plasmid resembles pIPO2 and pSB102 in replication, maintenance and transfer functions, but carries an additional genetic load consisting of various transposable elements, including a class I integron and a composite transposon coding for tetracycline resistance. Plasmid pFBAOT6 was isolated from the bacterium *Aeromonas caviae* in a clinical setting, which challenges the notion that the pIPO2/pSB102 family of environmental plasmids is exclusive to plant-associated bacteria (200).

Here, we introduce a new member of the pIPO2/pSB102 family: plasmid pTer331. We report on its isolation from the natural host *Collimonas fungivorans* strain Ter331 (201). This bacterium is one of 22 *Collimonas* strains originally isolated from the rhizosphere of Marram grass (*Ammophila arenaria*) as dominant microorganisms among the cultivable, chitinolytic bacterial population (34). Strains of *C. fungivorans* exhibit antifungal activity (30) and were shown to be mycophagous, i.e. able to use living fungi as a growth substrate (30, 34, 201). In addition, *C. fungivorans* Ter331 showed *in vivo* biocontrol activity towards the plant-pathogenic fungus *Fusarium oxysporum* f. sp. *radicis lycopersici*, which causes tomato foot and root rot (42). It has been suggested that this activity is linked to its efficient ability to colonize the tomato rhizosphere (42).

We present here the complete nucleotide sequence of pTer331, and provide an analysis of its coding capacity in the context of its demonstrable ability to replicate, (retro)mobilize, and stably maintain itself in host *C. fungivorans*

Ter331. In addition, we describe an experimental assessment of the contribution of pTer331 to the rhizosphere competency of its host. Furthermore, we exploit the high degree of identity between pTer331 and pIPO2 to reveal possible mechanisms of divergence since these plasmids split from their common ancestor and to hypothesize on the evolutionary events that shaped the diversity of known members of the pIPO2/pSB102 family of environmental plasmids.

Materials and methods

Strains, plasmids, and culture conditions

Strains and plasmids used in this study are listed in Table 1. Collimonads were grown at 25 °C in liquid or on solid 0.1x TSB medium, pH 6.5 (201) or on KB medium (202). For solid TSB or KB medium, 15 g agar was added per liter. In mating experiments, LB medium (203) was used to grow collimonads and pseudomonads at 28 °C and *E. coli* at 37 °C.

Table 1: Strains and plasmids used in this study.

Strain	Relevant characteristics	Reference
Collimonas fungivorans Ter331	β-Proteobacterium isolated from the rhizosphere of marram grass, with demonstrated antifungal activity and rhizosphere competency; harbors plasmid pTer331	(42, 201)
C. fungivorans Ter331R	spontaneous rifampicin-resistant derivative of *C. fungivorans* Ter331	(55)
C. fungivorans Ter331PC	plasmid-cured derivative of *C. fungivorans* Ter331	this study
Escherichia coli CV601	strain used as donor in bi- and tri-parental matings	(195)
Pseudomonas fluorescens R2f	strain used as recipient in tri-parental matings; rifampicin-resistant	(258)
P. fluorescens (pIPO2T)	strain used as positive control instead of *C. fungivorans* Ter331 in bi- and tri-parental matings	(206)
P. fluorescens PCL1285	rhizosphere-competent, kanamycin-resistant derivative of *Pseudomonas fluorescens* WCS365	(250)

Plasmid	Relevant characteristics	Reference
pTer331	plasmid native to *C. fungivorans* Ter331	this study
pTer331Δ	deletion derivative of pTer331, constructed by replacement of the 27.6-kb *BsaI*/*SacI* fragment with a kanamycin resistance marker	this study
pSM1890	mobilisable but not self-transmissable plasmid, confers resistance to gentamycin and streptomycin	(193)
pIPO2T	mini-Tn*5*-*tet* derivative of pIPO2, a self-transferable plasmid isolated exogenously from the wheat rhizosphere; confers resistance to tetracyclin	(196, 206)

Isolation of genomic and plasmid DNA from C. fungivorans Ter331

Genomic DNA was isolated from TSB-grown *C. fungivorans* Ter331 according to a protocol described elsewhere (204) with minor modifications. In short, cells were centrifuged and washed in 1 volume of buffer A, concentrated in the same solution to an optical density at 600 nm (OD_{600}) of 10-20, mixed with one volume of 2% low melting point agarose (Bio-Rad, Veenendaal, The Netherlands), and poured into plug molds (Bio-Rad). Solidified agarose plugs were gently shaken at room temperature in buffer B^* (buffer B lacking sodium deoxycholate and Brij-58). After 30 min, lysozyme was added to a final concentration of 2 mg per ml, and incubation was continued at 37 °C for 20 h. After two washes of 30 minutes each in solution B^* at room temperature, the plugs were transferred to solution C (solution B^* containing 0.2 mg proteinase K per ml) and incubated at 50 °C overnight. This step was repeated for an additional 5 h, after which the plugs were washed extensively in TE buffer.

Plasmid pTer331 was isolated from *C. fungivorans* Ter331 using a QIAprep Spin Miniprep Kit (Qiagen, Venlo, The Netherlands). For this, cells were grown in a 20-ml TSB liquid culture to an OD_{600} of 1.1, harvested and resuspended in 750 µl buffer P1 provided with the kit. Three aliquots of 250 µl were lysed and neutralized according to the manufacturer's instructions. After centrifugation, supernatants of the three aliquots were combined and

loaded on a single spin column, followed by washing and elution of the plasmid DNA with 50 µl preheated (70 °C) elution buffer.

Analysis of genomic and plasmid DNA by gel electrophoresis

Genomic DNA in agarose plugs was loaded on a 1% PFC agarose gel (Bio-Rad) in 0.5x TBE buffer, and separated on a CHEF-Mapper III system (Bio-Rad) at 12 °C, with the following settings: 6V/cm, 120° angle, pulse intervals of 6-60 seconds or 0.98-12.91 seconds, with a linear ramping factor. For restriction analysis of the plasmid, 0.2 µg of purified DNA was digested with 10U *EcoRI*, *HindIII* or *PstI* and analyzed on a regular 1% MP agarose gel (Roche, Almere, The Netherlands) in 0.5x TBE.

Sequencing of plasmid pTer331 and DNA sequence analysis

A shotgun approach was taken to determine the complete nucleotide sequence of plasmid pTer331 (Macrogen, Seoul, Korea). In short, random DNA fragments of 1.5-3 kb were cloned into pCR4Blunt-TOPO (Invitrogen, Carlsbad, CA) and sequenced from both ends. In total, 344 shotgun sequences were assembled using Lasergene's Seqman (DNAstar, Madison, WI). Remaining gaps were filled in by primer walking, representing an additional 18 sequence reads. The complete nucleotide sequence of pTer331 (40,457 bp) was searched for open reading frames using FGENESB (www.softberry.com) and by the automated genome interpretation system GenDB (205). Sequence similarity searches were performed using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information. BPROM (www.softberry.com) was used for prediction of σ^{70}-dependent promoters, and FindTerm (www.softberry.com) for finding rho-independent terminator sequences. Repeat regions within the pTer331 sequence were identified with Lasergene's Megalign (DNAstar). The annotated nucleotide sequence of pTer331 has been submitted to the DDBJ/EMBL/Genbank database under accession number EU315244. To allow comparison to pIPO2 at the DNA level, we reconstructed the original pIPO2 sequence (39,815 bp) *in silico* from that of pIPO2T (45,319 bp; accession number AJ297913) by removal of nucleotides 38238-43741.

Detection of pTer331 in other Collimonas isolates

To test the presence of plasmid pTer331 in other collimonads, we used a pTer331-specific PCR assay on 44 strains in our *Collimonas* collection. Twenty-one of these strains (Ter6, Ter10, Ter14, Ter72, Ter90, Ter91, Ter94, Ter113, Ter118, Ter146, Ter165, Ter166, Ter227, Ter228, Ter252, Ter266, Ter282, Ter291, Ter299, Ter300, and Ter330) have been described before by de Boer *et al.* (201) and 23 strains (R35505, R35506, R35507, R35508, R35509, R35510, R35511, R35512, R35513, R35515, R35516, R35517, R35518, R35520, R35521, R35522, R35523, R35524, R35525, R35526, R35529, LMG23976 and LMG23968) by Höppener-Ogawa *et al.* (39). From each strain, genomic DNA was isolated with a MO BIO Soil DNA Extraction Kit (MO BIO laboratories ; Carlsbad, CA) and used as template in a PCR using primers pIPO2 forward and pIPO2 reverse (206). This set was originally designed to be specific for pIPO2 but based on sequence similarity also detects pTer331, producing a 307-bp PCR product (see Fig. 2 for location on pTer331). PCR amplification was performed on a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) in a total volume of 15 µl containing 50 ng template DNA, 1.5 µl primer mix (final concentration 12.5 µM), 7.5 µl 2x ABsolute qPCR mix (ABgene, Epsom, UK), and using the following temperature profile: 15 min at 95 °C, 40 cycles of 45 sec at 95 °C, 45 sec at 55 °C, and 90 sec at 72 °C. End-point fluorescence measurements were used to establish the presence or absence of a pTer331-derived PCR amplicon, using genomic DNA from *C. fungivorans* Ter331 as a positive control.

Quantifying plasmid stability.

To determine the stability of pTer331 in *C. fungivorans* Ter331, the latter was cultivated for 35 generations in liquid KB by daily dilutions into fresh medium. Samples from the last generation were diluted and plated on KB agar to obtain individual colonies which were tested for possession of pTer331 by PCR as described above. Plasmid stability was estimated from the fraction of colony forming units that had retained the plasmid and expressed as M, i.e. the frequency of plasmid loss per cell per generation, calculated as $M=(\pi-\pi_0)/\ln(p/p_0)$, where π_0 and p_0 are, respectively, the

proportion of plasmid-free cells and the total number of cells at the start of the experiment, and π the proportion of plasmid-free cells after the total number of cells has risen to p (207). It should be noted that estimating plasmid stability by mean of this formula does not represent an accurate measurement of plasmid loss per generation as this formula does not take into account phenomena such as for example plasmid conjugation transfer rate. Since we tested 93 colony forming units (cells) from generation 35 for possession of plasmid pTer331, our detection limit for M was $(1/93\text{-}0)/\ln(2^{35}\cdot p_0/p_0)=0.00044$.

Mobilizing and retromobilizing activity of pTer331.

To assess the mobilizing and retromobilizing capacities of plasmid pTer331, we performed tri- and biparental mating experiments with *C. fungivorans* Ter331. The triparental mating mixture consisted of *C. fungivorans* Ter331 as helper, *P. fluorescens* R2f as recipient and *E. coli* CV601 (pSM1890) as donor. In the biparental mating, the mixture consisted of *C. fungivorans* Ter331R as recipient and *E. coli* CV601 (pSM1890) as donor. In control experiments, *P. fluorescens* (pIPO2T) was used instead of *C. fungivorans* Ter331. Overnight cultures of donor, recipient and/or helper strains were washed twice in 0.85% NaCl, mixed in equal amounts, pipetted as a 100-µl drop on LB agar, and incubated overnight at 28 ºC. Following incubation, 1-by-1 cm agar plugs containing the mating mixtures were cut out and vortexed for 5 min in 9 ml 0.85% NaCl. A ten-fold dilution series was plated on LB agar containing gentamicin (25 µg/ml) and rifampicin (15 µg/ml) to enumerate pSM1890-containing *P. fluorescens* R2f or *C. fungivorans* Ter331 transconjugants from the tri- and biparental matings, respectively. Transconjugants were verified by testing for growth on LB agar containing streptomycin (20 µg/ml) and rifampicin (15µg/ml) and by PCR amplification targeting *oriV* of pSM1890 as described elsewhere (208). Transfer frequencies were calculated as the ratio of transconjugants to recipients. We also tested for the presence of pTer331 in triparental transconjugants by PCR amplification using primers VirB10f (5'-CGSATCTTYGTGCTSTGG-3') and VirB10r (5'-AGKGTTGGCGGAATRTTGA-3') (see Fig. 2 for location on pTer331).

Construction of a pTer331 deletion derivative

For the construction of deletion derivative pTer331Δ, the kanamycin resistance gene from pCR-TOPO (Invitrogen, Breda, The Netherlands) was amplified with primers
Km_UP (5'- TTTTCGAGACCGGAAAACGCAAGCGCAAAGAGAAA-3'; the recognition site for enzyme *BsaI* is underlined) and
Km_LP (5'-GAGCTCGGGAATAAGGGCGACACGGAAATG-3'; *SacI* recognition site underlined), and ligated as a 1,085-bp *BsaI-SacI* fragment to *BsaI*/*SacI* double-digested plasmid pTer331. The architecture of pTer331Δ was confirmed by restriction enzyme digestion.

Plasmid curing of C. fungivorans Ter331

We cured *C. fungivorans* Ter331 from plasmid pTer331 exploiting the principle of plasmid incompatibility (209). For this, we introduced pTer331Δ as curative plasmid into *C. fungivorans* Ter331 by electroporation (210). Electrotransformants were selected for growth on KB agar supplemented with kanamycin at a concentration of 600 µg/ml. Plasmid DNA isolated from kanamycin-resistant transformants was identified as pTer331Δ by restriction analysis. Furthermore, the absence of pTer331 in these transformants was confirmed by PCR using primers 222f (5'-ACAAGGGCAAGCCAGTCAAG-3')
and 842r (5'-TCTGCCGACGAACGCTGTGT-3'), which amplify a 1.1-kb DNA fragment that is present on pTer331 but missing from pTer331Δ (Fig. 2). One *C. fungivorans* Ter331 (pTer331Δ) transformant was grown for several generations on KB in the absence of kanamycin to allow spontaneous curing of plasmid pTer331Δ. Plasmid-free derivatives were detected by their inability to grow on KB agar supplemented with kanamycin. The absence of plasmid pTer331Δ in these colonies was confirmed by our inability to 1) isolate plasmid DNA and 2) obtain a PCR product using primers Kan_UP and Kan_LP, which are specific for the kanamycin resistance locus on pTer331Δ. This plasmid-cured (PC) derivative of *C. fungivorans* Ter331 is referred to in the text as *C. fungivorans* Ter331PC.

Competitive root tip colonization assay

The ability of wild-type *C. fungivorans* Ter331 and plasmid-cured *C. fungivorans* Ter331PC to colonize tomato root tips was compared through competition experiments of each strain with rhizosphere-competent *P. fluorescens* PCL1285 using a previously described protocol (42, 211). In short, 1:1 mixes of overnight KB cultures of *Pseudomonas fluorescens* PCL1285 with either Ter331 or Ter331PC were used to inoculate sterilized and germinated tomato seeds cultivar Caramello (Syngenta, Enkhuizen, The Netherlands). Seedlings were transferred to sterile quartz sand and allowed to grow for one week at 24 °C and 16 hours light per day, at which point tomato plantlets were harvested. One-cm segments of the root tips of 10 plants were recovered and placed into 1 ml of phosphate-buffered saline. After shaking for 20 minutes, root washings were diluted and plated on KB agar and on KB agar supplemented with rifampicin. Colony-forming units were counted to calculate the ratios of PCL1285 (rifampicin-resistant) to either Ter331 or Ter331PC (both rifampicin-sensitive). From these, the relative rhizosphere competency of Ter331 and Ter331PC could be indirectly estimated. Data were analyzed statistically by the non-parametric Wilcoxon-Mann-Whitney test (212).

Results and Discussion

Identification, isolation, and size estimation of plasmid pTer331

Pulsed-field gel electrophoresis (PFGE) of genomic DNA isolated from *C. fungivorans* Ter331 revealed two discrete bands (Fig. 1A, lane 1). The smaller one of these migrated to the same location on the gel as DNA that was prepared from *C. fungivorans* Ter331 using a QIAprep Spin Miniprep Kit for the isolation of plasmid DNA (Fig. 1A, lane 2). When different PFGE settings were applied, this band migrated differently relative to the linear marker fragments (not shown), suggesting (213-214) that the plasmid, which we designated pTer331, is circular. Digestion of purified pTer331 with *HindIII* or *PstI* revealed in both cases a single, linear fragment with an estimated size of 40 kb (Fig. 1B, lanes 4 and 5), while digestion with *EcoRI*

produced eight fragments (Fig. 1B, lane 3) adding up to a plasmid size of approximately 39.9 kb.

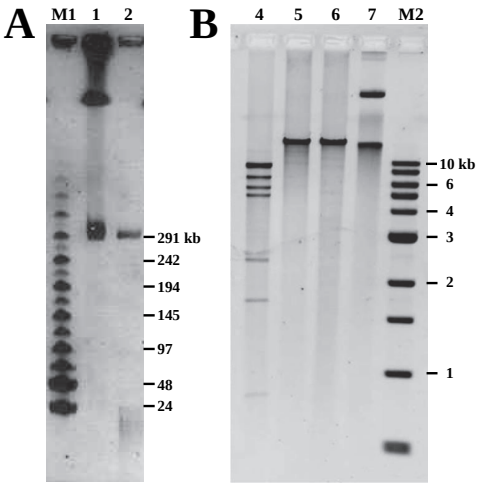

Figure 1. Gel electrophoresis of genomic and/or plasmid DNA isolated from *C. fungivorans* Ter331. (A) PFGE of *C. fungivorans* Ter331 genomic DNA prepared in agarose plugs (lane 1) and of plasmid pTer331 isolated with a QIAprep Spin Miniprep Kit (lane 2). PFGE conditions were as follows: 24 h run time with 6-60 s pulse times (left-hand gel). Lane M1: MidRange II PFG Marker (New England Biolabs). (B) Regular agarose gel (1%) showing 0.2 µg of purified pTer331 DNA digested with *EcoRI* (lane 3), *HindIII* (lane 4), or *PstI* (lane 5). Lane 6: undigested plasmid DNA. Lane M2: 1-kb marker (New England Biolabs).

Complete nucleotide sequence of plasmid pTer331

The complete nucleotide sequence of plasmid pTer331 was obtained from assembly of 362 shotgun sequence reads with an average length of 878 bp. The mean coverage was 7.9 per consensus base. Plasmid pTer331 has a size of 40,457 bp and a G+C content of 60.6% (Fig. 2). *In silico* digestion of pTer331 with *EcoRI* produced 9 fragments with sizes of 9319, 8914, 6662, 5532, 4873, 2390, 1720, 801, and 250 bp, which was consistent with the observed *EcoRI* banding pattern (Fig. 1B, lane 3). Also, as expected, we identified on pTer331 single recognition sites for *HindIII* and *PstI* (Fig. 2).

Analysis of the pTer331 DNA sequence revealed 44 open reading frames (ORFs), 39 of which were predicted to be organized in 11 operons of 2 or more genes. Table 2 lists all ORFs, their proposed gene names, locations, operonic organization, and G+C content, as well as the length and size of predicted gene products and highest similarity to proteins in the DDBJ/EMBL/Genbank databases.

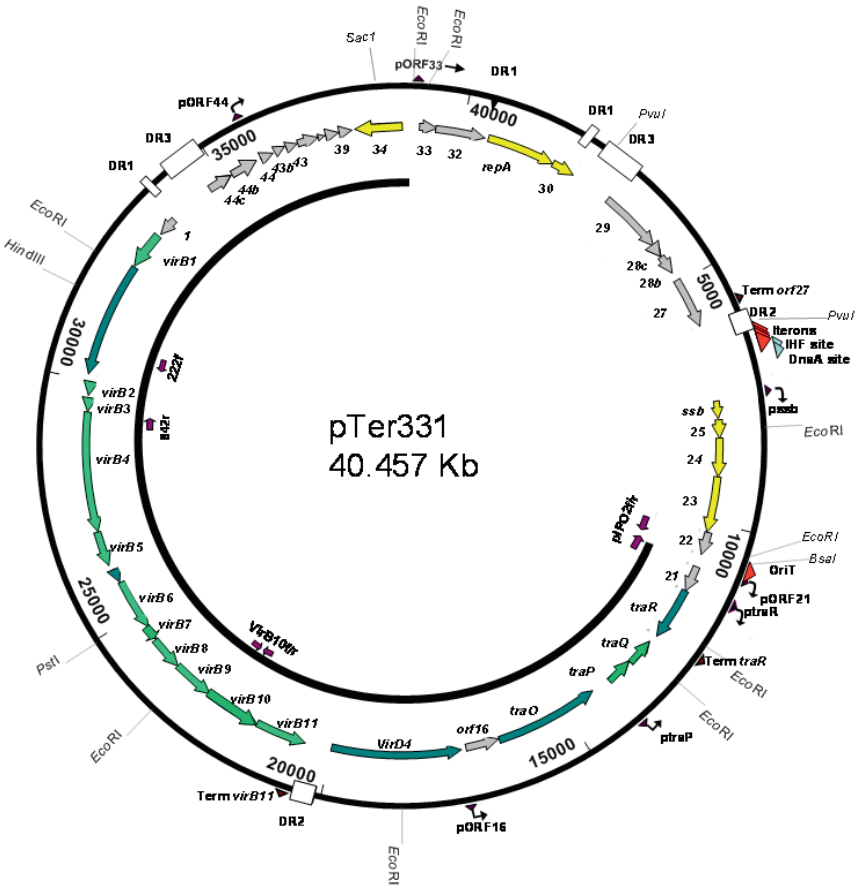

Figure 2. Genetic map of plasmid pTer331. The arrows indicate the position and direction of transcription of the putative ORFs. Different colors indicate a presumed function in replication and maintenance (yellow), mating pair formation (light green), DNA processing (dark green). ORFs with unknown function are colored grey. Also indicated are the positions of the putative origin of transfer (*oriT*), putative promoter sequences (P), long range direct repeats (DR1–DR3), positions of primers 222f, 842r, pIPO2 forward, pIPO2 reverse, virB10f, and virB10r, the putative IHF site, DnaA box and four iterons.

The overall genetic organization of pTer331 (Fig. 2) was highly similar to that of plasmid pIPO2 (196). For all but four genes on pTer331, we found homologs on plasmid pIPO2. Conversely, all but three ORFs previously identified on pIPO2 were also found on pTer331. The percentage identity between shared homologs was remarkably high and varied from 77%

(ORF15 or *virD*4) to 99% (ORF28b). Three of the seven ORFs that were identified as apparently unique to either pTer331 (i.e. ORF43b) or pIPO2 (i.e. ORF28a and ORF38) were indeed specific for one plasmid only, because the DNA fragment corresponding to each of these ORFs was deleted at least partially in the other plasmid. The likely cause of some of these deletions will be addressed in a later section. The other discrepancies between plasmids pTer331 and pIPO2 could be attributed to differences in annotation. For example, Tauch *et al* (196) interpreted the region upstream of ORF44 on pIPO2 to contain a divergently transcribed ORF45, while we assigned two ORFs (i.e. 44b and 44c) on the opposite strand of the same region on pTer331. The latter interpretation is most likely correct, based on evidence that will be presented later (Fig. 4). Also, a clear homolog of pTer331 ORF28c appears to exist on pIPO2 (positions 4172-4354), but it was not recognized earlier (196). Pairwise comparison of pTer331 to plasmids of the pIPO2/pSB102 family showed various degrees of sequence conservation (Fig. 3). Evidently, plasmid pSB102 has diverged more from pTer331 than did pIPO2 and (the only partially sequenced) pMOL98. Plasmid pSB102 further distinguished itself from pTer331 and pIPO2 by the acquisition of a transposon, Tn*5718*, which confers mercury resistance (197). Clearly, sequence similarity between pTer331 and pFBAOT6 or pXF51 was much lower and was mostly restricted to the region on pTer331 that carries genes required for plasmid transfer (see below). The pairwise comparison also revealed that pIPO2 and pMOL98 carry sequences with similarity to long direct repeats DR1, DR2, and DR3 found on pTer331 (Fig. 2 and 3), whereas on pSB102, only two partially conserved homologs of DR3 were identified. BLAST searches of the pTer331 repeats revealed the presence of a DR1 homolog on plasmid pBFp1 (215) and of partial DR2 homologs on R388 (216) and IncW pSa (217). The significance of these repeats occurring on different plasmids remains unclear. For pIPO2, it has been suggested that these repeats act as centromere-like sequences, involved in proper plasmid partitioning upon cell division (196).

Table 2. Putative coding regions of plasmid pTer331, their possible function and closest relationship to other proteins

ORF	Gene name*	Start position (nt)	Stop position (nt)	Predicted operon	G+C content (%)	Putative function	Protein length (aa).	Protein size (kDa)	Amino acid identity to pIPO2	Percentage identity to other plasmid gene products	Accession
31	*repA*	1	1389	1	62.2	replication initiation	462	50.9	95% repA	38% to RepA, plasmid pSa17	S30121
30	*parB*	1389	1853	1	65.6	involvement in plasmid partition	154	16.9	94% ORF30	57% to ParB, plasmid RP4	AAA26416
29	*ardC*	2699	4009	2	61.2	antirestriction	436	47.7	93% ORF29	91% to ArdC, plasmid pSa	AAD52160
28c	*orf28c*	4097	4279	2	56.8	probable transmembrane protein, function unknown	60	6.2	n/a	n/a	n/a
28b	*orf28b*	4283	4675	2	65.9	unknown	130	14.5	99% ORF28b	61% to ORF5, plasmid pSB102	CAC79150
27	*krfA*	4849	5883	3	69.9	regulation of plasmid segregation	344	36.9	90% ORF27	38% to KrfA, plasmid/mobile genomic island pKLC102	AAP22622
26	*ssb*	7396	7800	4	56.3	single stranded DNA binding activity, function unknown	182	20.3	97% ssb	33% to P116, plasmid RK2	CAD58038
25	*orf25*	7804	8202	4	55.9	partition gene repressor	132	14.7	96% ORF25	48% to XACb0052, plasmid pXAC64	AAM39298
24	*incC*	8199	8990	4	55.9	IncC-like protein	263	28.7	98% ORF24	35% to IncC, plasmid pMBA19a	AAX19280
23	*korB*	8991	10136	4	63.4	KorB-like transcriptional repressor	381	40.2	94% ORF23	35% to KorB, plasmid pBP136	BAF33443

ORF	Gene name*	Start position (nt)	Stop position (nt)	Predicted operon	G+C content (%)	Putative function	Protein length (aa).	Protein size (kDa)	Amino acid identity to pIPO2	Percentage identity to other plasmid gene products	Accession
22	*orf22*	10100	10609	4	57.1	unknown	169	18.4	94% ORF22	43% to Neut_2600, plasmid 2	ABI60803
21	*mobC*	10877	11416	5	58.9	unknown	179	20.2	97% ORF21	31% to MobC, plasmid pRA3	ABD64841
20	*traR/virD2*	11413	12507	5	61.7	nickase/relaxase activity	364	40.4	96% TraR	40% to Nic, plasmid pRA3	ABD64842
19	*traQ*	12678	13256	6	62	Type IV secretion channel, structural component	192	20.5	94% TraQ	63% to TrbM, plasmid pB3	CAG26010
18	*traP*	13270	13830	6	57.7	outer membrane protein	218	23.9	95% TraP	52% to Upf30.5, plasmid pA1	BAE19699
17	*traO*	14227	16419	6	60.8	DNA primase activity	730	80.9	95% TraO	31% to TraC4, plasmid pRA3	ABD64845
16	*orf16*	16431	17129	7	55.8	unknown	232	25.3	95% ORF16	27% to Neut_2626, plasmid 2	ABI60828
15	*traN/virD4*	17230	19932	8	58.9	ATPase activity, coupling the relaxosome with the transfer machinery	917	100.2	77% TraN	30% to VirD4, plasmid pTiA6NC	P09817
14	*virB11*	20480	21580	9	58.8	mating pair formation, ATPase	355	39.9	97% TraM	55% to VirB11, plasmid pES100	AAW88285
13	*virB10*	21531	22691	9	62.4	Type IV secretion channel, structural component	386	39.4	87% TraL	32% to VirB10, plasmid pTiC58	P17800
12	*virB9*	22691	23563	9	60	Type IV secretion channel, structural component	290	31.6	98% TraK	28% to VirB9, plasmid pTi15955	P0A3W7
11	*virB8*	23560	24270	9	58.8	Type IV secretion channel, structural component	236	26.2	98% TraJ	29% to VirB8, plasmid pTiC58	P17798

ORF	Gene name*	Start position (nt)	Stop position (nt)	Predicted operon	G+C content (%)	Putative function	Protein length (aa).	Protein size (kDa)	Amino acid identity to pIPO2	Percentage identity to other plasmid gene products	Accession
10	*virB7*	24276	24440	9	56.9	Type IV secretion channel, structural component	54	5.6	98% TraI	36% to XF_a0011, plasmid pXF51	P58337
9	*virB6*	24577	25659	10	59.6	Type IV secretion channel, structural component	360	37.9	96% TraH	31% to VirB6, plasmid pES100	AAW88297
8	*traG*	25671	25967	10	60.9	entry exclusion	98	10.2	95% TraG	41% to BBta_p0253, plasmid pBBta01	ABQ39891
7	*virB5*	26071	26790	11	58.7	Type IV secretion channel, structural component	224	24.6	97% TraF	40% to VFB54, plasmid pES100	AAW88296
6	*virB4*	26796	29276	11	56.5	mating pair formation, ATPase	826	93.9	98% TraE	46% to VFB39, plasmid pES100	AAW88281
5	*virB3*	29283	29606	11	58	Type IV secretion channel, structural component	107	12.1	98% TraD	34% to Neut_2637, plasmid 2	ABI60839
4	*virB2*	29616	30086	11	56.9	pilin precursor	152	16.1	93% TraC	44% to VFB38, plasmid pES100	AAW88280
3	*traB*	30089	32404	12	60.6	DNA topoisomerase activity	771	85.5	87% TraB	43% to ORF31, plasmid pRA3	ABD64859
2	*virB1*	32414	33184	12	63	involved in the local enzymatic disruption of the peptidoglycan layer	256	26.6	94% TraA	56% to VirB1, plasmid pXcB	AAO72105
1	*orf1*	33242	33637	12	51.5	unknown	131	14.9	93% ORF1	33% to XF_a0004, plasmid pXF51	AAF85573
44c	*orf44c*	34789	35097	13	56.6	unknown	102	11.6	n/a	n/a	n/a

ORF	Gene name*	Start position (nt)	Stop position (nt)	Predicted operon	G+C content (%)	Putative function	Protein length (aa).	Protein size (kDa)	Amino acid identity to pIPO2	Percentage identity to other plasmid gene products	Accession
44b	*orf44b*	35119	35667	13	66.7	unknown	182	20	n/a	72% to Neut_2597, plasmid 2	ABI60800
44	*orf44*	35776	36042	14	61	unknown	88	9.8	98% ORF44	27% to Oant_4534, plasmid pOANT01	ABS17221
43b	*orf43b*	36130	36270	14	58.2	unknown	46	5.2	n/a	n/a	n/a
43	*orf43*	36327	36563	14	65.4	unknown	78	8.6	96% ORF43	48% to ORF41, plasmid pSB102	CAC79186
42	*orf42*	36574	37002	14	68.8	unknown	142	16.5	93% ORF42	n/a	n/a
41	*orf41*	36999	37136	14	60.9	unknown	45	5.2	97% ORF41	n/a	n/a
40	*orf40*	37157	37423	14	66.3	unknown	88	9.5	97% ORF40	60% to Orf45	CAC79190
39	*orf39*	37420	37695	14	64.1	unknown	64	9.7	95% ORF39	40% to ORF1, plasmid pBFp1	AAQ94180
34	*parA*	37792	38688	15	68.1	resolvase activity	298	32	93% ORF 34	77% to ParA, plasmid pBP136	BAF33474
33	*yacA*	39053	39406	1	63.3	regulation of plasmid replication	117	13.1	97% ORF33	60% to YacA plasmid pXcB	AAO72122
32	*orf32*	39381	40382	1	62.2	cell filamentation	333	37.9	96% ORF32	30% to Ajs_4242, plasmid pAOVO01	ABM44342

* regarding the virB genes of the bacterial type IV secretion systems we chose to follow the nomenclature proposed by Christie (Christie, Atmakuri et al. 2005)

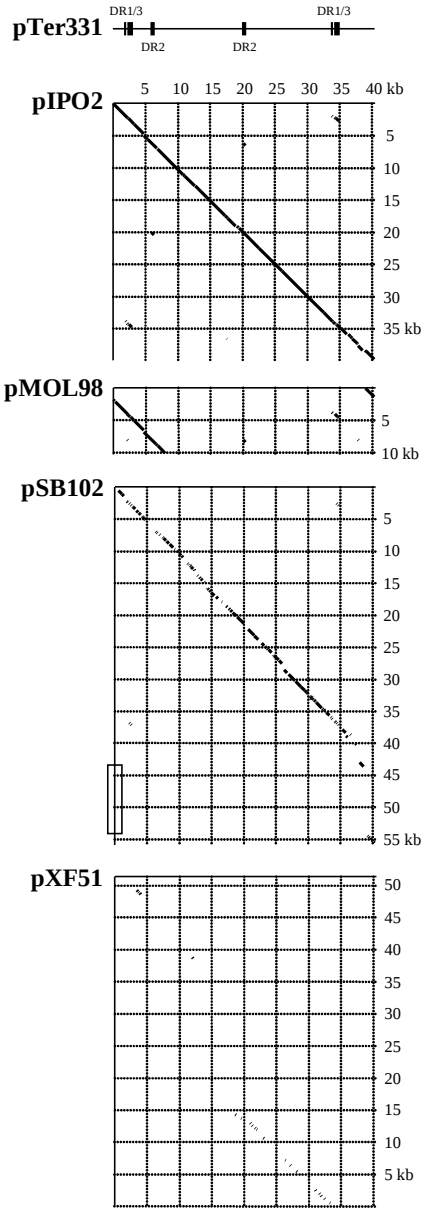

Figure 3. Pairwise comparison of the pTer331 DNA sequence to those of pIPO2, pMOL98, pSB102, pFBAOT6, and pXF51. Dot plots were created using Lasergene's module MegAlign using a 65% match cut-off and a window size of 50. The alignment of pMOL98 only included the 10,264-bp sequence reported for this plasmid (199). As reference points, the long direct repeats DR1, DR2 and DR3 are indicated on the linear representation of pTer331 at the top of the graph. The hatched bar on the Y-axis of the pSB102 alignment indicates the position of the 10,414-bp transposon Tn*5718* (197), and those on the Y-axis of the pFBAOT6 alignment the location of an In*4*-like integron and the left and right ends of Tn*1721* (200). Note that the Y-axis in the comparison with pXF51 has been reversed.

Functional annotation of plasmid pTer331

Following the example of pIPO2 (196), we can group the predicted gene products of pTer331 into 2 categories: 1) replication and partitioning functions, 2) transfer functions, including mating pair formation and DNA processing. A third category consists of genes and their products for which no function could be predicted based on sequence homology (Table 2).

1) Replication and partitioning functions

As has been demonstrated for other members of the pIPO2/pSB102 family (199), plasmid pTer331 has several characteristics that suggest a Θ-type mode of replication (218-220). First, ORF31 showed homology to several RepA replication initiator proteins. Second, in an *oriV*-like region approximately 6 kb downstream of the *repA* gene, four putative iterons, representing binding sites for RepA, were identified as three identical 20-bp direct repeats (5'-CACGCTGAAAGTGTCTTGCG-3') and one 19-bp imperfect repeat (5'-ACGCTGAAACTGTCTTGCG-3'). These repeats overlap in sequence and approximate location with iteron sequences identified on pSB102, pMOL98, and pIPO2 (199).

Furthermore, we located an AT-rich (83%) region (5'-TTTAGGTTTTTTTCCCTTTAAAAATATA-3') possibly representing the site of DNA strand opening (221), as well as a putative DnaA binding site (5'-CTATCCACA-3') which conforms to the consensus sequence of the DnaA protein of *E. coli* (222) and a potential IHF site (5'-TTAAAGCCTTATGAATCAATGGCTTGCGCGCAAGA-3'; IHF consen-sus bases underlined) (Figure 2), the last two sites representing potential binding sequences for host encoded factors which help altering the plasmid DNA helix during the first steps of plasmid replication.

The *repA* gene is the third in a putative operon that contains other genes with probable involvement in plasmid replication. The predicted product of ORF33 features a ribbon-helix-helix motif typical of CopG-like transcriptional repressor proteins involved in the regulation of plasmid replication (221). ORF32 revealed partial homology to the *fic* gene involved in cell cycling (223), suggesting that replication of pTer331 might be closely linked to host cell division.

To identify the origin of replication of pTer331, i.e. the minimal *cis*-acting region that can support its autonomous replication, we constructed a deletion derivative of pTer331. Plasmid pTer331Δ consists of a kanamycin resistance gene ligated to the 12.9-kb *BsaI-SacI* fragment of plasmid pTer331 (Fig. 2). This construct could be readily introduced and maintained in hosts *Escherichia coli* EP-max 10B (Bio-Rad) and *P. putida* KT2440 (224), suggesting that the 27.6 kb fragment deleted from pTer331 have no contribution in replication function. The remaining 12.9-kb fragment of pTer331 largely overlaps with a sequenced 10.2-kb region of pMOL98 which has previously been shown to be sufficient for autonomous replication of this plasmid (199). This further confirms the functionality of pTer331Δ as a mini-replicon.

Additional deletion from pTer331Δ of an internal 4.1-kb *Pvu*I fragment carrying ORFs 29, 28c, 28b and 27 (Fig. 2) diminished the plasmid's ability to replicate or be maintained in *E. coli* (results not shown). The functional annotation of these ORFs offers several possible explanations for the apparent requirement of this operon for plasmid replication/maintenance. The predicted product of ORF29 showed considerable homology (31% identity in the N-terminus) to the antirestriction protein ArdC from plasmid pSa (217). This protein has been shown to protect single-stranded DNA from host endonuclease activity (217) and might safeguard plasmid pTer331 during stages of the replication process when the DNA strands are separated. The ORF27 product resembled KfrA from plasmid pKLC102 (225), which has a predicted role as a transcriptional repressor in plasmid segregation during cell division (226).

Plasmid stability is a measure of the likelihood with which a plasmid is inherited by daughter cells at cell division (227). We estimated the stability of plasmid pTer331 in *C. fungivorans* Ter331 experimentally (see Materials and methods) and found that the frequency of plasmid loss per generation (M) was lower than the detection limit of 0.00044. Such a low value for M is typical for plasmids with an active stable partitioning system (207, 228-229). Besides ORF27 (see above), we identified several other genes on pTer331 with a possible contribution to plasmid stability.

ORFs 25, 24, and 23 are homologous to the KorA-IncC-KorB system which dictates the segregational stabilization of IncP-1 plasmids (230-231). In the same putative operon, ORF26 is likely to encode a single-stranded DNA-binding protein with helix-destabilizing activity, but its role, if any, in partitioning is unclear. Additional genes on pTer331 with possible involvement in plasmid stabilization are ORF30, whose product resembles ParB which has an essential role in the partitioning of plasmid RK4 (232) and ORF34, which codes for a putative resolvase with homology to ParA of RP4 (232) and which might resolve plasmid multimers as suggested for its homolog on pSB102 (197). It was suggested that the ORF34 homolog of pIPO2 is not essential for plasmid stability as a knockout in this gene had no effect in surrogate host *P. fluorescens* (196).

2) Transfer functions: mating pair formation and DNA processing

In tri- and bi-parental mating experiments, we established that pTer331 possesses both mobilizing and retro-mobilizing properties. Triparental matings involved *C. fungivorans* Ter331 as a helper strain, facilitating through pTer331 the transfer of plasmid pSM1890 from *E. coli* CV601 to *P. fluorescens* R2f. Under the circumstances tested (see Experimental procedures), this transfer occurred at a frequency (defined as the number of transconjugants per recipient) of 1.8×10^{-4}. In a control experiment with pIPO2, the rate was 233-fold higher at 4.2×10^{-2}. About ten percent of the transconjugants in these mating experiments tested positive with primers for pTer331, indicating that co-transfer of pTer331 occurred during triparental mating. In biparental matings, we determined the rate with which pTer331 mediated the acquisition of plasmid pSM1890 by *C. fungivorans* Ter331 from *E. coli* CV601. With pTer331, the transfer frequency was 2.22×10^{-8} compared to 7.87×10^{-8} with pIPO2. Higher values of transfer rate have been reported independently for pIPO2 by Tauch *et al.* (196) and for other plasmids (233), nevertheless the discrepancy could be due to a difference in the mobilizing vector and in the experimental conditions.

The (retro)mobilizing activity of plasmid pTer331 can be attributed to two groups of genes: 1) those involved in mating pair formation

(indicated in light green in Fig. 2) and 2) those involved in processing of the plasmid DNA for transfer to, and establishment in, the recipient cell (indicated in dark green in Fig. 2). On plasmid pTer331, these genes occur interspersed, which is characteristic for plasmids from the pIPO2/pSB102 family (196). Similar to pIPO2 and pSB102, the mating pair formation genes of pTer331 showed a high degree of similarity in synteny and coding sequence to the type IV secretion system encoded by the *virB* gene cluster of several different *Brucella* species. These bacteria use the secretion system to inject effector proteins into host macrophages (234). In the context of plasmids, the *virB* genes are responsible for establishing initial contact with the recipient cell and for assembling the secretion structure that allows the transfer of plasmid DNA through a pilus appendage into the recipient (235). DNA processing in the donor cell involves nicking of the plasmid DNA at the origin of transfer (*oriT*) by a nickase that - together with accessory proteins and the plasmid DNA - constitutes the relaxosome. This protein-DNA complex is then presented by a so-called coupling protein to the membrane-located type IV secretion system (235-236). Upstream of ORF21 on pTer331, we identified a putative *oriT* site (5'-GTGTGGGCTATTGCA
GGAT-3') with 100% identity to that described for pIPO2 (196). The ORF downstream of ORF21 codes for a putative protein that is homologous to VirD2-like proteins with DNA relaxase/nickase activity (237), while ORF15 is the most likely candidate coding for a relaxosome-coupling function based on homology to *traN*/*virD4* genes from other plasmids (238). The involvement of other pTer331 genes in plasmid DNA processing is unclear. Some, for example the TraB product of ORF3 (a putative DNA topoisomerase), might be part of the relaxosome. The predicted product of ORF17 (TraO) shows similarity to DNA primases and it has been suggested (197, 235) that it is co-transferred with the plasmid DNA into the recipient to convert the single-stranded DNA to double-stranded DNA by lagging strand DNA synthesis. TraG, the product of ORF8, shows similarity to the entry exclusion protein from R388 (239), which specifically prevents transfer to recipient bacteria that already carry the plasmid (240).

Comparison of pTer331 and pIPO2 at the DNA level: evidence for past evolutionary events

A ClustalW alignment of the 40,457-bp pTer331 and 39,815-bp pIPO2 sequences revealed that the two plasmids share 36,411 identical nucleotides, corresponding to 90.0 and 91.5% identity, respectively. We identified a total of 2869 substitutions (7.1 and 7.2%, respectively): 1663 transitions (A↔G or C↔T) and 1206 transversions (A↔C, A↔T, G↔C, or G↔T). Furthermore, 1177 nucleotides on pTer331 (2.9%) were not found on pIPO2, and 535 nucleotides on pIPO2 (1.3%) were absent from pTer331. This assortment of substitutions and indels offered several lines of indirect evidence for the evolutionary forces that shaped pTer331 and pIPO2 since the presumed split from their common ancestor.

First, a comparison of nucleotide identity along the aligned plasmid sequences (Fig. 4A) revealed that the similarity between pTer331 and pIPO2 deviated significantly from the average of 92% along the aligned sequences. In several parts of the DNA alignment, identity dropped to below 70%, e.g. in the central part of ORF27, upstream of ORF26, and upstream of ORF1. The two most prominent stretches with reduced identity were within the genes *virD4* (*traN*, ORF15) and *traB* (ORF3). At the amino acid level, the products of these genes showed a low degree of identity with their pIPO2 counterparts (77% and 87%, respectively). In comparison, most other proteins that are shared between the two plasmids feature identity scores of 90% or higher. At present, we cannot explain why the *virD4* and *traB* genes and products of pTer331 diverged from their pIPO2 homologs at a comparable and apparently much higher rate. One interesting hypothesis is that VirD4 and TraB interact with each other directly and that the introduction of mutations in one of the proteins selected for compensatory mutations in the other and vice versa. This hypothesis, which assumes a high degree of specificity in the interaction, still needs testing, but the predicted functions of VirD4 and TraB do not preclude an interaction between the two. In fact, as a coupling protein, VirD4 is responsible for recruiting the relaxosome and presenting it to the membrane-located proteins involved in mating pair formation (236). The *traB* gene product is annotated as a DNA topoisomerase I

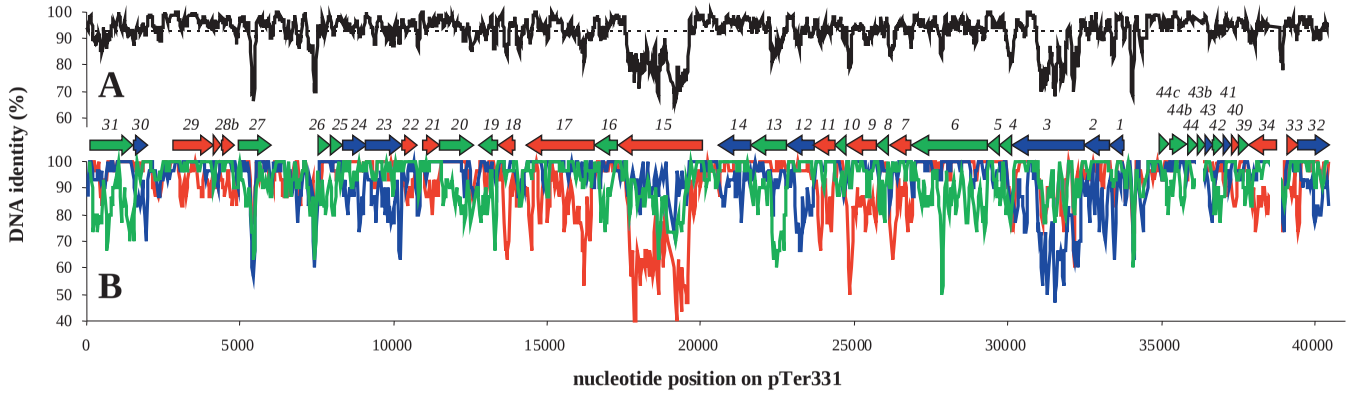

Figure 4. Percentages of DNA identity along the aligned DNA sequences of plasmids pTer331 and pIPO2. (A) For this graph, pTer331 and pIPO2 were aligned, and with 10-bp steps the percentage of identical nucleotides in every 100-bp window of shared DNA sequence was plotted as a function of the position in the pTer331 sequence. (B) A similar analysis was done on three new alignments obtained after splitting the alignment of panel A three-ways as follows: frame 1: nucleotides 1, 4, 7, 10, etc. (red line in the graph), frame 2: nucleotides 2, 5, 8, 11, etc. (blue line), and frame 3: nucleotides 3, 6, 9, 12, etc. (green line). Also indicated are the position and orientation of each of the ORFs we identified on pTer331. The color of each ORF corresponds to its wobble frame.

with as-yet undetermined function in pTer331/pIPO2 biology, but it might well be part of the relaxosome and responsible for or accessory to nicking and/or relaxation of the plasmid DNA. Direct interaction between VirD4 -like proteins and enzymes with relaxase/topoisomerase activity has been demonstrated for other plasmid systems (241). To address this hypothesis in greater detail and to determine the degree of specificity underlying the interaction between VirD4 and TraB, future experiments will include a swap of *virD4* and/or *traB* genes between plasmids pTer331 and pIPO2.

We also observed that substitutions in the pTer331/pIPO2 alignment were not randomly distributed between each of the reading frames (Fig. 4B). Instead, substitutions occurred with greater frequency in one particular frame over stretches that clearly co-localized with ORFs (Fig. 4B). In all cases, this frame corresponded to the wobble-frame of the corresponding ORF, i.e. the sequence containing every third nucleotide of the ORF. This suggests that accumulation of point mutations has not been random since the two plasmids diverged, and that there apparently has been selection for functional conservation of all ORFs shared between both plasmids. Interestingly, this not only applies to genes with predicted function, but also for ORFs to which no clear function could be assigned, including the ORF44c-39 cluster of hypothetical genes. Thus, the wobble-frame analysis of two genes from this cluster, ORFs 44c and 44b, would favor their annotation over an ORF45-like gene present on the reverse-complement strand, as has been done for pIPO2 (196) and pSB102 (197). Further support for the authenticity of ORF44b comes from the high degree of identity (up to 72%) of its gene product to hypothetical conserved proteins encoded on other plasmids, including plasmid2 from *Nitrosomonas eutropha* C91 (accession number CP000452), pAgK84 from *Agrobacterium* K84 (242), pVEIS01 from *Verminephrobacter eiseniae* EF01-2 (accession number CP000543), pLPP from *Legionella pneumophila* str. Paris (243), R721 from *E. coli* K-12 (244), and pEL60 from *Erwinia amylovora* LebB66 (245). We verified that for most of these plasmid-localized genes, annotation was unambiguous, i.e. no significant ORFs were present in the reverse-

complement strand. Interestingly, ORFs 44c through 39 all have the same orientation and as a cluster are preceded by a region upstream of ORF44c that overlaps with the large direct repeat DR3 (Fig. 2) which is duplicated upstream of the putative operon consisting of ORFs 29 through 28b. The significance of this finding and whether ORFs 44c-39 and ORFs 29-28b (and possibly ORF27) share an identical promoter and thus transcriptional profile remains to be elucidated.

While comparison of the ORF44c-39 clusters from pTer331 and pIPO2 suggests functional conservation, a different picture emerges in a three-way comparison with pSB102. The corresponding region of this plasmid features numerous frame shifts over the entire length of comparison to pTer331 (Fig. 5). This dictates that the coding potential of pSB102 in this region cannot be the same as that of pTer331 and pIPO2. By comparison, frame shifts in the pIPO2 sequence were much less frequent and furthermore restricted to three intergenic regions and an out-of-frame/back-in-frame shift in ORF42 (Fig. 5). Based on this, it may be suggested that the ORF44c-39 region on pSB102 is no longer functional and has started to rapidly diverge from the pTer331/pIPO2 sequences. Still, several regions in the pTer331/pSB102 alignment could be identified that are devoid of frame shifts (Fig. 5). The largest one of these covered exactly the length of ORF44b, suggesting that at least this gene escaped degeneration and was conserved between pTer331, pIPO2, and pSB102. This targeted conservation suggests an important, as-yet unknown role for this ORF.

Plasmids pTer331 and pIPO2 were also analyzed for differences in GC3 content (246-247) and synonymous/non-synonymous substitution rates (248). However, these analyses did not reveal significant differences, suggesting that, since their split from a common ancestor, these plasmids have been maintained in hosts with similar codon usage. Alternatively, if they did not, there has not been enough time for them to adapt to the codon usage of their respective natural hosts.

The alignment of pTer331 and pIPO2 revealed 51 gaps of varying length: twenty-one 1-bp indels, four 2-bp indels, four 3-bp indels, three 6-bp

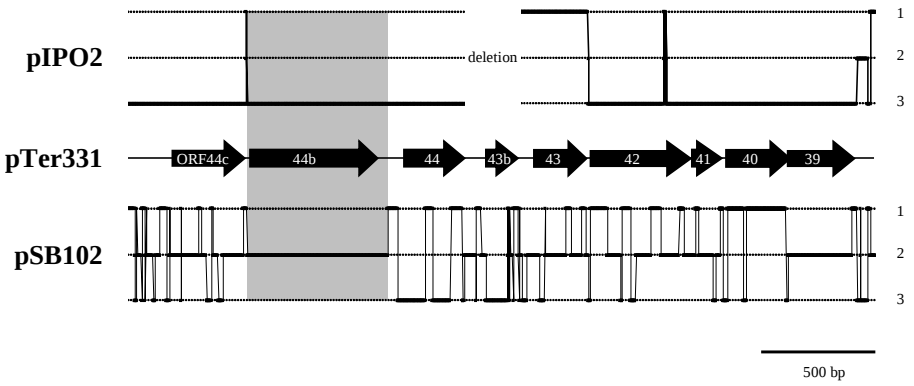

Figure 5. Alignment of the ORF44c-39 clusters from pTer331, pIPO2, and pSB102. Shown are the locations of ORFs 44c, 44b, 44, 43b, 43, 42, 41, 40, and 39 on pTer331 in relation to 1-bp gaps and resultant frame shifts in the alignment to pIPO2 (top) and pSB102 (bottom). For pIPO2 and pSB102, each vertical line represents a shift from one frame (arbitrarily numbered 1, 2, or 3) to another. The largest section devoid of frame shifts in both pIPO2 and pSB102 is indicated by a grey box. The region encompassing ORF43b is absent from pIPO2 and has been marked as 'deletion'.

indels, two indels each of 9, 18, 21, 33, and 60 bp, and nine single indels with a length of 12, 39, 62, 79, 90, 223, 235, 249, or 382 bp, respectively. Eighteen of these gaps were located in ORFs (i.e. one in each of the ORFs 21, 23, 29, 42, and in *traA* and *traL*, two in ORFs 27 and 32, and eight in *traN*). Of these gaps, sixteen had a length that was divisible by three, which would add/delete amino acids from the encoded protein, but prevent a shift in the open reading frame. The other two gaps both occurred in ORF27 as 1-bp indels, one representing an out-of-frame shift and the other a back-in-frame shift. These results again suggest a positive selection for conservation of gene function during divergence from the pTer331/pIPO2 ancestor. The ORF that was most affected by indels was *virD4*/*traN*: we identified one 3-bp sequence in the *virD4*/*traN* sequence of pIPO2 that was not present on pTer331, and 7 sequences (between 6 and 60 bp in length) that were present in *traN* of pTer331 but not pIPO2. This explains the overall smaller size of TraN encoded by pIPO2 (837 aa) compared to pTer331 (917 aa).

A third insight into the divergent evolution of plasmids pTer331 and pIPO2 comes from comparison of long and short direct repeats (DRs). As mentioned already, we identified three pairs of long DRs on pTer331: a 649-bp perfect repeat (DR3 copies A and B), a 367-bp perfect repeat (DR2 copies A and B) and a 93-bp imperfect repeat with one mismatch (DR1 copies A and B). These repeats correspond to direct repeats DR3 (403 bp), DR2 (386 bp), and DR1 (96 bp), respectively, on plasmid pIPO2. By aligning the DR3 sequences of pTer331 with the corresponding regions of pIPO2, we observed that three distinct gaps on pIPO2 explain the difference in size between the DR3 direct repeats of pTer331 and pIPO2 (Fig. 6A). Closer examination revealed the presence of short direct repeats (7, 10, and 8 bp) flanking these gaps on the corresponding DNA of pTer331 (Fig. 6B). In a plasmid ancestral to pIPO2, such repeats may have facilitated a 62-bp deletion from DR3 copy A and deletion of 60 and 79 bp from DR3 copy B. We are confident that these gaps in the alignment represent deletions from an ancestor of pIPO2 rather than acquisition by an ancestral form of pTer331: BLAST analysis of the three DNA fragments absent from pIPO2 revealed their presence on plasmid pMOL98 embedded in a region with homology to DR3 from pTer331.We found at least five other gaps in the pTer331/pIPO2 alignment that were flanked by such short direct repeats. In one case (Fig. 6B, example 4), the gap consisted of a 9-bp string that occurred immediately duplicated in *traA* on pTer331 and only once on pIPO2, which explains why the *traA* gene product of pTer331 is 3 amino acids shorter than that of pIPO2. In at least 2 cases, repeat-flanked DNA on pIPO2 did not occur on pTer331 (see example 5, Fig. 6B). A possible mechanism for deletion of DNA fragments that are flanked by short direct -repeats is ‘replication slippage’ (249), which involves mispairing between a replicating strand and its template at sites of repetitive DNA sequences. In theory, such an event can lead to a deletion or duplication of the flanked DNA, and in *E. coli*, little or no bias seems to exist towards deletion- versus duplication-producing misalignments (249). However, we observed no obvious tandem-duplicated regions flanked and separated by direct repeats on pTer331 and pIPO2, suggesting that,

in the evolution of these plasmids, either deletion occurred more frequently or duplication was selected against.

As a final clue towards understanding the evolutionary forces that shaped pTer331 and pIPO2, we counted 7 mismatches in the 403-bp overlap between the DR3 sequences of pTer331 and pIPO2 (Fig. 6A), and 10 mismatches in the 367-bp overlap between the DR2 sequences (not shown). We conclude for both DR3 and DR2 that 1) on either plasmid, copies A and B are identical, and 2) copy A on pTer331 differs from copy A on pIPO2 in exactly the same way as does copy B on pTer331 from copy B on pIPO2. This seems to suggest a mechanism that keeps the two copies of these long direct repeats on the same plasmid identical. The mechanism or function underlying this conservation remains to be elucidated. We note that the region of pIPO2 corresponding to subfragment *c* from pTer331 (Fig. 6A) is no longer considered part of DR3 on pIPO2 (196) and has diverged differently upstream of copy A compared to copy B (Fig. 6A). This would suggest that contiguity is a prerequisite for conservation. Whatever the mechanism, it is apparently not at work in the much shorter DR1 sequences of pTer331 and pIPO2: in a 90-bp overlap, we identified 2 mismatches between copies A, 2 mismatches between copies B, 3 mismatches between copy A on pTer331 and copy B on pIPO2, and 3 mismatches between copy B on pTer331 and copy A on pIPO2, in addition to the 1-bp mismatch between copy A and B on pTer331, and 1-bp mismatch between copy A and B on pIPO2.

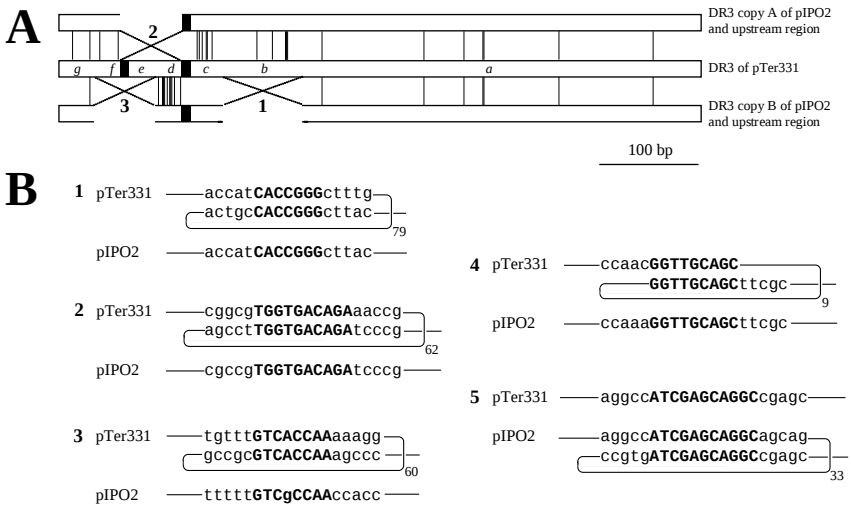

Figure 6. Indels in the pTer331/pIPO2 alignment. (A) Shown is a schematic comparison of the DR3 long repeat of pTer331 with DR3 copies A and B of pIPO2 and their upstream regions. The DR3 region of pIPO2 is shaded and corresponds to the region on pTer331 labeled as fragment *a*. Short direct repeats in DR3 of pTer331 are indicated by boxes with similar shading and were transposed onto the DNA upstream of DR3 copy A and B of pIPO2 to reveal the absence of corresponding fragments *d* and *e* from the DNA upstream of DR3 copy A and of fragments *b*, *e*, and *f* from the DNA upstream of DR3 copy B. Indicated by vertical lines are single nucleotide differences between DR3 of pTer331 and copy A or B or their upstream regions on pIPOs. (B) Detail of short direct repeats flanking DNA fragments that are unique to pTer331 or pIPO2 and comparison with the corresponding region on pIPO2 or pTer331, respectively. Examples 1, 2 and 3 correspond to fragments/gaps *b*, *d-e* and *e-f* in panel A, respectively. Examples 4 and 5 are discussed in the text. Repeats are shown in bold, together with 5 nucleotides upstream and 5 downstream of the repeat. The number in each example represents the length of the DNA fragment (in bp) that is unique to the respective plasmid (so for example 1, the alignment of pTer331 and pIPO2 features a 79-bp gap in the pIPO2 sequence).

Hypotheses on the ecological role of plasmid pTer331

We exploited the fact that pTer331 is available in its native host to test the contribution of the plasmid to some of the phenotypes that characterize *C. fungivorans* Ter331. One of these is the ability of *C. fungivorans* Ter331 to efficiently colonize the root system of tomato (42). This is particularly relevant since, for pIPO2, it was suggested that it might confer rhizosphere competency to its (unknown) host, based on the observation that pIPO2-like sequences showed a biased detection in soils associated with roots of different plants, including tomato (196). We tested this hypothesis for pTer331 by comparing the root-colonizing ability of wild-type *C. fungivorans* Ter331 with an otherwise isogenic but plasmid-cured derivative, Ter331PC. For this, tomato seeds were inoculated with mixtures of established root colonizer *Pseudomonas fluorescens* PCL1285 (250) and either *C. fungivorans* Ter331 or *C. fungivorans* Ter331PC, and developing roots were examined for absolute and relative bacterial abundances. In this indirect comparison, both *C. fungivorans* Ter331 and *C. fungivorans* Ter331PC competed equally well with *P. fluorescens* PCL1285, colonizing root tips at log10 densities of 5.12 ± 0.2 (CFU+1)/cm and 5.07 ±0.4 (CFU+1)/cm, respectively. From this, we conclude that pTer311 does not contribute significantly to the rhizosphere competency of its host under the conditions tested. However we can not exclude that in a more natural setting the plasmid may reveal an advantage to its host that could not be measured under laboratory conditions. Additional phenotypes of *C. fungivorans* Ter331 are its ability to hydrolyze chitin (30), to inhibit fungal growth (34), to live at the expense of living fungi (201), and to weather biotite (251). We rule out an involvement of plasmid pTer331 in these activities, given our observations that no PCR products were obtained with pTer331/pIPO2-specific primers from the genomic DNA of 44 *Collimonas* strains in our collection, which for the most part showed the same phenotypes as pTer331-carrying *C. fungivorans* Ter331. This leaves us to conclude that pTer331 is a cryptic plasmid, as defined by our inability to assign, either based on analysis of gene content or on experimental evidence, an obvious advantage of the plasmid to its host *C. fungivorans* Ter331. The

same was concluded for pIPO2 based on its coding potential (196). Most, if not all, genes on pTer331 seem to be dedicated to the plasmid's spread and survival, given their homology to or association with previously described plasmid-located genes. Even the ORF44c-39 cluster, which has been suggestively linked to the plant-associated occurrence of plasmids pIPO2 (196) and pSB102 (197), contains at least one gene (ORF44b) which is conserved in plasmids besides pIPO2 and pSB102, and which thus might not contribute to or have an involvement in functions other than those related to plasmid biology. Thus, plasmids like pTer331 (and pIPO2) can be considered canonical genetic parasites, with highly developed replication, maintenance and self-transfer systems to ensure their persistence in different bacterial hosts and natural environments. However, it has also been suggested (252) that cryptic plasmids eventually go extinct unless they acquire genes that benefit their host. Perhaps plasmids pTer331 and pIPO2 once, like pSB102 now, carried useful genes, but recently lost them. This 'nonequilibrium' interpretation would predict that the ability of a plasmid to 'attract' conditionally useful genes would enhance survival of the plasmid. In this context, we note that plasmids pSB102, pIPO2, and pMOL98 apparently feature a putative, not previously recognized, hotspot for transposon insertions. Plasmids pIPO2T and pMOL98 are mini-Tn*5*::luxABtet- and mini-Tn*5*-Km1-tagged derivatives of pIPO2 and pES1, respectively. In both cases, like in pSB102, the transposon occurs inserted upstream of the gene that corresponds to ORF33 on pTer331, i.e. ORF68 on pSB102, ORF33 on pIPO2, and *orf1* on pMOL98. It will be of quite some interest to determine experimentally whether these hotspots are genuine and act as 'magnets' for the insertion of transposons and the genes associated with them. If this hypothesis would be confirmed the pIPO2-pSB102 family would have a parallel in the IncP-1 family where a recent work has shown that the presence of two hot spot for transposon insertion in combination with selection accounts for the common architectural feature of the family (253). But even without accessory genes, plasmids such as pTer331 and pIPO2 may confer a clear benefit to bacteria. Through their (retro)mobilizing activity, they are potential catalysts of the

dissemination of the mobile gene pool ('mobilome') within a bacterial community. In this capacity, they could effectively increase the adaptability of an entire bacterial community to a changing environmental condition. The rhizosphere, from which plasmids pTer331, pIPO2 and pSB102 were isolated, represents a microbial habitat that has been recognized as a natural hotspot for plasmid transfer (254-257). It is unclear whether (retro)mobilizing, but otherwise cryptic, plasmids are relatively more common in such hotspot environments. Some of the metagenomic approaches that are currently underway to reveal the diversity of mobile DNA in the rhizosphere and other microbial habitats will undoubtedly answer this question. To test whether such plasmids play a role in the acceleration of intracommunal gene transfer and of adaptation at the population level, the pIPO2/pSB102 family presents an obvious and attractive model system.

The confrontation of *C. fungivorans* Ter331 with the fungus *A. niger* (presented in Chapter 2), indicated that the genes encoded on plasmid pTer331 are upregulated during this interaction between the fungus and the bacterium. This finding seems to support the assumption that plasmid pTer331 plays a role in accelerating bacterial adaptation to changing environmental conditions. Through its retromobilizing property, the plasmid facilitates the genetic exchange with other bacteria present in the environment and may help the bacterium to gain functions useful for adaptation. This might also be the case for the confrontation of *C. fungivorans* Ter331 with *A. niger*, when, after some time, the fungus causes a modification in the environment which may be detrimental to the bacterium (Chapter 2).

Acknowledgements

Strain *E. coli* CV601 (pSM1890) was provided by Dr. K. Smalla (Biologische Bundesanstalt, Braunschweig). We also thank Faina Kamilova and Shamil Validov for their help with the tomato root colonization experiments.

Chapter 4

Comparative genomics of *Collimonas* bacteria

F. Mela, K. Fritsche, W. de Boer, M. van den Berg, J. A. van Veen, J. H. J. Leveau

Abstract

Collimonas is a genus of soil bacteria which comprises three recognized species: *C. fungivorans*, *C. pratensis* and *C. arenae*. The bacteria belonging to this genus share the ability to lyse chitin (chitinolysis) and feed on living fungal hyphae (mycophagy), but they differ in colony morphology, physiological properties and antifungal activity. In order to gain a better insight into the genetic background underlying this phenotypic variability of collimonads, we investigated the variability in the genomic content of five strains representing the three formally recognized *Collimonas* species. The genomic content of four test strains was hybridized on an array representing the reference strain *C. fungivorans* Ter331. The analysis yielded a set of genes common to all strains, a set of genes present in some but not all the analyzed strains, and a set of genes unique to strain Ter331. Also several of the genetic determinants putatively underlying mycophagy showed an irregular distribution among *Collimonas* strains, including genes for motility, production of antifungals, and secretion systems. We hypothesize that the possession of a different collection of these genetic determinants might be at the base of specialization of *Collimonas* strains towards different fungal hosts.

Introduction

The bacterial genus *Collimonas* belongs to the family *Oxalobacteraceae* in the order *Burkholderiales* of the β-proteobacteria. The study of the taxonomy of this genus led to the identification of three species: *C. fungivorans*, *C. arenae* and *C. pratensis* (259). All three *Collimonas* species were isolated from slightly acidic dune soils from the Dutch Wadden island Terschelling, where they were dominant among the cultivable chitinolytic bacteria (30, 34). Later studies revealed that these bacteria have a widespread occurrence in terrestrial environments and that their distribution encompasses a wide range of natural and semi-natural environments, albeit at relatively low abundances (32, 39). *Collimonas* bacteria are known for their ability to grow at the expenses of living fungal hyphae, a trophic behavior called mycophagy (29), which was demonstrated for the first time in a soil-like microcosms (30, 201). A subsequent study demonstrated that mycophagous growth of *Collimonas* bacteria is not restricted to the artificial laboratory environment, but can also take place in natural soils (31). All *Collimonas* strains described so far are mycophagous and share certain features, such as chitinolysis. However, they differ for other traits such as colony morphology, the ability to oxidize various carbon sources, and their antifungal activity against several fungal species (Table 1). In this study we aimed at gaining insight into the genomic differences that underlie the phenotypic variability of this genus. To achieve this goal we compared our reference strain, *C. fungivorans* Ter331, with four other *Collimonas* strains using array based comparative genomics. The comparison involved representatives of the three species identified in the genus. We discuss the implications of our results for the mechanistic definition of bacterial mycophagy and the species-specific interaction of *Collimonas* bacteria and fungi.

Table 1. Overview of some of the features and mycophagous determinants possessed by the *Collimonas* strains used in this study. Present, +; not present, -; not determined, nd.

	Ter6	Ter10	Ter14	Ter91	Ter331	Reference *
Species:						
C. fungivorans	+	-	+	-	+	1
C. pratensis	-	-	-	+	-	2
C. arenae	-	+	-	-	-	2
Plasmid pTer331	-	-	-	-	+	3
Mycophagy:						
Chaetomium globosum	+	+	+	+	+	4
Fusarium culmorum	+	+	+	+	+	4
Mucor hiemalis	+	+	+	+	+	4
Antifungal activity:						
Chaetomium globosum	-	+	-	nd	+	5
Fusarium culmorum	+	-	-	nd	+	5
Fusarium oxysporum	-	-	-	nd	-	5
Idriella bolleyi	+	-	+	nd	+	5
Mucor hiemalis	+	+	+	nd	+	5
Phoma exigua	+	+	+	nd	+	5
Ulocladium sp.	+	+	+	nd	+	5
Aspergillus niger	-	-	+	-	+	6
Colony type	I	II	I	III	I	1
Swimming motility	+	+	+	-	+	1
Assimilation of D-trehalose	+	-	+	+	+	1
Chitinolytic activity	+	+	+	+	+	1

* References:1, de Boer, Leveau *et al.* (201); 2, Höppener-Ogawa, de Boer *et al.* (259); 3, Mela, Fritsche *et al.* (286); 4, de Boer, Klein Gunnewiek *et al.* (105); 5, de Boer, Klein Gunnewiek *et al.* (34); 6, this study.

Materials and Methods

Strains used in this study. The strains used in this study have been previously described (201, 259): Ter6, Ter14 and Ter331 belong to the species *C. fungivorans*, while Ter10 and Ter91 belong to the species *C. arenae* and *C. pratensis*, respectively.

Antifungal activity. The antifungal activity of *Collimonas* strains against the fungus *Aspergillus niger* was measured on WYA [1 g KH2PO4 , 5 g NaCl, 0.1 g BactoTM Yeast-Extract (Becton, Dickinson and Company, Breda, The Netherlands), and 20 g agar (Boom BC, Meppel, The Netherlands) per liter] supplemented with 2mM N-Acetylglucosamine.

Genomic DNA preparation. Bacterial cells were grown overnight at 25 °C in King's B (KB) medium (260), subsequently centrifuged and the total genomic DNA was extracted using QIAGEN Genomic-tip (QIAGEN, Venlo, The Netherlands) following the manufacturer's instructions.

Comparative genomic hybridization (CGH) microarrays. The *Collimonas* CGH microarray is a custom microarray manufactured by NimbleGen (Roche NimbleGen Systems, Iceland) based on the sequences of *C. fungivorans* Ter331 chromosome (32) and plasmid pTer331 (Chapter3). The microarray features 385536 tiling probes covering both coding and non coding regions of the sequence. The probe length ranges from 50 to 74 bases with an average tiling interval of 11 bases. 7242 internal control probes are present on the microarray, resulting in a total of 392778 probes. *Collimonas* CGH array hybridization and scanning were performed by NimbleGen. Briefly genomic DNA from the test strain and the reference Ter331 strain were labelled with fluorescent Cy3 and Cy5 labels, respectively, and the two samples were co-hybridized to the microarray. Each array was performed in 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, genomic DNA isolated from strain *C. fungivorans* Ter331 was hybridized in duplicate to the microarray. Description of the platform with probe information as well as the hybridization data is available from the

Arrayexpress database of the European Bioinformatics Institute (EBI) through the accession number A-MEXP-1876.

Data analysis. Within-array fluorescence ratios were normalized by NimbleGen using qspline normalization (261). Between-array normalization was obtained by dividing signal intensities in each array by the mode of their distribution (262). The normalized $\log_2$ (test/reference) hybridization values of the two dye-swap replicate arrays were averaged and used for subsequent analysis. The presence of a gene in *Collimonas* strains Ter6, Ter10, Ter14, Ter91 was inferred using the intensity of the hybridization signal. The procedure adopted was the following: we selected the P_i set of all probes targeting each g_i gene of the reference strain and calculated the m_i mode from the distribution of the hybridization values obtained by the test strain for the P_i probes. The gene was considered present if $m_i \geq$ threshold T and absent if $m_i <$ threshold T (see below for the value of T). The procedure is analogous to the one described for the PanCGH algorithm (263), and corresponds to the situation in which the orthologous group g_i contains a single gene and the presence score $S_i = m_i$. The mode m_i of each gene is calculated using the half.range.mode algorithm from the Genefilter package available in the Bioconductor suite (264). In order to reduce the error rate the genes having <13 matching probes were left out of the analysis. Under these criteria we were left with a total of 4283 genes: 4239 encoded on the chromosome and 44 encoded on the plasmid pTer331. To determine the T threshold value we used as positive control the presence score S_i distribution obtained with the self hybridizations of the strain *C. fungivorans* Ter331. The S_i distribution of the plasmid pTer331, which was confirmed to be absent from all the strains except *C. fungivorans* Ter331(Chapter 3), was used as negative control. The best T threshold value was established by testing the performance of all possible thresholds when confronted with the S_i distribution of the positive control, the negative control and the S_i values of a subset of 12 genes examined by PCR analysis. We generated a Receiver Operating Characteristics (ROC) curve plotting on the *Y* axis the true positive rate and on the *X* axis the false positive rate of all possible

thresholds and chose the threshold corresponding to the maximum value of accuracy (265).

PCR experiment. We performed polymerase chain reaction (PCR) analysis on a subset of 12 genes. The list of the primers used with the corresponding targeted genes is presented in Table 2. The primers were designed to target conserved gene regions. PCR amplification was performed in 25 µl reaction mixtures containing: 10 ng genomic DNA, 1X FastStart High Fidelity Reaction buffer (Roche Applied Science, Almere, Netherlands), 1.8 mM $MgCl_2$, 200 µM dNTPs, 400 nM of each forward and reverse primer and 1.25 U FastStart High Fidelity Taq polymerase (Roche Applied Science). The reaction mixtures were incubated in a PTC-200 Peltier thermal cycler (MJ Research, Waltham, MA) using the following parameters: 94 °C for 3 min, 30 cycles at 94 °C for 30 sec, primer specific annealing temperature (see Table 2) for 30 sec, and 72 °C for 1 min followed by a final extension period of 10 min at 72 °C. The genomic DNA of strain Ter331 and no DNA template were used as positive and negative controls, respectively. Visual detection on agarose gel of a band corresponding to a DNA fragment of the expected size indicated the presence of the gene in the analyzed strain.

Results and Discussion

We compared the genomic content of the reference strain *C. fungivorans* Ter331 to the genomic content of 4 test strains (*C. fungivorans* Ter6 and 14, *C. arenae* Ter10 and *C. pratensis* Ter91) representing the three known *Collimonas* species (Figure 1).

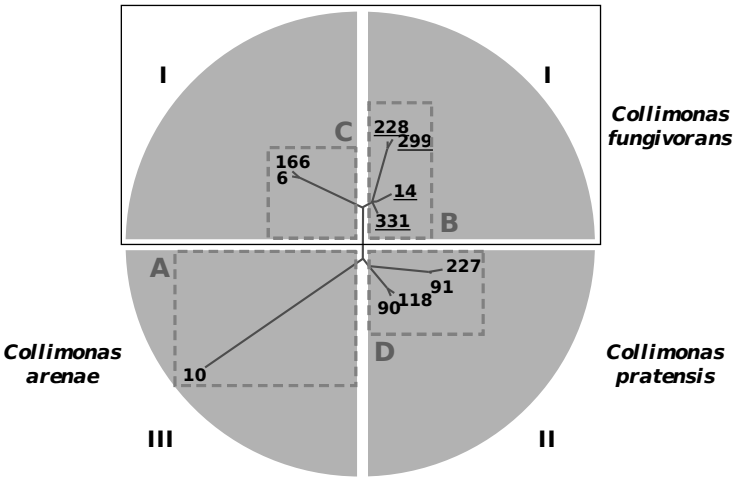

Figure 1. Phylogenetic tree of *Collimonas* strains based on 16S rRNA gene sequences, consistent with the results of BOX-PCR (groups A to D) and electropherovar analysis (roman numbers I to III) (259). The variation of antifungal activity is indicated as follows: the strains reported as underlined are positive for inhibition of *A. niger*; the strains not underlined scored negative.

The presence/absence of a target gene was determined by comparison of its hybridization value with that of the corresponding gene in the reference strain. A target gene was considered as present if its hybridization value was equal or greater than the threshold. To determine the best threshold value, we tested all possible values between the minimum and maximum presence scores (Figure 2) and we chose the value corresponding to the minimum total error rate.

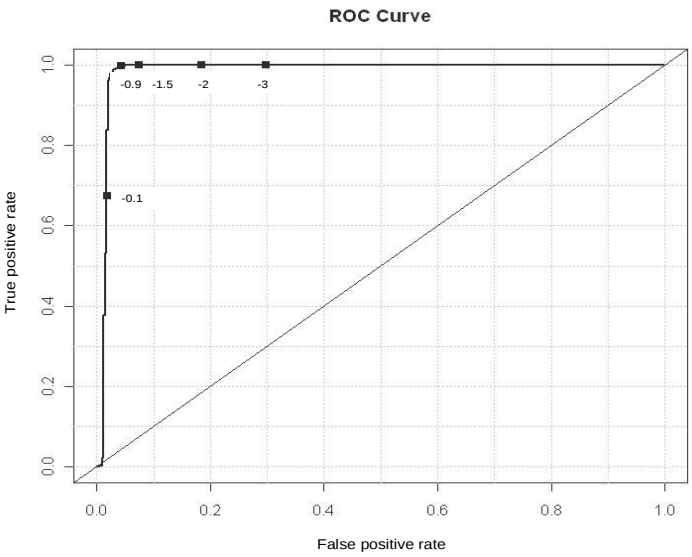

Figure 2. ROC (relative operating characteristic) curve indicating different presence score thresholds used to separate true-positive from false-positive calls. The points on the curve represent true-positive and false-positive rates at various thresholds, including the chosen threshold of -0.9.

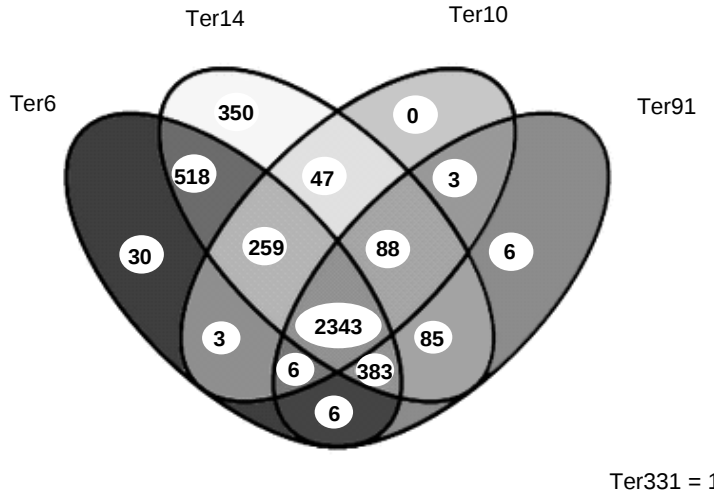

Figure 3. Venn diagram illustrating the number of genes shared by the four test strains (Ter6, Ter10, Ter14 and Ter91) in reference to the Ter331 genome. 2343 Ter331 genes are shared by all four strains. 156 genes, exclusive of Ter331, are reported outside of the diagram.

A subset of 12 genes was selected to confirm the presence/absence based on the array hybridization values by visual detection of amplified fragments on agarose gel (Table 2).

Our results indicate that 2343 genes (54.7%) were conserved in all *Collimonas* strains tested (Figure 3). The percentage of *C. fungivorans* Ter331 genes detected in the other strains ranged from 64.2% in Ter10, to 95.1% in Ter14 (Figure 4). Most of these core genes encode housekeeping functions, including all the genes encoding the ribosomal subunit proteins and genes involved in the synthesis of peptidoglycan, a result validating the array analysis. Included in the core there are also the genes underlying the chitinolytic system of *Collimonas* (47), in agreement with the fact that the ability to lyse chitin, a structural component of the fungal cell wall, is a distinctive trait shared among all *Collimonas* strains (Table 1). Based on the hybridization data we built a phylogenetic tree using hierarchical clustering and average linkage method to report on the relationship among the analyzed strains. The tree is in agreement with the taxonomic topology established using other methods (15, 201), further validating the microarray results (Figure 4).

Genes that underlie traits characterizing *Collimonas* and distinguishing it from other genera can be called *Collimonas*-signature genes. These genes will be part of the genes conserved by all *Collimonas* species and are likely to be important in shaping *Collimonas* specific functional traits and ecological niche (32). The number of candidates for *Collimonas*-signature genes can be reduced by subtracting from the core all the genes that *Collimonas* has in common with non-*Collimonas* species, such as genes involved in the basic cell metabolism. With the support of the Seed environment for comparative genomics (266), we compared the genomic content of *C. fungivorans* Ter331 with the genomic data from two sequenced non-mycophagous bacteria of the family *Oxalobacteraceae*: *Herminiimonas arsenicoxydans* and *Janthinobacterium* sp. Marseille (*Minibacterium massiliensis).* The first was isolated from the activated sludge of an industrial treatment plant contaminated with heavy metals and

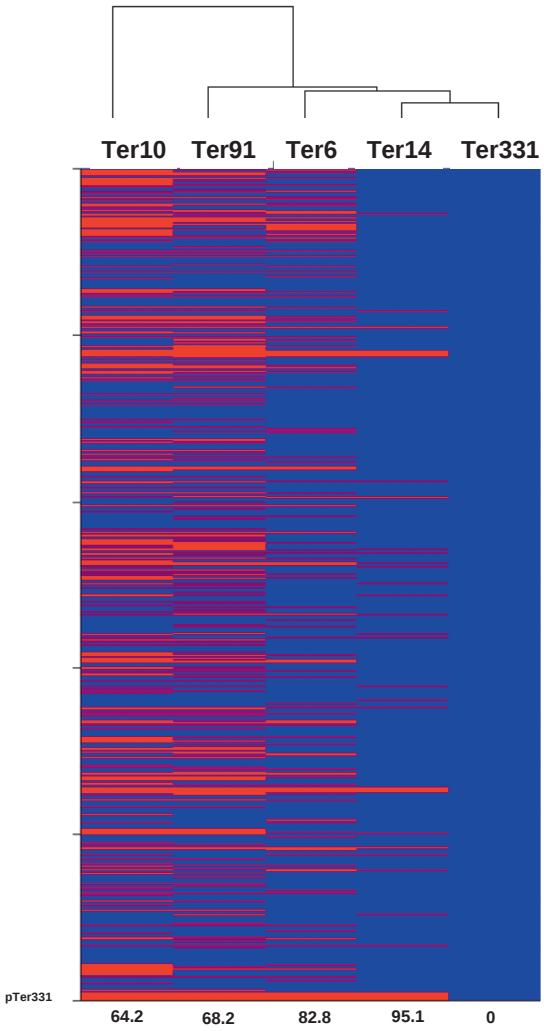

Figure 4. Presence and absence of *C. fungivorans* Ter331 genes in other *Collimonas* strains. The gene status is color coded: blue, present; red, absent. The genes on the chromosome are represented vertically in order of position in *C. fungivorans* Ter331; the genes located on the plasmid are indicated at the bottom of the figure and reported according to their location on plasmid pTer331. The number at the bottom of the figure indicates the percentage of genes present in each strain. On top of the figure the phylogenetic tree is presented which is constructed with the gene hybridization value using hierarchical clustering and average linkage method. The strains are sorted from left to right in accordance with the tree (Ter331, Ter14, Ter6 = *C. fungivorans*; Ter91 = *C. pratensis*; Ter10 = *C. arenae*).

Table 2. PCR analysis of gene presence in *Collimonas* strains. Present, +, absent, -.

Gene	Description	Primer	Sequence	Ann. T (°C)	Ter 6	Ter1 0	Ter1 4	Ter9 1	Ter331
Cf_866	Capsular polysaccharide biosynthesis protein capD	pp331R frag4_f	GTGGCTGCGCCGTTTATTTC						
		pp331Rfrag4_r	CTTGACCCGCGCCATAAATC	51	-	-	+	-	+
Cf_1131	fatty acid desaturase	Colli1131F	GCACGATTGCGGGCACAA						
		Colli1131R	CGCCGAAGCTGAAATCCT	55	+	-	+	-	+
Cf_1135	probable peptide synthetase protein	Colli1135F	GCACTGCTGCTGTCCGTAT						
		Colli1135R	GCTGGTTGTCAGCGGAAT	50	-	-	+	-	+
Cf_1136	Possible mltidrug resistance protein B	1136F3	ATCCCGACTATCTGCACACC						
		1136R3	CGAGCACCGATCCCATCT	51	-	-	-	-	+
Cf_1139	fatty acid desaturase	Colli1139F	CACGCCCTCGCATTCTTC						
		Colli1139R	TCGTGTCCAACAAAGGTCA	50	-	-	+	-	+
Cf_1140	Monooxygenase	Colli1140F	TGTCCACCCACTGGATTTC						
		Colli1140R	AAGAAAAAGCGCAGGTTCAA	50	-	-	+	-	+

Gene	Description	Primer	Sequence	Ann. T (°C)	Ter 6	Ter1 0	Ter1 4	Ter9 1	Ter331
Cf_114 1	3-oxoacyl-acyl carrier protein synthase II	1141F2	GTCAACGCCCATGCTACATCGA						
		1141R2	CGAACCCGAACCCGTTGGA	55	-	-	+	-	+
Cf_167 6	Endochitinase B precursor (EC 3.2.1.14) (CHN-B)	Q304_f	GCCTGCCATCTCCCAAAAC						
		Q304_r	CGTGCCAATCGACCATTCTG	51	-	-	+	-	+
Cf_234 3	GlcNac-binding protein A precursor.	Q652_f	AACCCAGCCTCTGAAATGGA						
		Q652_r	CACTGCCACCTCAAACTGGAA	51	-	-	-	-	+
Cf_266 7	ABC transporter, extracellular-binding protein PH1039 precursor	pp331R frag2_f	GTGGGAAACCGTGCTGATCC						
		pp331Rfrag2_r	TGGCTGTCAATCTGTATCTAACTG	51	+	+	+	+	+
f_3039	chitinase	ChB(591)f	GAT GAC TCA CCT GAA TTA TGC G						
		ChB(5D7E3.0)r	GTATCTGATCTTGTAGTCCAGC	51	+	-	-	+	+
Cf_304 2	beta-N-acetylhexosaminidase	Q591_f	GAACATGGTGAACCCCGAAC						
		Q591_r	TTCCTGGTCGATGCCTATCA	51	+	-	+	-	+

is able to metabolize arsenic (267-268); the second is a water-born bacterium showing heavy metal and antibiotic resistance (269). 637 core genes were unique for *Collimonas* and represent candidate *Collimonas* signature-genes (Appendix Table A4). 40 of these genes were differentially expressed in the confrontation between *C. fungivorans* Ter331 and the fungus *A. niger* (Chapter 2).

Out of the total genes, 1939 genes (45.3%) were absent or diverged too extensively to be detected in at least one of the other *Collimonas* strains and constitute a set of variable genes. Many of the variable genes appeared clustered in genomic regions constituted of genes functioning in the same metabolic pathway and often they showed species-specific pattern of conservation. We analyzed the pattern of gene cluster conservation in more detail (Figure 5). We named the gene clusters with alphabetic letters, consistently with a description presented in a previous work (Chapter 2).

Out of the total genes, 156 were not detected in any strain other than *C. fungivorans* Ter331. The majority of these genes, as expected, were related to the mobile genetic pool, such as the genes encoded on plasmid pTer331 (Chapter 3) and the ORFs belonging to putative prophages, e.g. cluster N (Cf_1041 to Cf_1075), R (Cf_2197 to Cf_2205) and S (Cf_3425 to Cf_3453).

383 genes were detected in all strains except *C. arenae* Ter10. These genes comprise clusters L, T, U and V, encoding four bacterial secretion systems. Cluster L, spanning gene Cf_2276 to Cf_2288, encodes a type II secretion system (T2SS) (143), cluster T (Cf_4382 to Cf_4403) and U (Cf_4415 to Cf_4435) encode two type III secretion systems (T3SS) (270-271) and cluster V (Cf_116 to gene Cf_144) encodes a type VI secretion system (T6SS) (272-273). 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 (274). During the confrontation of *C. fungivorans* Ter331 with the fungus *A. niger* we observed the activation of the T2SS encoded in cluster L (Chapter 2). This result adds to an increasing body of evidence suggesting that secretion systems may play a role in the interaction between bacteria and fungi (68,

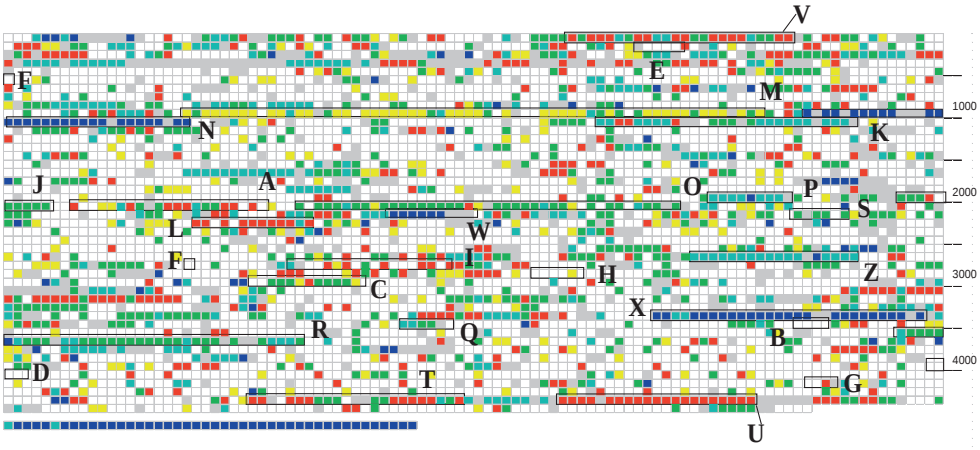

Figure 5. Representation of *C. fungivorans* Ter331 gene conservation. Each gene is represented by a square in the order as it appears on the genome. The color of a square indicates in which strains the gene was detected: blue, genes detected just in *C. fungivorans* Ter331; light blue, genes detected in *C. fungivorans* Ter331 and 14; green, genes detected in all *C. fungivorans* strains (Ter6, 14 and 331); yellow, genes detected in all strains except *C. pratensis* Ter91; red, genes detected in all strains except *C. arenae* Ter10; white, genes detected in all strains; grey, other genes and non calculated. Boxed are gene clusters that are referred to in the text. The bottom row represents genes present on plasmid pTer331.

83-85). In the present study we detected a considerable variability in the secretion systems possessed by *Collimonas* strains: in addition to the four secretion systems mentioned above, we noticed a T1SS (cluster Y) that *C. fungivorans* Ter331 shares only with Ter14; and a second T1SS (cluster W) specific for all *C. fungivorans* strains (see following discussion). Furthermore, considering that the technique used in this study lacks information on the loci not represented on the reference genome, it is possible that additional SSs present in the genome of the tested *Collimonas* strains remain undetected. It has been hypothesized that possession of different SSs might influence host specificity (275) and it is tempting to speculate that the different SSs possessed by the *Collimonas* strains might play a role in their strain and species-specific interaction with fungi. Gene Cf_228 was detected in all strains except *C. arenae* Ter10. This gene encodes a trehalase, an enzyme that catalyses the hydrolysis of trehalose to

two molecules of glucose. This enzyme enables bacteria to grow on trehalose, a compound that many fungi accumulate as a reserve compound and stress protectant (276). The ability to use trehalose is expected to be advantageous for mycophagous bacteria, nevertheless this property is not universal for all *Collimonas* strains. Indeed *C. arenae* Ter10, which does not have gene Cf_228, does not grow on trehalose (Table 1).

259 genes were conserved in all *C. fungivorans* and *C. arenae* strains, but they were not detected in *C. pratensis* Ter91. These genes comprise cluster M, which covers more than 56 kb (Cf_975 to Cf_1036) and encodes chemotaxis-related genes and the flagellar apparatus (277). Accordingly, while most *Collimonas* isolates inoculated into low-strength agar are highly motile, isolate *C. fungivorans* Ter91 showed reduced motility (Table 1). It is likely that the lack of motility has a negative effect on the ability of bacteria to establish a contact with the mycelium and diminish their possibility to obtain nutrients from the fungus. Given that strain *C. fungivorans* Ter91 is not impaired in the mycophagous behavior, it seems plausible that this strain may still be motile, but propels its movement with structures different than flagella. A possibility would be that pili mediate twitching motility in this strain. Twitching movement relies on pilus extension, attachment to a surface and retraction and is not effective in liquid media (278).

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). These genes comprise cluster O (Cf_2087 to Cf_2127), encoding a putative prophage, cluster W (Cf_3651 to Cf_3687), encoding a T1SS (274) and cluster X (Cf_2240 to Cf_2245) encoding genes homologous to the ones belonging to the Syringomycin and Syringopeptin gene cluster of *Pseudomonas syringae* pv. s*yringae* strain B301D (279), two non ribosomal peptides associated with antibacterial and antifungal activity (280). Many of the *C. fungivorans*-specific genes encode functions related to cell wall and membrane biogenesis. Changes in the bacterial cell envelope are related to colony morphological variations in several bacteria (281-283). The three *Collimonas* species differ in colony morphology (Table 1) and possession of *fungivorans*-specific outer-bound elements is

likely to be important in determining the *C. fungivorans* morphology type. Genes of cluster J, also conserved preferentially in *C. fungivorans* species, are homologues to the ones coding for the synthesis of the exopolysaccharides colanic acid in *Escherichia coli* and are likely to play a role in the *C. fungivorans* morphology type as well. Exopolysaccharides aid bacterial adhesion to solid surfaces, including fungal hyphae (70-71) and can also have a role as species-specific signals during cell to cell interactions. The latter was shown during the initiation of symbiosis between the bacterium *Ensifer meliloti* and the plant *Medicago truncatula* when symbiotically active exopolysaccharides function as species-specific signals inducing the plant host to permit rhizobial invasion (284).
Out of the total, 350 *C. fungivorans* Ter331 genes were only shared with *C. fungivorans* Ter14, the strain most closely related to *C. fungivorans* Ter331. This group of genes comprises cluster Y (Cf_2729 to Cf_2745) encoding a T1SS (274), cluster P (Cf_2031 to Cf_2039), involved in the general stress response (Chapter 2), and cluster K (Cf_1127 to Cf_1146) encoding a putative antifungal compound (285). Synthesis of compounds with antifungal activity is likely to constitute an important trait for bacterial mycophagy. Yet, there is evidence suggesting a certain degree of variability in the antifungal activity of different *Collimonas* strains and species towards fungi (Table 1). This variability may be linked to the possession of genes encoding different antifungal compounds and may be important for determining an ecological niche differentiation of the strains.

Antifungal activity of different *Collimonas* strains against *A. niger*

Agar plate confrontation assays involving 20 *Collimonas* strains showed that several *Collimonas* strains are able to inhibit the growth of the fungus *A. niger*, while others do not inhibit the fungus (Figure 1). The five strains used for the comparative genomic study were also included in the test. While *C. fungivorans* Ter331 and Ter14 scored positive, the other three strains showed no antifungal activity. We hypothesize that the genetic determinants of the antifungal activity against *A. niger* should be common to strains *C. fungivorans* Ter331 and Ter14 but absent from the others. We

analyzed the gene clusters differentially expressed in *C. fungivorans* Ter331 during the confrontation with *A. niger* (Chapter 2) and we observed that 2 out of 18 differentially expressed clusters, cluster K, and P, are conserved in strains *C. fungivorans* Ter331 and Ter14, but are mainly undetected in the other strains (Appendix Table A5). Cluster P is involved in the general stress response, while cluster K encodes a putative antifungal compound. As a consequence we consider genes of cluster K as obvious candidates for encoding the determinant essential for the antifungal activity against *A. niger*. The compound encoded by this gene cluster and its range of activity is currently under investigation.

Conclusions

Analyzing the genomic content of test strains with a microarray targeting one reference strain presents some challenges that deserve consideration. Most importantly we have to emphasize that when a gene is not detected it is not possible to distinguish between actual absence of that gene in the test strain and reduced hybridization due to nucleotide polymorphism. In the latter case, the gene may still be functionally equivalent. Nevertheless it seems plausible that the functional significance of a negative detection is high when the absent/divergent genes cluster on the genome and function in the same metabolic pathway.

This study identified a set of genes present in all strains and a set of genes whose presence varied depending on the strain considered, providing a list of candidate genes underlying the common and variable features of *Collimonas* bacteria. Even though mycophagy is a trait characterizing all *Collimonas* strains, several genetic determinants putatively involved in bacterial mycophagy, presented a patchy distribution among the analyzed strains. These determinants include possession of motility, secretion of bioactive compounds and ability to grow on fungal derived substrates. This finding suggests that some genetic determinants putatively underlying mycophagy in *C. fungivorans* Ter331 might be absent in other strains and that other determinants might be present in these strains. An increasing body of evidence indicates that several genes and gene functions contribute

incrementally to the mycophagous behavior and that none of the genetic determinants is strictly necessary for mycophagy. Indeed, attempts to identify mycophagous related genes in *C. fungivorans* Ter331 adopting a loss of function approach were not successful, as the mycophagous activity was not completely suppressed by the loss of any determinant (30, 32, 47). In addition, *Collimonas* bacteria have species and strain specific interactions with fungi (15, 30, 34), reinforcing the possibility that the *Collimonas* strains possess a different set of mycophagous determinants, towards which each fungus shows different sensitivity.

Chapter 5

General discussion

Bacteria and fungi live together in several terrestrial habitats where they have a range of interactions. Knowledge of these interactions is important for a better understanding of terrestrial ecosystem functioning e.g. nutrient cycling, plant nutrition and disease suppression. Nevertheless, the mechanisms and the genetic determinants that underlie bacterial-fungal interactions are still poorly understood. In this PhD study I aimed to improve the understanding of the events and the genes involved in bacterial mycophagy, a trophic interaction in which bacteria feed on living fungi. To achieve this goal I adopted a genomic approach for the study of *Collimonas* bacteria, the first bacteria that were demonstrated to possess mycophagous ability. In this study I addressed the following issues (1) which bacterial genes are up and downregulated when the bacterium is confronted with a fungus and, as a counterpart, which genes are differentially expressed in the fungus as a response to the presence of the bacterium (2) which role does plasmid pTer331 play in the interaction of *C. fungivorans* Ter331 with fungi and in the other phenotypes characterizing *Collimonas* bacteria (3) what is the level of conservation of the genes encoded in the model strain *C. fungivorans* Ter331, especially the genes involved in bacterial-fungal interactions.

Dual expression profiling of the interaction between the bacterium *C. fungivorans* Ter331 and the fungus *Aspergillus niger*

C. fungivorans Ter331 shows an antagonistic interaction towards the fungus *A. niger*. When the two organisms are confronted *in vitro* the fungal growth is inhibited and accumulation of bacterial biomass, in the form of slime, can be observed on the plate.

The relationship between antifungal activity and mycophagy has not been clarified, yet, even though it seems that there are common denominators among the two phenomena.

In order to understand the mechanisms and the genetic determinants involved in the antifungal activity of *C. fungivorans* Ter331 against *A. niger* and to elucidate its relationship with bacterial mycophagy, the expression profile of the two organisms during the confrontation was studied *in vitro*. The study resulted in a list of fungal and bacterial genes differentially expressed as a consequence of the confrontation. The fungus stimulated the expression of several bacterial genes, including genes involved in motility, synthesis of exopolysaccharides and of a putative antimicrobial agent, providing evidence for a role played by these activities in bacterial-fungal interactions. The activation of these mechanisms supports also the existence of an overlap between the determinants of antifungal activity and mycophagy. In addition the presence of the fungus activated genes involved in the consumption of fungal derived substrates, suggesting that production of bacterial slime observed on plate may originate from a conversion of fungal biomass into bacterial biomass. We hypothesize that the presence of a fungus coupled with a scarcity of nutrients stimulated the expression of the determinants of mycophagy in *C. fungivorans* Ter331. The fungus responded to the presence of the bacterium by activating genes involved in metabolism of lipid and cell wall. This finding corresponds well with the observation of hyphal deformations such as swelling and hyperbranching. In addition to the medium acidification, which was present also on the control plate, the presence of the bacterium stimulated the expression of genes involved in secondary metabolites, suggesting a possible self-defense reaction of the fungus to the presence of the bacterium. The analysis of differentially expressed genes during this confrontation indicated that both organisms presented signs of distress: the fungus showed upregulation of genes involved in sporulation and endoplasmic reticulum stress and the bacterium showed downregulation of genes encoding ribosomal proteins and upregulation of mobile genetic elements, furthermore both organisms showed sign of nitrogen limitation. Overall, our results indicate that the

interaction between *Collimonas* and *Aspergillus* is characterized by a complex interplay between trophism, antibiosis, and competition for nutrients. The choice of *A. niger* as fungal partner for this study was determined by the fact that this fungus shows a marked inhibition in the presence of *Collimonas*, coupled with a visible accumulation of bacterial biomass. In addition, *A. niger* is well known thanks to its economical and medical relevance and tools such as the genomic sequence and an expression microarray are available for this fungus. This experiment seems to indicate that the fungal reaction to the presence of the bacterium blocks the expression of the full mycophagous potential of *Collimonas*. This might explain the failure to detect an upregulation of the chitinolytic genes, which are expected to be activated when chitin originating from the fungal cell wall is available to the bacterium. Future experiments confronting *Collimonas* with other fungal species will expand our understanding of the genetic determinants of mycophagy.

Sequence, evolution and function of plasmid pTer331

Plasmid pTer331 was isolated from its natural host *C. fungivorans* Ter331. Sequencing of the plasmid revealed 91% identity with the sequence of plasmid pIPO2 (196). I compared the sequences of the two plasmids and found that nucleotide substitution and insertion/deletions events were the mechanisms of sequence divergence since pTer331 and PIPO2 split from their common ancestor. Sequence annotation of pTer331 yielded 44 putative genes, mostly involved in replication, partitioning and transfer of the plasmid itself, suggesting that pTer331 is a cryptic plasmid that does not confer any evident phenotypic trait to its host. The failure to detect pTer331 in strains other than *C. fungivorans* Ter331 indicated that the plasmid does not play a role in traits that are common to all *Collimonas* strains, including antifungal activity, mycophagy, weathering and chitinolysis. Afterwards I tested experimentally the hypothesis that pTer331 could confer a selective advantage for the colonization of the plant rhizosphere. This hypothesis was assessed by obtaining a plasmid-free strain and comparing the performance of this strain and the wild type in colonizing the rhizosphere of tomato

plants. I found that the plasmid had no significant contribution in the rhizosphere competence of *C. fungivorans* Ter331. Thus pTer331 is likely to be a selfish genetic element, maintained in the bacterial host thanks to its ability to self replicate and spread, rather than to the positive effect on the host fitness. Nevertheless the presence on the plasmid of a hot-spot for insertion of additional genetic modules, suggest that this plasmid might incidentally acquire genes useful for the host survival and enhance its survival and spread in the bacterial population.

Recently pTer331 has been proposed to be a member of a new family of broad host range plasmids named "PromA" (95). Besides pIPO2 and pTer331, the family includes plasmid pMOL 98 (199), pSB102 (197) and pMRAD02 (287). These five plasmids were isolated from either rhizosphere or soil in distinct locations in The Netherlands, Germany and Japan. The five plasmids show extensive conservation of the plasmid backbone constituted by the genes necessary for plasmid self replication, maintenance and transfer. Van der Auwera and colleagues compared the accessory genes of these five plasmids and found that natural transposons and transposable elements engineered into the plasmids are inserted in the *parA* locus, confirming the presence on plasmid pTer331 of a hot-spot for the insertion of transposable elements (95). The existence of pTer33-related plasmids carrying accessory genes beneficial to their host, supports the hypothesis that this selfish element might constitute a minimized form of PromA plasmids, which, in certain instances, may acquire genes useful for its host and favour their dissemination in the bacterial population. During the confrontation of *C. fungivorans* Ter331 and the fungus *A. niger* (Chapter 3), when the bacterium manifested signs of distress, the upregulation of the plasmid genes was observed. This finding also hints at the possibility that the plasmid plays a role in facilitating the acquisition of new genes useful for bacterial survival in an unfavorable environment.

Comparative genomic study of *Collimonas* strains

All *Collimonas* bacteria share characteristics such as the ability to lyse chitin and the ability to feed on hyphae of living fungi, but they differ with

respect to the possession of several traits such as colony morphology and antifungal activity. I investigated the variability in the genomic content of five strains, representatives of the three species formally recognized in the genus *Collimonas*: *C. fungivorans*, *C. pratensis* and *C. arenae*. With the aid of microarray technology I compared the genomic content of the reference strain *C. fungivorans* Ter331 to the genomic content of four tested strains. The genes encoded in the reference genome were divided into two categories: the genes conserved in all strains and the genes conserved in some but not all strains. I expected to find among the genes conserved in all strains the ones determining characteristics common to all *Collimonas* strains and to find among the variable genes the ones responsible for the traits differentiating the *Collimonas* strains from one another. This hypothesis was partially true, indeed genes such as the ones constituting the chitinolytic system were conserved in all strains, in agreement with the fact that chitinolysis is a property characterizing all collimonads. Nevertheless I found that several genes underlying putative determinants of bacterial mycophagy, such as motility, ability to grow on trehalose and secretion systems, were not conserved in all strains. More detailed studies are needed to give evidence that these traits are indeed important for mycophagous growth, e.g. by comparing mycophagous growth yields of different strains or of mutants defective in one of these traits. Given the fact that all *Collimonas* strains are mycophagous, the variability observed in the possession of putative mycophagous determinants is in line with the hypothesis that bacterial mycophagy is a complex phenotypic trait that is built on the possession of several determinants with additive effects, none of which is strictly necessary for the mycophagous phenotype. This hypothesis is also supported by the fact that attempts to trace individual mycophagous genes and gene functions via a loss of function approach were not successful (32). Possession of a variable set of mycophagous determinants may, in addition, explain the variability observed in the interactions between *Collimonas* strains and fungi (30, 34), possibly indicating that different fungal species have variable susceptibility towards various mycophagous determinants.

Perspectives for future study of *Collimonas* bacteria

This thesis has addressed important issues regarding the genetic determinants and the mechanisms involved in the interaction between bacteria and fungi. The study of the confrontation between *C. fungivorans* Ter331 and the fungus *A. niger* yielded a set of genes differentially expressed during this bacterial-fungal interaction. This study confirmed the activation of bacterial mechanisms such as motility and chemotaxis, production of antifungals and degradation of fungal derived substrates and indicated that the fungus reacted with mechanisms of cell self defense and secondary metabolites production. The different sensitivity of the fungus to the determinants activated by the bacterium and the different sensitivity of the bacterium to the strategies of fungal response might determine the outcome of bacterial-fungal interactions and might contribute to the species-specific interaction observed between *Collimonas* strains and fungi, possibly reflecting a niche differentiation among *Collimonas* strains. A comparative genomic study of *Collimonas* strains detected that several potential mycophagous determinants are not present in all strains, suggesting that additional, as yet undetected determinants might be present in different *Collimonas* strains. Exploiting the existence of several mycophagous strains, it is possible to further explore the range of existing mycophagous determinants, as well as the relationship between the set of mycophagous determinants possessed by a bacterium and its interaction with specific fungi. Elucidating the relationship between mycophagous determinants and the susceptibility of target pathogens will open the way for potential application of *Collimonas* strains as biocontrol agents of plant pathogenic fungi.

The availability of non-mycophagous bacterial genera closely related to *Collimonas*, such as *Herbaspirillum* and *Janthinobacterium*, can be exploited for comparative genomic studies aimed at understanding the evolutionary adaptations that led to the mycophagous behavior. The existence of bacterial selected fungal communities and fungal selected bacterial communities supports the existence of a reciprocal influence of bacterial and fungal communities. Future studies on the interactions between

Collimonas and fungi and potential niche differentiation of different *Collimonas* strains will help clarifying the role played by fungi on the evolution of mycophagous traits and the mechanism of bacterial and fungal coevolution. The evolution of mycophagy as a survival strategy should be studied in the context of the relationship of collimonads with other soil microorganisms. Growth of soil microbes is mostly carbon-limited. This will favour the evolution of strategies to exploit new carbon resources (in this case fungal carbon). Hence, collimonad mycophagy may have evolved and be activated to obtain carbon in a carbon-limited environment. Experiments investigating the competitive ability of *Collimonas* for non-fungal substrates will clarify the role played by competition from other soil bacteria in the evolution and activation of *Collimonas* mycophagy.

Currently, it is not clear which environmental conditions determine the outcome of the bacterial-fungal interaction. Future studies to assess the effect of different abiotic conditions applied in the *in vitro* studies will aid in the identification of the conditions that stimulate the activation of the mycophagous behavior. Mycophagy might represent one of the possible trophic strategies of *Collimonas* bacteria. Whether or not *Collimonas* bacteria obtain nutrients from a fungus might depend on the availability of other more accessible food sources and on the proximity of a suitable fungal match. More easily accessible food sources might be preferred when they are available and mycophagy might represent an additional resource in nutrient poor environment.

Bibliography

1. de Boer W, Folman LB, Summerbell RC, & Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *Fems Microbiology Reviews* 29(4):795-811.
2. Compant S, Duffy B, Nowak J, Clement C, & Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* 71(9):4951-4959.
3. Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* 52(Roots Special Issue):487-511.
4. Frey-Klett P, Garbaye J, & Tarkka M (2007) The mycorrhiza helper bacteria revisited. *New Phytol.* 176(1):22-36.
5. Shirtliff ME, Peters BM, & Jabra-Rizk MA (2009) Cross-kingdom interactions: Candida albicans and bacteria. *Fems Microbiology Letters* 299(1):1-8.
6. Straight PD & Kolter R (2009) Interspecies Chemical Communication in Bacterial Development. *Annual Review of Microbiology* 63:99-118.
7. Wargo MJ & Hogan DA (2006) Fungal-bacterial interactions: a mixed bag of mingling microbes. *Current Opinion in Microbiology* 9:359-364.
8. Chamilos G, Lewis RE, & Kontoyiannis DP (2007) Multidrug-resistant endosymbiotic bacteria account for the emergence of zygomycosis: A hypothesis. *Fungal Genetics and Biology* 44(2):88-92.
9. Lackner G, Partida-Martinez LP, & Hertweck C (2009) Endofungal bacteria as producers of mycotoxins. *Trends in Microbiology* 17(12):570-576.
10. Partida-Martinez LP & Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature* 437(7060):884-888.

11. Artursson V, Finlay RD, & Jansson JK (2005) Combined bromodeoxyuridine immunocapture and terminal-restriction fragment length polymorphism analysis highlights differences in the active soil bacterial metagenome due to *Glomus mosseae* inoculation or plant species. *Environmental Microbiology* 7(12):1952-1966.
12. Christensen H & Jakobsen I (1993) Reduction of bacterial-growth by a vesicular-arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L.). *Biology and Fertility of Soils* 15(4):253-258.
13. de Boer W, de Ridder-Duine AS, Klein Gunnewiek PJA, Smant W, & van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. *Soil Biology & Biochemistry* 40(6):1542-1544 .
14. Folman LB, Klein Gunnewiek PJA, Boddy L, & de Boer W (2008) Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil. *Fems Microbiology Ecology* 63(2):181-191 .
15. Höppener-Ogawa S, Leveau JHJ, Hundscheid MPJ, van Veen JA, & de Boer W (2009) Impact of *Collimonas* bacteria on community composition of soil fungi. *Environmental Microbiology* 11(6):1444-1452.
16. Buee M, de Boer W, Martin F, van Overbeek L, & Jurkevitch E (2009) The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil* 321(1-2):189-212 .
17. Treonis AM, *et al.* (2004) Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. *Soil Biology & Biochemistry* 36(3):533-537 .
18. Butler JL, Williams MA, Bottomley PJ, & Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. *Applied and Environmental Microbiology* 69:6793-6800.

19. de Boer W, de Ridder-Duine AS, Gunnewiek P, Smant W, & van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. *Soil Biology & Biochemistry* 40(6):1542-1544 .
20. Céspedes R, González B, & Vicuña R (1997) Characterization of a bacterial consortium degrading the lignin model compound vanillyl-beta-D-glucopyranoside. *Journal of Basic Microbiology* 37(3):175-180.
21. Vicuña R, González B, Seelenfreund D, Ruttimann C, & Salas L (1993) Ability of natural bacterial isolates to metabolize high and low-molecular-weight lignin-derived molecules. *J. Biotechnol.* 30 (1):9-13 .
22. Murray AC & Woodward S (2003) In vitro interactions between bacteria isolated from Sitka spruce stumps and *Heterobasidion annosum*. *Forest Pathology* 33(1):53-67 .
23. Hildebrandt U, Ouziad F, Marner FJ, & Bothe H (2006) The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. *Fems Microbiology Letters* 254(2):258-267.
24. Barron GL (1988) Microcolonies of bacteria as a nutrient source for lignicolous and other fungi. *Canadian Journal of Botany-Revue Canadienne de Botanique* 66(12):2505-2510 .
25. Fermor TR, Wood DA, Lincoln SP, & Fenlon JS (1991) Bacteriolysis by *Agaricus bisporus*. *Journal of General Microbiology* 137:15-22 .
26. Denef K, *et al.* (2007) Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. *Biogeosciences* 4(5):769-779.
27. Rillig MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters* 7(8):740-754 .
28. de Boer W, *et al.* (2004) *Collimonas fungivorans* gen. nov., sp nov., a chitinolytic soil bacterium with the ability to grow on living fungal

hyphae. *International Journal of Systematic and Evolutionary Microbiology* 54:857-864.

29. Leveau JHJ & Preston GM (2008) Bacterial mycophagy: definition and diagnosis of a unique bacterial-fungal interaction. *New Phytologist* 177(4):859-876.
30. de Boer W, Klein Gunnewiek PJA, Kowalchuk GA, & van Veen JA (2001) Growth of chitinolytic dune soil beta-subclass Proteobacteria in response to invading fungal hyphae. *Applied and Environmental Microbiology* 67(8):3358-3362.
31. Höppener-Ogawa S, Leveau JHJ, van Veen JA, & de Boer W (2009) Mycophagous growth of *Collimonas* bacteria in natural soils, impact on fungal biomass turnover and interactions with mycophagous *Trichoderma* fungi. *Isme Journal* 3(2):190-198.
32. Leveau JHJ, Uroz S, & de Boer W (2009) The bacterial genus *Collimonas*: mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments. *Environmental Microbiology* 12:281-292.
33. Höppener-Ogawa S, *et al.* (2008) *Collimonas arenae* sp nov. and *Collimonas pratensis* sp nov., isolated from (semi-)natural grassland soils. *International Journal of Systematic and Evolutionary Microbiology* 58:414-419.
34. de Boer W, *et al.* (1998) Anti-fungal properties of chitinolytic dune soil bacteria. *Soil Biology & Biochemistry* 30(2):193-203.
35. Höppener-Ogawa S, Leveau JHJ, Smant W, van Veen JA, & de Boer W (2007) Specific detection and real-time PCR quantification of potentially mycophagous bacteria belonging to the genus *Collimonas* in different soil ecosystems. *Applied and Environmental Microbiology* 73(13):4191-4197.
36. Sogin ML, *et al.* (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America* 103:12115-12120.

37. Opelt K & Berg G (2004) Diversity and antagonistic potential of bacteria associated with bryophytes from nutrient-poor habitats of the Baltic Sea coast. *Applied and Environmental Microbiology* 70(11):6569-6579.
38. Hakvag S, *et al.* (2009) Violacein-Producing *Collimonas* sp from the Sea Surface Microlayer of Costal Waters in Trondelag, Norway. *Marine Drugs* 7(4):576-588.
39. Höppener-Ogawa S, Leveau JHJ, Smant W, van Veen JA, & de Boer W (2007) Specific detection and real-time PCR quantification of potentially mycophagous bacteria belonging to the genus *Collimonas* in different soil ecosystems. *Applied and Environmental Microbiology* 73(13):4191-4197.
40. Benítez T, Rincón AM, Limón MC, & Codón AC (2004) Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* 7(4):249-260 .
41. Harman GE, Howell CR, Viterbo A, Chet I, & Lorito M (2004) *Trichoderma* species - Opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology* 2(1):43-56.
42. Kamilova F, Leveau JHJ, & Lugtenberg B (2007) *Collimonas fungivorans*, an unpredicted in vitro but efficient in vivo biocontrol agent for the suppression of tomato foot and root rot. *Environmental Microbiology* 9(6):1597-1603.
43. Aspray TJ, Hansen SK, & Burns RG (2005) A soil-based microbial biofilm exposed to 2,4-D: bacterial community development and establishment of conjugative plasmid pJP4. *Fems Microbiology Ecology* 54(2):317-327.
44. Mahmood S, Paton GI, & Prosser JI (2005) Cultivation-independent in situ molecular analysis of bacteria involved in degradation of pentachlorophenol in soil. *Environmental Microbiology* 7(9):1349-1360.
45. Wilson MS, Herrick JB, Jeon CO, Hinman DE, & Madsen EL (2003) Horizontal transfer of phnAc dioxygenase genes within one of two phenotypically and genotypically distinctive naphthalene-

degrading guilds from adjacent soil environments. *Applied and Environmental Microbiology* 69(4):2172-2181.

46. Uroz S, *et al.* (2009) Efficient mineral weathering is a distinctive functional trait of the bacterial genus *Collimonas*. *Soil Biology & Biochemistry* 41(10):2178-2186.
47. Fritsche K, *et al.* (2008) Identification and characterization of genes underlying chitinolysis in *Collimonas fungivorans* Ter331. *Fems Microbiology Ecology* 66(1):123-135.
48. Adesina MF, Lembke A, Costa R, Speksnijder A, & Smalla K (2007) Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: site-dependent composition and diversity revealed. *Soil Biology & Biochemistry* 39:2818-2828.
49. Deveau A, *et al.* (2007) The mycorrhiza helper *Pseudomonas fluorescens* BBc6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *New Phytologist* 175:743-755.
50. Pivato B, *et al.* (2009) Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza* 19(2):81-90 .
51. Hoppener-Ogawa S, Leveau JHJ, Hundscheid MPJ, van Veen JA, & de Boer W (2009) Impact of *Collimonas* bacteria on community composition of soil fungi. *Environmental Microbiology* 11(6):1444-1452.
52. Höppener-Ogawa S, Leveau JHJ, van Veen JA, & De Boer W (2009) Mycophagous growth of *Collimonas* bacteria in natural soils, impact on fungal biomass turnover and interactions with mycophagous *Trichoderma* fungi. *Isme Journal* 3(2):190-198.
53. Leveau JHJ *et al.* Manuscript in preparation.
54. Leveau JHJ, Gerards S, de Boer W, & van Veen JA (2004) Phylogeny-function analysis of (meta)genomic libraries: screening for expression of ribosomal RNA genes by large-insert library

fluorescent in situ hybridization (LIL-FISH). *Environmental Microbiology* 6(9):990-998.

55. Leveau JHJ, Gerards S, Fritsche K, Zondag G, & van Veen JA (2006) Genomic flank-sequencing of plasposon insertion sites for rapid identification of functional genes. *Journal of Microbiolical Methods* 66(2):276-285 .
56. Soler-Rivas C, Jolivet S, Arpin N, Olivier JM, & Wichers HJ (1999) Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. *Fems Microbiology Reviews* 23(5):591-614.
57. Barret M, *et al.* (2009) The plant pathogenic fungus *Gaeumannomyces graminis* var. tritici improves bacterial growth and triggers early gene regulations in the biocontrol strain *Pseudomonas fluorescens* Pf29Arp. *New Phytologist* 181(2):435 -447.
58. Schrey SD, Schellhammer M, Ecke M, Hampp R, & Tarkka MT (2005) Mycorrhiza helper bacterium *Streptomyces* AcH 505 induces differential gene expression in the ectomycorrhizal fungus *Amanita muscaria*. *New Phytologist* 168(1):205-216.
59. Lehr NA, Adomas A, Asiegbu FO, Hampp R, & Tarkka MT (2009) WS-5995 B, an antifungal agent inducing differential gene expression in the conifer pathogen *Heterobasidion annosum* but not in *Heterobasidion abietinum*. *Applied Microbiology and Biotechnology* 85(2):347-358 .
60. Roesti D, *et al.* (2005) Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. *Applied and Environmental Microbiology* 71(11):6673-6679.
61. Bonfante P & Anca IA (2009) Plants, Mycorrhizal Fungi, and Bacteria: A Network of Interactions. *Annual Review of Microbiology* 63:363-383.
62. Artursson V, Finlay RD, & Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental Microbiology* 8(1):1-10.

63. Bianciotto V, *et al.* (1996) An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Applied and Environmental Microbiology* 62(8):3005-3010.
64. de Weert S, Kuiper I, Lagendijk EL, Lamers GEM, & Lugtenberg BJJ (2004) Role of chemotaxis toward fusaric acid in colonization of hyphae of *Fusarium oxysporum* f. sp *radicis-lycopersici* by *Pseudomonas fluorescens* WCS365. *Molecular Plant-Microbe Interactions* 17(11):1185-1191.
65. Kamilova F, Lamers G, & Lugtenberg B (2008) Biocontrol strain *Pseudomonas fluorescens* WCS365 inhibits germination of *Fusarium oxysporum* spores in tomato root exudate as well as subsequent formation of new spores. *Environmental Microbiology* 10(9):2455-2461.
66. Park SY, *et al.* (2008) Citrinin, a mycotoxin from *Penicillium citrinum*, plays a role in inducing motility of *Paenibacillus polymyxa*. *Fems Microbiology Ecology* 65(2):229-237.
67. Warmink JA & van Elsas JD (2009) Migratory Response of Soil Bacteria to *Lyophyllum* sp Strain Karsten in Soil Microcosms. *Applied and Environmental Microbiology* 75(9):2820-2830.
68. Nazir R, Warmink JA, Boersma H, & van Elsas JD (2010) Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *Fems Microbiology Ecology* 71(2):169-185.
69. Yang CH, Menge JA, & Cooksey DA (1994) Mutations affecting hyphal colonization and pyoverdine production in Pseudomonads antagonistic toward *Phytophthora parasitica*. *Applied and Environmental Microbiology* 60(2):473-481.
70. Bianciotto V, Andreotti S, Balestrini R, Bonfante P, & Perotto S (2001) Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscular mycorrhizal structures. *European Journal of Histochemistry* 45(1):39-49.

71. Broek AV & Vanderleyden J (1995) The role of bacterial motility, chemotaxis, and attachment in bacteria plant interactions. *Molecular Plant-Microbe Interactions* 8(6):800-810.
72. Rainey PB (1991) Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects attachment to *Agaricus bisporus* mycelium. *Journal of General Microbiology* 137:2769-2779 .
73. Russo A, *et al.* (2003) Interaction between gfp-tagged *Pseudomonas tolaasii* P12 and *Pleurotus eryngii*. *Microbiological Research* 158(3):265-270.
74. Bolwerk A & Lugtenberg BJJ (2006) *Visualization of interactions of microbial biocontrol agents and phytopathogenic fungus* Fusarium oxysporum *f. sp* radicis-lycopersici *on tomato roots* (Springer, Dordrecht) pp 217-231.
75. Schroeckh V, *et al.* (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences of the United States of America* 106(34):14558-14563.
76. Ikeda R & Sawamura K (2008) Bacterial and H_2O_2 stress-induced apoptosis-like events in *Cryptococcus neoformans*. *Research in Microbiology* 159(9-10):628-634 .
77. Raaijmakers JM, Vlami M, & de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81(1-4):537-547.
78. Li SJ, Calvo AM, Yuen GY, Du LC, & Harris SD (2009) Induction of Cell Wall Thickening by the Antifungal Compound Dihydromaltophilin Disrupts Fungal Growth and is Mediated by Sphingolipid Biosynthesis. *Journal of Eukaryotic Microbiology* 56(2):182-187.
79. Li SJ, Du LC, Yuen G, & Harris SD (2006) Distinct ceramide synthases regulate polarized growth in the filamentous fungus *Aspergillus nidulans*. *Molecular Biology of the Cell* 17(3):1218-1227.

80. Bhattacharya D, Nagpure A, & Gupta RK (2007) Bacterial chitinases: properties and potential. *Critical Reviews in Biotechnology* 27(1):21-28.
81. Preston GM (2007) Metropolitan microbes: Type III secretion in multihost symbionts. *Cell Host & Microbe* 2:291-294.
82. Clarke M (2010) Recent insights into host-pathogen interactions from *Dictyostelium*. *Cellular Microbiology* 12(3):283-291.
83. Warmink JA & van Elsas JD (2008) Selection of bacterial populations in the mycosphere of *Laccaria proxima*: is type III secretion involved? *Isme Journal* 2(8):887-900.
84. Rezzonico F, Binder C, Defago G, & Moenne-Loccoz Y (2005) The type III secretion system of biocontrol *Pseudomonas fluorescens* KD targets the phytopathogenic chromista *Pythium ultimum* and promotes cucumber protection. *Molecular Plant-Microbe Interactions* 18(9):991-1001.
85. Chowdhury PR & Heinemann JA (2006) The general secretory pathway of *Burkholderia gladioli* pv. agaricicola BG164R is necessary for for cavity disease in white button mushrooms. *Applied and Environmental Microbiology* 72(5):3558-3565.
86. Toljander JF, Lindahl BD, Paul LR, Elfstrand M, & Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *Fems Microbiology Ecology* 61(2):295-304 .
87. Danell E, Alstrom S, & Ternstrom A (1993) *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycological Research* 97:1148-1152 .
88. Frey P, FreyKlett P, Garbaye J, Berge O, & Heulin T (1997) Metabolic and genotypic fingerprinting of fluorescent pseudomonads associated with the Douglas fir *Laccaria bicolor* mycorrhizosphere. *Applied and Environmental Microbiology* 63(5):1852-1860.

89. Frost LS, Leplae R, Summers AO, & Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. *Nature Reviews Microbiology* 3(9):722-732.
90. Smalla K, Osborn AM, & Wellington EMH (2000) Isolation and characterization of plasmids from bacteria. *The Horizontal Gene Pool, Bacterial Plasmids and Gene Spread* ed Thomas CM (Harwood Academic Publishers, Amsterdam, The Netherlands), pp 207–248.
91. Szpirer C, Top E, Couturier M, & Mergeay M (1999) Retrotransfer or gene capture: a feature of conjugative plasmids, with ecological and evolutionary significance. *Microbiology-(UK)* 145:3321-3329 .
92. Ronchel MC, Ramos-Díaz MA, & Ramos JL (2000) Retrotransfer of DNA in the rhizosphere. *Environmental Microbiology* 2(3):319 -323.
93. Young JPW, *et al.* (2006) The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. *Genome Biology* 7(4).
94. Okinaka RT, *et al.* (1999) Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. *Journal of Bacteriology* 181(20):6509-6515.
95. Van der Auwera GA, *et al.* (2009) Plasmids captured in *C. metallidurans* CH34: defining the PromA family of broad-host-range plasmids. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 96(2):193-204.
96. Kobayashi DY & Crouch JA (2009) Bacterial/Fungal Interactions: From Pathogens to Mutualistic Endosymbionts. *Annual Review of Phytopathology* 47:63-82.
97. Duffy B, Schouten A, & Raaijmakers JM (2003) Pathogen self-defense: mechanisms to counteract microbial antagonism. *Annual Review of Phytopathology* 41:501-538.
98. Rainey PB, Cole ALJ, Fermor TR, & Wood DA (1990) A model system for examining involvement of bacteria in basidiome initiation of *Agaricus bisporus*. *Mycological Research* 94:191-195.

99. Xavier LJC & Germida JJ (2003) Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. *Soil Biology & Biochemistry* 35(3):471-478.
100. Bell AA & Wheeler MH (1986) Biosynthesis and Functions of Fungal Melanins. *Annual Review of Phytopathology* 24:411-451.
101. Butler MJ, Gardiner RB, & Day AW (2005) Degradation of melanin or inhibition of its synthesis: are these a significant approach as a biological control of phytopathogenic fungi? *Biological Control* 32(2):326-336.
102. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, & Moenne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321(1-2):341-361.
103. Jamieson DJ (1998) Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast* 14(16):1511-1527.
104. Tarkka MT, Sarniguet A, & Frey-Klett P (2009) Inter-kingdom encounters: recent advances in molecular bacterium-fungus interactions. *Current Genetics* 55(3):233-243.
105. de Boer W, Gunnewiek P, Kowalchuk GA, & van Veen JA (2001) Growth of chitinolytic dune soil beta-subclass Proteobacteria in response to invading fungal hyphae. *Applied and Environmental Microbiology* 67(8):3358-3362.
106. Pel HJ, *et al.* (2007) Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nature Biotechnology* 25(2):221-231.
107. van Rij ET, Girard G, Lugtenberg BJJ, & Bloemberg GV (2005) Influence of fusaric acid on phenazine-1-carboxamide synthesis and gene expression of *Pseudomonas chlororaphis* strain PCL1391. *Microbiology-Sgm* 151:2805-2814.
108. Maligoy M, Mercade M, Cocaign-Bousquet M, & Loubiere P (2008) Transcriptome analysis of *Lactococcus lactis* in coculture with *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology* 74(2):485-494.

109. Barnett MJ, Tolman CJ, Fisher RF, & Long SR (2004) A dual-genome Symbiosis Chip for coordinate study of signal exchange and development in a prokaryote-host interaction. *Proceedings of the National Academy of Sciences of the United States of America* 101(47):16636-16641.
110. Tailleux L, *et al.* (2008) Probing Host Pathogen Cross-Talk by Transcriptional Profiling of Both *Mycobacterium tuberculosis* and Infected Human Dendritic Cells and Macrophages. *Plos One* 3(1):e1403.
111. van der Veen D, Oliveira JM, van den Berg WAM, & de Graaff LH (2009) Analysis of Variance Components Reveals the Contribution of Sample Processing to Transcript Variation. *Applied and Environmental Microbiology* 75(8):2414-2422.
112. Affymetrix (2004) GeneChip Expression Analysis, technical manual 701021 Rev. 5. http://jaxservices.jax.org/Affymetrix_Gene_expression_manual_430.pdf
113. Bolstad BM, Irizarry RA, Astrand M, & Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19(2):185-193.
114. Irizarry RA, *et al.* (2003) Summaries of affymetrix GeneChip probe level data. *Nucleic Acids Research* 31(4):e15.
115. Irizarry RA, *et al.* (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4(2):249-264.
116. Gautier L, Cope L, Bolstad BM, & Irizarry RA (2004) affy - analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20(3):307-315.
117. Smyth GK (2004) Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray. *Statistical Applications in Genetics and Molecular Biology* 3(1):Article 3.

118. Applied Biosystems (2008) Guide to Performing Relative Quantitation of Gene Expression Using Real-Time Quantitative PCR. Part Number 4371095 Rev B.
119. Anantharam V, Allison MJ, & Maloney PC (1989) Oxalate:formate exchange. The basis for energy coupling in *Oxalobacter*. *Journal of Biological Chemistry* 264(13):7244-7250 .
120. Dimroth P & Schink B (1998) Energy conservation in the decarboxylation of dicarboxylic acids by fermenting bacteria. *Archives of Microbiology* 170(2):69-77.
121. Oh JI & Bowien B (1998) Structural analysis of the fds operon encoding the NAD(+)-linked formate dehydrogenase of *Ralstonia eutropha*. *Journal of Biological Chemistry* 273(41):26349-26360.
122. Svedruzic D, *et al.* (2005) The enzymes of oxalate metabolism: unexpected structures and mechanisms. *Archives of Biochemistry and Biophysics* 433(1):176-192.
123. Sahin N (2003) Oxalotrophic bacteria. *Research in Microbiology* 154(6):399-407.
124. Quayle JR (1961) Metabolism of C_1 compounds in autotrophic and heterotrophic microorganisms. *Annual Review of Microbiology* 15:119-152.
125. Cornick NA & Allison MJ (1996) Assimilation of oxalate, acetate, and CO_2 by *Oxalobacter formigenes*. *Canadian Journal of Microbiology* 42(11):1081-1086.
126. Dawson KA, Allison MJ, & Hartman PA (1980) Isolation and some characteristics of anaerobic oxalate-degrading bacteria from the rumen. *Applied and Environmental Microbiology* 40(4):833-839.
127. Wyborn NR, Mills J, Williams SG, & Jones CW (1996) Molecular characterisation of formamidase from *Methylophilus methylotrophus*. *European Journal of Biochemistry* 240(2):314-322.
128. Skouloubris S, Labigne A, & De Reuse H (2001) The AmiE aliphatic amidase and AmiF formamidase of *Helicobacter pylori*: natural evolution of two enzyme paralogues. *Molecular Microbiology* 40(3):596-609.

129. Wu QT & Stewart V (1998) NasFED proteins mediate assimilatory nitrate and nitrite transport in *Klebsiella oxytoca* (*pneumoniae*) M5al. *Journal of Bacteriology* 180(5):1311-1322.
130. Mantsala P & Zalkin H (1976) Active subunits of *Escherichia coli* glutamate synthase. *Journal of Bacteriology* 126(1):539-541.
131. Young GM, Amid D, & Miller VL (1996) A bifunctional urease enhances survival of pathogenic *Yersinia enterocolitica* and *Morganella morganii* at low pH. *Journal of Bacteriology* 178(22):6487-6495.
132. Thomas G, Coutts G, & Merrick M (2000) The gInKamtB operon - a conserved gene pair in prokaryotes. *Trends in Genetics* 16(1):11-14.
133. Khademi S & Stroud RM (2006) The Amt/MEP/Rh family: structure of AmtB and the mechanism of ammonia gas conduction. *Physiology* 21:419-429.
134. Valladares A, Montesinos ML, Herrero A, & Flores E (2002) An ABC-type, high-affinity urea permease identified in cyanobacteria. *Molecular Microbiology* 43(3):703-715.
135. Mao Y, Doyle MP, & Chen JR (2001) Insertion mutagenesis of wca reduces acid and heat tolerance of enterohemorrhagic *Escherichia coli* O157:H7. *Journal of Bacteriology* 183(12):3811-3815.
136. Becker A & Vorhölter FJ eds (2009) *Xanthan biosynthesis by Xanthomonas bacteria: an overview of the current biochemical and genomic data* (Caister Academic Press), pp 1-13.
137. Roseiro JC, Esgalhado ME, Emery AN, & AmaralCollaco MT (1996) Technological and kinetic aspects of sublethal acid toxicity in microbial gum production. *Journal of Chemical Technology and Biotechnology* 65(3):258-264.
138. Esgalhado ME, Caldeira AT, Roseiro JC, & Emery AN (2001) Polysaccharide synthesis as a carbon dissipation mechanism in metabolically uncoupled *Xanthomonas campestris* cells. *Journal of Biotechnology* 89(1):55-63.
139. Costerton JW, *et al.* (1987) Bacterial biofilms in nature and disease. *Annual Review of Microbiology* 41:435-464.

140. Stewart PS (2002) Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology* 292(2):107-113.
141. Stewart PS & Franklin MJ (2008) Physiological heterogeneity in biofilms. *Nature Reviews Microbiology* 6(3):199-210.
142. Beeckman DSA & Vanrompay DCG (2009) Bacterial Secretion Systems with an Emphasis on the Chlamydial Type III Secretion System. *Current Issues in Molecular Biology* 12:17-41.
143. Cianciotto NP (2005) Type II secretion: a protein secretion system for all seasons. *Trends in Microbiology* 13(12):581-588 .
144. Foster PL (2007) Stress-induced mutagenesis in bacteria. *Critical Reviews in Biochemistry and Molecular Biology* 42:373-397.
145. Wilson DN & Nierhaus KH (2005) Ribosomal proteins in the spotlight. *Critical Reviews in Biochemistry and Molecular Biology* 40(5):243-267.
146. Brodersen DE & Nissen P (2005) The social life of ribosomal proteins. *Febs Journal* 272(9):2098-2108.
147. Ishige T, Krause M, Bott M, Wendisch VF, & Sahm H (2003) The phosphate starvation stimulon of *Corynebacterium glutamicum* determined by DNA microarray analyses. *Journal of Bacteriology* 185(15):4519-4529.
148. Lawrence CL, Botting CH, Antrobus R, & Coote PJ (2004) Evidence of a new role for the high-osmolarity glycerol mitogen-activated protein kinase pathway in yeast: regulating adaptation to citric acid stress. *Molecular and Cellular Biology* 24(8):3307-3323.
149. Silberbach M & Burkovski A (2006) Application of global analysis techniques to *Corynebacterium glutamicum*: new insights into nitrogen regulation. *Journal of Biotechnology* 126(1):101-110.
150. Silberbach M, *et al.* (2005) DNA microarray analysis of the nitrogen starvation response of *Corynebacterium glutamicum*. *Journal of Biotechnology* 119(4):357-367.

151. Stintzi A (2003) Gene expression profile of *Campylobacter jejuni* in response to growth temperature variation. *Journal of Bacteriology* 185(6):2009-2016.
152. Akbar S, Lee SY, Boylan SA, & Price CW (1999) Two genes from *Bacillus subtilis* under the sole control of the general stress transcription factor σ^B. *Microbiology-Uk* 145:1069-1078.
153. Pragai Z & Harwood CR (2002) Regulatory interactions between the Pho and σ^B-dependent general stress regulons of *Bacillus subtilis*. *Microbiology-Sgm* 148:1593-1602.
154. Hecker M & Volker U (1998) Non-specific, general and multiple stress resistance of growth-restricted Bacillus subtilis cells by the expression of the sigma(B) regulon. *Molecular Microbiology* 29(5):1129-1136 .
155. Aravind L & Koonin EV (2001) Prokaryotic homologs of the eukaryotic DNA-end-binding protein Ku, novel domains in the Ku protein and prediction of a prokaryotic double-strand break repair system. *Genome Research* 11(8):1365-1374.
156. Stammers DK, *et al.* (2001) The structure of the negative transcriptional regulator NmrA reveals a structural superfamily which includes the short-chain dehydrogenase/reductases. *Embo Journal* 20(23):6619-6626.
157. Andrianopoulos A, Kourambas S, Sharp JA, Davis MA, & Hynes MJ (1998) Characterization of the *Aspergillus nidulans* nmrA gene involved in nitrogen metabolite repression. *Journal of Bacteriology* 180(7):1973-1977.
158. Gorfinkiel L, Diallinas G, & Scazzocchio C (1993) Sequence and regulation of the uapa gene encoding a uric acid-xanthine permease in the fungus *Aspergillus nidulans*. *Journal of Biological Chemistry* 268(31):23376-23381.
159. de Alba E & Tjandra N (2004) Structural studies on the Ca^{2+}-binding domain of human nucleobindin (calnuc). *Biochemistry* 43(31):10039-10049.

160. Tsukumo Y, *et al.* (2007) Nucleobindin 1 controls the unfolded protein response by inhibiting ATF6 activation. *Journal of Biological Chemistry* 282(40):29264-29272.
161. Corrochano LM, Lauter FR, Ebbole DJ, & Yanofsky C (1995) Light and developmental regulation of the gene con-10 of *Neurospora crassa*. *Developmental Biology* 167(1):190-200.
162. Roberts AN, Berlin V, Hager KM, & Yanofsky C (1988) Molecular Analysis of a *Neurospora crassa* Gene Expressed During Conidiation. *Molecular and Cellular Biology* 8(6):2411-2418.
163. Wilson RA, *et al.* (2004) Two Δ9-stearic acid desaturases are required for *Aspergillus nidulans* growth and development. *Fungal Genetics and Biology* 41(5):501-509.
164. van den Brink HJM, van Gorcom RFM, van den Hondel C, & Punt PJ (1998) Cytochrome P450 enzyme systems in fungi. *Fungal Genetics and Biology* 23(1):1-17.
165. Gautam P, *et al.* (2008) Proteomic and Transcriptomic Analysis of *Aspergillus fumigatus* on Exposure to Amphotericin B. *Antimicrobial Agents and Chemotherapy* 52(12):4220-4227.
166. Hamilton JM (1972) Sterols from polyene-resistant mutants of *Candida albicans*. *Journal of General Microbiology* 73:201-203.
167. Moore CB, Sayers N, Mosquera J, Slaven J, & Denning DW (2000) Antifungal drug resistance in *Aspergillus*. *Journal of Infection* 41(3):203-220.
168. DeZwaan TM, Carroll AM, Valent B, & Sweigard JA (1999) Magnaporthe grisea Pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues. *Plant Cell* 11(10):2013-2030.
169. Lin XR & Momany M (2004) Identification and complementation of abnormal hyphal branch mutants ahbA1 and ahbB1 in *Aspergillus nidulans*. *Fungal Genetics and Biology* 41(11):998-1006.
170. Meyer V, *et al.* (2007) Survival in the presence of antifungals - Genome-wide expression profiling of *Aspergillus niger* in response

to sublethal concentrations of caspofungin and fenpropimorph. *Journal of Biological Chemistry* 282:32935-32948.

171. Yuen KY, *et al.* (2001) Characterization of AFMP1: a novel target for serodiagnosis of aspergillosis. *Journal of Clinical Microbiology* 39(11):3830-3837.
172. Yamazaki H, Tanaka A, Kaneko J, Ohta A, & Horiuchi H (2008) *Aspergillus nidulans* ChiA is a glycosylphosphatidylinositol (GPI)-anchored chitinase specifically localized at polarized growth sites. *Fungal Genetics and Biology* 45(6):963-972.
173. Meyer V, *et al.* (2009) Reconstruction of Signaling Networks Regulating Fungal Morphogenesis by Transcriptomics. *Eukaryotic Cell* 8(11):1677-1691 .
174. Mao YQ, Varoglu M, & Sherman DH (1999) Molecular characterization and analysis of the biosynthetic gene cluster for the antitumor antibiotic mitomycin C from *Streptomyces lavendulae* NRRL 2564. *Chemistry & Biology* 6(4):251-263.
175. Almeida PF & Pokorny A (2009) Mechanisms of Antimicrobial, Cytolytic, and Cell-Penetrating Peptides: From Kinetics to Thermodynamics. *Biochemistry* 48(34):8083-8093 .
176. Lewis JA, Roberts DP, & Hollenbeck MD (1991) Induction of cytoplasmic leakage from Rhizoctonia solani hyphae by *Gliocladium virens* and partial characterization of a leakage factor *Biocontrol Science and Technology* 1(1):21-29.
177. Riedlinger J, *et al.* (2006) Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the mycorrhiza helper bacterium *Streptomyces* strain AcH 505. *Applied and Environmental Microbiology* 72(5):3550-3557.
178. Ahn SJ, Yang CH, & Cooksey DA (2007) *Pseudomonas putida* 06909 genes expressed during colonization on mycelial surfaces and phenotypic characterization of mutants. *Journal of Applied Microbiology* 103(1):120-132.

179. Dutton MV & Evans CS (1996) Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Canadian Journal of Microbiology* 42(9):881-895 .
180. Horner HT & Wagner BL (1995) Calcium oxalate formation in higher plants. *Calcium oxalate in biological systems*, ed Khan SR (CRC Press, Boca Raton, Florida), pp 53-72.
181. Rosling A (2009) Trees, Mycorrhiza and Minerals - Field Relevance of in vitro Experiments. *Geomicrobiology Journal* 26(6):389-401.
182. Folman LB, Gunnewiek P, Boddy L, & de Boer W (2008) Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil. *Fems Microbiology Ecology* 63(2):181-191.
183. Smalla K, *et al.* (2000) Exogenous isolation of antibiotic resistance plasmids from piggery manure slurries reveals a high prevalence and diversity of IncQ-like plasmids. *Applied and Environmental Microbiology* 66(11):4854-4862.
184. Rensing C, Newby DT, & Pepper IL (2002) The role of selective pressure and selfish DNA in horizontal gene transfer and soil microbial community adaptation. *Soil Biology & Biochemistry* 34(3):285-296.
185. Orgel LE, Crick FHC, & Sapienza C (1980) Selfish DNA. *Nature* 288(5792):645-646 .
186. Doolittle WF & Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. *Nature* 284(5757):601-603 .
187. Velmurugan S, Mehta S, Uzri D, & Jayaram M (2003) Stable propagation of 'selfish' genetic elements. *Journal of Biosciences* 28(5):623-636.
188. Lilley A., Young P, & M. B (2000) Bacterial population genetics: do plasmids maintain bacterial diversity and adaptation? *The horizontal gene pool, bacterial plasmids and gene spread*, ed Thomas CM (Harwood Academic Publishers), pp 287-300.
189. Joshi BD, Berg M, Rogers J, Fletcher J, & Melcher U (2005) Sequence comparisons of plasmids pBJS-O of *Spiroplasma citri* and

pSKU146 of *Spiroplasma kunkelii*: implications for plasmid evolution. *BMC Genomics* 6:175.

190. Price CW, *et al.* (2001) Genome-wide analysis of the general stress response in *Bacillus subtilis*. *Molecular Microbiology* 41(4):757-774.
191. Dennis JJ (2005) The evolution of IncP catabolic plasmids. *Current Opinion in Biotechnology* 16(3):291-298.
192. Osborn AM & Boltner D (2002) When phage, plasmids, and transposons collide: genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. *Plasmid* 48(3):202-212.
193. Haagensen JAJ, Hansen SK, Johansen T, & Molin S (2002) In situ detection of horizontal transfer of mobile genetic elements. *Fems Microbiology Ecology* 42(2):261-268.
194. Jones BV & Marchesi JR (2007) Transposon-aided capture (TRACA) of plasmids resident in the human gut mobile metagenome. *Nature Methods* 4(1):55-61.
195. Smalla K & Sobecky PA (2002) The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: insights gained from different methodological approaches. *Fems Microbiology Ecology* 42(2): 165-175.
196. Tauch A, *et al.* (2002) The complete nucleotide sequence and environmental distribution of the cryptic, conjugative, broad-host-range plasmid pIPO2 isolated from bacteria of the wheat rhizosphere. *Microbiology-Sgm* 148:1637-1653.
197. Schneiker S, *et al.* (2001) The genetic organization and evolution of the broad host range mercury resistance plasmid pSB102 isolated from a microbial population residing in the rhizosphere of alfalfa. *Nucleic Acids Research* 29(24):5169-5181.
198. Marques MV, da Silva AM, & Gomes SL (2001) Genetic organization of plasmid pXF51 from the plant pathogen *Xylella fastidiosa*. *Plasmid* 45(3):184-199.

199. Gstalder ME, *et al.* (2003) Replication functions of new broad host range plasmids isolated from polluted soils. *Research in Microbiology* 154(7):499-509.
200. Rhodes G, *et al.* (2004) Complete nucleotide sequence of the conjugative tetracycline resistance plasmid pFBAOT6, a member of a group of IncU plasmids with global ubiquity. *Applied and Environmental Microbiology* 70(12):7497-7510.
201. de Boer W, *et al.* (2004) *Collimonas fungivorans* gen. nov., sp nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae. *International Journal of Systematic and Evolutionary Microbiology* 54:857-864.
202. King EO, Ward MK, & Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* 44(2):301-307.
203. Bertani G (1951) Studies on lysogenesis .1. The mode of phage liberation by lysogenic *Escherichia coli*. *Journal of Bacteriology* 62(3):293-300.
204. Rondon MR, Raffel SJ, Goodman RM, & Handelsman J (1999) Toward functional genomics in bacteria: analysis of gene expression in *Escherichia coli* from a bacterial artificial chromosome library of *Bacillus cereus*. *Proceedings of the National Academy of Sciences of the United States of America* 96(11):6451-6455.
205. Meyer F, *et al.* (2003) GenDB - an open source genome annotation system for prokaryote genomes. *Nucleic Acids Research* 31(8):2187-2195.
206. van Elsas JD, Gardener BBM, Wolters AC, & Smit E (1998) Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. *Applied and Environmental Microbiology* 64(3):880-889.
207. Ike Y, Hashimoto H, & Mitsuhashi S (1981) A Mutant Defective in Partitioning of Composite Plasmid Rms201. *Journal of Bacteriology* 147(2):578-588.

208. Gotz A, *et al.* (1996) Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. *Applied and Environmental Microbiology* 62(7):2621-2628.
209. Uraji M, Suzuki K, & Yoshida K (2002) A novel plasmid curing method using incompatibility of plant pathogenic Ti plasmids in *Agrobacterium tumefaciens*. *Genes & Genetic Systems* 77(1):1-9.
210. Sambrook J, Fritsch EF, & Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) second ed.
211. Simons M, *et al.* (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Molecular Plant-Microbe Interactions* 9(7):600-607.
212. Zar JH (1999) *Biostatistical analysis* (Prentice Hall) 4th ed.
213. Brito L & Paveia H (1999) Presence and analysis of large plasmids in *Oenococcus oeni*. *Plasmid* 41(3):260-267.
214. Nakamura H, *et al.* (2003) Usefulness of a combination of pulsed-field gel electrophoresis and enrichment culture in laboratory investigation of a foodborne outbreak due to *Clostridium perfringens*. *Diagnostic Microbiology and Infectious Disease* 47(3):471-475.
215. Bergstrom M, Hermansson M, & Dahlberg C (2004) Isolation and sequencing of the replication region of plasmid pBFp1 isolated from a marine biofilm. *Plasmid* 51(3):179-184.
216. Francia MV, Delacruz F, & Lobo JMG (1993) Secondary sites for integration mediated by the tn21 integrase. *Molecular Microbiology* 10(4):823-828.
217. Belogurov AA, *et al.* (2000) Antirestriction protein ard (Type C) encoded by IncW plasmid pSa has a high similarity to the "protein transport" domain of TraC1 primase of promiscuous plasmid RP4. *Journal of Molecular Biology* 296(4):969-977.
218. Espinosa M, *et al.* eds (2000) *Plasmid replication and copy number control* (Harwood academic publishers), pp 1-47.

219. Kruger R, Rakowsky SA, & Filutowics M eds (2004) *Participating elements in replication of iteron-containing plasmids* (ASM press, American Society for Microbiology), pp 25-45.
220. Summers DK (1996) *The biology of plasmids* (Blackwell science).
221. del Solar G, Hernández-Arriaga AM, Gomis-Ruth FX, Coll M, & Espinosa M (2002) A genetically economical family of plasmid-encoded transcriptional repressors involved in control of plasmid copy number. *Journal of Bacteriology* 184(18):4943-4951.
222. Schaefer C & Messer W (1991) DnaA protein DNA interaction - modulation of the recognition sequence. *Molecular & General Genetics* 226(1-2):34-40.
223. Kawamukai M, Matsuda H, Fujii W, Utsumi R, & Komano T (1989) Nucleotide-sequences of fic and fic-1 genes involved in cell filamentation induced by cyclic-amp in *Escherichia-coli*. *Journal of Bacteriology* 171(8):4525-4529.
224. Nelson KE, *et al.* (2003) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440 (vol 4, pg 799, 2002). *Environmental Microbiology* 5(7):630-630.
225. Klockgether J, Reva O, Larbig K, & Tummler B (2004) Sequence analysis of the mobile genome island pKLC102 of Pseudomonas aeruginosa C. *Journal of Bacteriology* 186(2):518-534.
226. Kornacki JA, Chang CH, & Figurski DH (1993) Kil-kor regulon of promiscuous plasmid-rk2 - structure, products, and regulation of 2 operons that constitute the kilE locus. *Journal of Bacteriology* 175(16):5078-5090.
227. Cooper TF & Heinemann JA (2000) Postsegregational killing does not increase plasmid stability but acts to mediate the exclusion of competing plasmids. *Proceedings of the National Academy of Sciences of the United States of America* 97(23):12643-12648.
228. Huang J, *et al.* (2006) Molecular characterization of a DNA fragment harboring the replicon of pBMB165 from *Bacillus thuringiensis* subsp *tenebrionis*. *BMC Genomics* 7.

229. Sanchis V, Agaisse H, Chaufaux J, & Lereclus D (1997) A recombinase-mediated system for elimination of antibiotic resistance gene markers from genetically engineered *Bacillus thuringiensis* strains. *Applied and Environmental Microbiology* 63(2):779-784.
230. Bignell C & Thomas CM (2001) The bacterial ParA-ParB partitioning proteins. *J. Biotechnol.* 91(1):1-34.
231. Pansegrau W, *et al.* (1994) Complete Nucleotide-Sequence of Birmingham Incp-Alpha Plasmids - Compilation and Comparative-Analysis. *Journal of Molecular Biology* 239(5):623-663.
232. Gerlitz M, Hrabak O, & Schwab H (1990) Partitioning of broad-host-range plasmid rp4 is a complex system involving site-specific recombination. *Journal of Bacteriology* 172(11):6194-6203.
233. Top E, Vanrolleghem P, Mergeay M, & Verstraete W (1992) Determination of the mechanism of retrotransfer by mechanistic mathematical-modeling. *Journal of Bacteriology* 174(18):5953-5960.
234. Boschiroli ML, *et al.* (2002) The Brucella suis virB operon is induced intracellularly in macrophages. *Proceedings of the National Academy of Sciences of the United States of America* 99(3):1544-1549.
235. Llosa M, Gomis-Ruth FX, Coll M, & de la Cruz F (2002) Bacterial conjugation: a two-step mechanism for DNA transport. *Molecular Microbiology* 45(1):1-8.
236. Christie PJ, Atmakuri K, Krishnamoorthy V, Jakubowski S, & Cascales E (2005) Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annual Review of Microbiology* 59:451-485.
237. Avila P, Nunez N, & de la Cruz F (1996) Plasmid R6K contains two functional oriTs which can assemble simultaneously in relaxosomes in vivo. *Journal of Molecular Biology* 261(2):135-143.
238. Porter SG, Yanofsky MF, & Nester EW (1987) Molecular characterization of the vird operon from agrobacterium-tumefaciens. *Nucleic Acids Research* 15(18):7503-7517.

239. Llosa M, Bolland S, & Delacruz F (1994) Genetic organization of the conjugal dna processing region of the incw plasmid-r388. *Journal of Molecular Biology* 235(2):448-464.
240. Fernandez-Lopez R, *et al.* (2005) Unsaturated fatty acids are inhibitors of bacterial conjugation. *Microbiology-Sgm* 151:3517-3526.
241. Szpirer CY, Faelen M, & Couturier M (2000) Interaction between the RP4 coupling protein TraG and the pBHR1 mobilization protein Mob. *Molecular Microbiology* 37(6):1283-1292.
242. Kim JG, *et al.* (2006) Bases of biocontrol: Sequence predicts synthesis and mode of action of agrocin 84, the Trojan Horse antibiotic that controls crown gall. *Proceedings of the National Academy of Sciences of the United States of America* 103(23):8846-8851.
243. Cazalet C, *et al.* (2004) Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nature Genetics* 36(11):1165-1173.
244. Kim SR & Komano T (1992) Nucleotide-sequence of the r721 shufflon. *Journal of Bacteriology* 174(21):7053-7058.
245. Foster GC, McGhee GC, Jones AL, & Sundin GW (2004) Nucleotide sequences, genetic organization, and distribution of pEU30 and pEL60 from *Erwinia amylovora*. *Applied and Environmental Microbiology* 70(12):7539-7544.
246. Bellgard M, Schibeci D, Trifonov E, & Gojobori T (2001) Early detection of G+C differences in bacterial species inferred from the comparative analysis of the two completely sequenced *Helicobacter pylori* strains. *Journal of Molecular Evolution* 53(4-5):465-468.
247. Sueoka N (1988) Directional mutation pressure and neutral molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America* 85(8):2653-2657.
248. Nei M & Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Molecular Biology and Evolution* 3(5):418-426.

249. Lovett ST (2004) Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences. *Molecular Microbiology* 52(5):1243-1253.
250. Lugtenberg BJJ, Dekkers L, & Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annual Review of Phytopathology* 39:461-490.
251. Uroz S, *et al.* (2007) Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology* 73(9):3019-3027.
252. Bergstrom CT, Lipsitch M, & Levin BR (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* 155(4):1505-1519.
253. Sota M, *et al.* (2007) Region-specific insertion of transposons in combination with selection for high plasmid transferability and stability accounts for the structural similarity of IncP-1 plasmids. *Journal of Bacteriology* 189(8):3091-3098.
254. Espinosa-Urgel M (2004) Plant-associated *Pseudomonas* populations: molecular biology, DNA dynamics, and gene transfer. *Plasmid* 52(3):139-150.
255. van Elsas JD & Bailey MJ (2002) The ecology of transfer of mobile genetic elements. *Fems Microbiology Ecology* 42(2):187-197.
256. van Elsas JD, Fry J, Hirsch P, & Molin S (2000) Ecology of plasmid transfer and spread. *The horizontal gene pool, bacterial plasmids and gene spread*, ed Thomas CM (Harwood academic publishers), pp 175-206.
257. van Elsas JD, Turner S, & Bailey MJ (2003) Horizontal gene transfer in the phytosphere. *New Phytologist* 157(3):525-537.
258. Smit E, van Elsas JD, van Veen JA, & de Vos WM (1991) Detection of plasmid transfer from *Pseudomonas fluorescens* to indigenous bacteria in soil by using bacteriophage Phi-r2f for donor counterselection. *Applied and Environmental Microbiology* 57(12):3482-3488.

259. Höppener-Ogawa S, *et al.* (2008) *Collimonas arenae* sp nov. and *Collimonas pratensis* sp nov., isolated from (semi-)natural grassland soils. *International Journal of Systematic and Evolutionary Microbiology* 58:414-419.
260. King E, Ward M, & Raney D (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* 44:301–307.
261. Workman C, *et al.* (2002) A new non-linear normalization method for reducing variability in DNA microarray experiments. *Genome Biology* 3(9):research0048.1–research0048.16.
262. Draghici S (2003) *Data analysis tools for DNA microarrays* (Chapman & Hall / CRC, Boca Raton, FL).
263. Bayjanov JR, *et al.* (2009) PanCGH: a genotype-calling algorithm for pangenome CGH data. *Bioinformatics* 25(3):309-314.
264. Gentleman RC, *et al.* (2004) Bioconductor: open software development for computational biology and bioinformatics. *Genome Biology* 5(10):R80.
265. Fawcett T (2006) An introduction to ROC analysis. *Pattern Recognition Letters* 27(8):861-874.
266. Overbeek R, *et al.* (2005) The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Research* 33(17):5691-5702.
267. Muller D, *et al.* (2007) A tale of two oxidation states: bacterial colonization of arsenic-rich environments. *Plos Genetics* 3(4).
268. Weiss S, *et al.* (2009) Enhanced structural and functional genome elucidation of the arsenite-oxidizing strain *Herminiimonas arsenicoxydans* by proteomics data. *Biochimie* 91(2):192-203.
269. Audic S, *et al.* (2007) Genome analysis of *Minibacterium massiliensis* highlights the convergent evolution of water-living bacteria. *Plos Genetics* 3:1454-1463.
270. Buttner D & He SY (2009) Type III Protein Secretion in Plant Pathogenic Bacteria. *Plant Physiology* 150(4):1656-1664.

271. Mukaihara T & Tamura N (2009) Identification of novel *Ralstonia solanacearum* type III effector proteins through translocation analysis of hrpB-regulated gene products. *Microbiology-Sgm* 155:2235-2244.
272. Cascales E (2008) The type VI secretion toolkit. *Embo Reports* 9(8):735-741.
273. Filloux A, Hachani A, & Bleves S (2008) The bacterial type VI secretion machine: yet another player for protein transport across membranes. *Microbiology-Sgm* 154:1570-1583.
274. Beeckman DSA & Vanrompay DCG (2010) Bacterial Secretion Systems with an Emphasis on the Chlamydial Type III Secretion System. *Current Issues in Molecular Biology* 12:17-41.
275. Fauvart M & Michiels J (2008) Rhizobial secreted proteins as determinants of host specificity in the rhizobium-legume symbiosis. *Fems Microbiology Letters* 285(1):1-9.
276. Argüelles JC (2000) Physiological roles of trehalose in bacteria and yeasts: a comparative analysis. *Archives of Microbiology* 174(4):217-224.
277. Terashima H, Kojima S, & Homma M (2008) Flagellar motility in bacteria: structure and function of flagellar motor. *International Review of Cell and Molecular Biology* Vol 270, pp 39-85.
278. Jarrell KF & McBride MJ (2008) The surprisingly diverse ways that prokaryotes move. *Nature Reviews Microbiology* 6(6):466-476 .
279. Scholz-Schroeder BK, Soule JD, Lu SE, Grgurina I, & Gross DC (2001) A physical map of the syringomycin and syringopeptin gene clusters localized to an approximately 145-kb DNA region of *Pseudomonas syringae* pv. syringae strain B301D. *Molecular Plant-Microbe Interactions* 14(12):1426-1435.
280. Raaijmakers JM, de Bruijn I, & de Kock MJD (2006) Cyclic lipopeptide production by plant-associated *Pseudomonas* spp.: diversity, activity, biosynthesis, and regulation. *Molecular Plant-Microbe Interactions* 19(7):699-710.

281. Hasman H, Schembri MA, & Klemm P (2000) Antigen 43 and type 1 fimbriae determine colony morphology of *Escherichia coli* K-12. *Journal of Bacteriology* 182(4):1089-1095.
282. Long CD, Madraswala RN, & Seifert HS (1998) Comparisons between colony phase variation of *Neisseria gonorrhoeae* FA1090 and pilus, pilin, and S-pilin expression. *Infection and Immunity* 66(5):1918-1927.
283. Poolman JT, Hopman CTP, & Zanen HC (1985) Colony variants of *Neisseria meningitidis* strain 2996 (b-2b-p1-2) - influence of class-5 outer-membrane proteins and lipopolysaccharides. *Journal of Medical Microbiology* 19(2):203-209.
284. Jones KM, *et al.* (2008) Differential response of the plant *Medicago truncatula* to its symbiont *Sinorhizobium meliloti* or an exopolysaccharide-deficient mutant. *Proceedings of the National Academy of Sciences of the United States of America* 105(2):704-709.
285. Fritsche K *et al.* Manuscript in preparation.
286. Mela F, *et al.* (2008) Comparative genomics of the pIPO2/pSB102 family of environmental plasmids: sequence, evolution, and ecology of pTer331 isolated from *Collimonas fungivorans* Ter331. *Fems Microbiology Ecology* 66(1):45-62.
287. Ito H & Iizuka H (1971) Taxonomic studies on a radio-resistant *Pseudomonas* . Part XII. studies on microorganisms of cereal grain. *Agricultural and Biological Chemistry* 35(10):1566-1571.

Appendix

Table A1. Subset of *C. fungivoran*s genes differentially expressed at T1

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster A	*Cf_2063*	acetolactate synthase large subunit	9.8	up	6.9	up
Cluster A	*Cf_2064*	NAD-dependent formate dehydrogenase gamma subunit	3.4	up	2.2	up
Cluster A	*Cf_2065*	NAD-dependent formate dehydrogenase beta subunit	2.5	up	2.3	up
Cluster A	*Cf_2066*	uncharacterized anaerobic dehydrogenase	5.3	up	3.9	up
Cluster A	*Cf_2067*	NAD-dependent formate dehydrogenase delta subunit	3.7	up	1.4	up
Cluster A	*Cf_2070*	Protein cbbY, plasmid	35.2	up	3.9	up
Cluster A	*Cf_2071*	hypothetical protein predicted by Glimmer/Critica	23.5	up	2.8	up
Cluster A	*Cf_2072*	Oxalate:formate antiporter	9.9	up	3.3	up
Cluster A	*Cf_2073*	fumarylacetoacetate hydrolase family protein	6.8	up	6.3	up
Cluster A	*Cf_2074*	2-dehydropantoate 2-reductase	7.2	up	3.5	up
Cluster A	*Cf_2075*	Monocarboxylate transporter 2	9.4	up	4.5	up
Cluster A	*Cf_2076*	Putative quinone oxidoreductase	2.9	up	1.4	up
Cluster A	*Cf_2079*	acetolactate synthase large subunit	14.0	up	8.5	up
Cluster A	*Cf_2080*	putative enzyme	13.4	up	6.1	up
Cluster A	*Cf_2081*	PAS	4.3	up	2.7	up
Cluster A	*Cf_2082*	putative enzyme	2.4	up	2.2	up
Cluster A	*Cf_2083*	Putative HTH-type transcriptional regulator yeaM	2.2	up	1.9	up
Cluster B	*Cf_3540*	2-hydroxy-3-oxopropionate reductase	4.9	up	5.4	up
Cluster B	*Cf_3541*	hydroxypyruvate isomerase	3.8	up	4.2	up
Cluster B	*Cf_3542*	glyoxylate carboligase	5.3	up	6.3	up
Cluster C	*Cf_2982*	hypothetical protein	2.6	up	18.3	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster C	*Cf_2983*	hypothetical protein	2.7	up	6.6	up
Cluster C	*Cf_2984*	hypothetical protein predicted by Glimmer/Critica	13.1	up	4.7	up
Cluster C	*Cf_2985*	formamidase	32.8	up	4.8	up
Cluster C	*Cf_2986*	aliphatic amidase	55.8	up	9.3	up
Cluster C	*Cf_2987*	ABC-type urea permease	17.0	up	2.0	up
Cluster C	*Cf_2988*	ABC-type urea permease	22.4	up	4.1	up
Cluster C	*Cf_2989*	ABC-type urea permease	48.5	up	10.7	up
Cluster C	*Cf_2990*	ABC-type urea permease	22.6	up	3.2	up
Cluster C	*Cf_2991*	ABC-type urea permease	70.9	up	6.8	up
Cluster C	*Cf_2992*	two-component hybrid sensor and regulator	2.3	up	3.4	up
Cluster C	*Cf_2993*	putative two-component response regulator protein	2.3	up	2.0	up
Cluster D	*Cf_4054*	uroporphyrin-III C-methyltransferase	5.1	up	3.1	up
Cluster D	*Cf_4055*	Nitrate transport protein nasD.	9.5	up	3.5	up
Cluster D	*Cf_4056*	Nitrate transport permease protein nrtB.	16.3	up	6.1	up
Cluster D	*Cf_4057*	Nitrate transport protein nrtA.	20.8	up	5.5	up
Cluster E	*Cf_223*	probable high affinity nitrate transporter transmembrane protein	7.2	up	7.0	up
Cluster E	*Cf_224*	nitrite reductase	17.1	up	5.9	up
Cluster E	*Cf_225*	nitrite reductase	12.6	up	5.2	up
Cluster E	*Cf_226*	nitrite reductase	4.6	up	2.1	up
Cluster E	*Cf_227*	nitrate reductase large subunit	6.1	up	4.5	up
Cluster F	*Cf_556*	glutamate synthase	2.2	up	1.6	down
Cluster F	*Cf_2775*	Allantoin permease (Allantoin transport protein).	5.9	up	7.0	up
Cluster G	*Cf_4241*	Acetyltransferase	2.8	up	1.8	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster G	*Cf_4242*	ammonium transporter	9.8	up	1.9	up
Cluster G	*Cf_4243*	Nitrogen regulatory protein P-II.	5.7	up	2.5	up
Cluster H	*Cf_2912*	ABC-type urea permease	11.6	up	6.8	up
Cluster H	*Cf_2913*	ABC-type urea permease	6.1	up	8.0	up
Cluster H	*Cf_2914*	ABC-type urea permease	6.8	up	6.3	up
Cluster H	*Cf_2915*	ABC-type urea permease	6.8	up	6.3	up
Cluster H	*Cf_2916*	ABC-type urea permease	9.2	up	7.0	up
Cluster I	*Cf_2786*	GumF protein	3.7	up	15.8	up
Cluster I	*Cf_2787*	phosphomannomutase	3.3	up	12.3	up
Cluster I	*Cf_2788*	mannose-6-phosphate isomerase / mannose-1-phosphate guanylyltransferase	5.5	up	54.3	up
Cluster I	*Cf_2789*	mannose-1-phosphate guanyltransferase	8.3	up	74.6	up
Cluster I	*Cf_2790*	GumM protein	7.7	up	50.2	up
Cluster I	*Cf_2791*	Hypothetical 55.0 kDa protein in cps region	3.8	up	24.4	up
Cluster I	*Cf_2792*	Polypeptide N-acetylgalactosaminyltransferase 1	5.4	up	34.8	up
Cluster I	*Cf_2793*	cellulase	2.5	up	15.1	up
Cluster I	*Cf_2794*	O-acetyltransferase oatA (EC 2.3.1.-).	5.3	up	28.7	up
Cluster I	*Cf_2795*	GumH protein	4.8	up	26.0	up
Cluster I	*Cf_2796*	hypothetical protein predicted by Glimmer/Critica	4.3	up	27.6	up
Cluster I	*Cf_2797*	hypothetical protein	4.5	up	33.8	up
Cluster I	*Cf_2798*	lipopolysaccharide biosynthesis	7.4	up	76.2	up
Cluster I	*Cf_2799*	EPS I polysaccharide export outer membrane protein epsA precursor.	5.1	up	56.5	up
Cluster I	*Cf_2800*	hypothetical protein	9.6	up	100.4	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster I	*Cf_2801*	hypothetical protein predicted by Glimmer/Critica	5.2	up	53.4	up
Cluster I	*Cf_2802*	Putative colanic biosynthesis UDP-glucose lipid carrier transferase.	3.9	up	16.7	up
Cluster I	*Cf_2803*	hypothetical protein	3.8	up	30.1	up
Cluster J	*Cf_2051*	Putative polysaccharide export protein wza precursor.	11.0	up	2.9	up
Cluster J	*Cf_2052*	Probable low molecular weight protein-tyrosine-phosphatase amsI	9.3	up	2.1	up
Cluster J	*Cf_2053*	exopolysaccharide transport protein	9.1	up	3.5	up
Cluster J	*Cf_2054*	hypothetical protein predicted by Glimmer/Critica	7.7	up	4.2	up
Cluster J	*Cf_2055*	hypothetical protein predicted by Glimmer/Critica	6.9	up	1.9	up
Cluster J	*Cf_2056*	hypothetical protein	4.6	up	1.7	up
Cluster J	*Cf_2057*	Hypothetical 41.2 kDa protein in cps region	5.0	up	1.2	up
Cluster J	*Cf_2058*	hypothetical protein	4.6	up	1.9	up
Cluster J	*Cf_2059*	UTP--glucose-1-phosphate uridylyltransferase	24.0	up	2.7	up
Cluster J	*Cf_2060*	sugar transferase	22.1	up	2.9	up
Cluster K	*Cf_1128*	hypothetical protein predicted by Glimmer/Critica	2.4	up	1.8	up
Cluster K	*Cf_1129*	High molecular weight rubredoxin	6.5	up	3.4	up
Cluster K	*Cf_1130*	Abhydrolase domain-containing protein 12.	6.7	up	3.9	up
Cluster K	*Cf_1131*	fatty acid desaturase	9.0	up	2.9	up
Cluster K	*Cf_1132*	Putative polyketide synthase pksM.	8.5	up	3.6	up
Cluster K	*Cf_1133*	Acyl-CoA desaturase 1	4.7	up	2.1	up
Cluster K	*Cf_1134*	Delta-9 acyl-lipid desaturase 2 (EC 1.14.19.-).	3.4	up	2.0	up
Cluster K	*Cf_1135*	probable peptide synthetase protein	4.7	up	2.9	up
Cluster K	*Cf_1136*	Possible Multidrug resistance protein B.	4.5	up	3.5	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster K	*Cf_1137*	hypothetical protein predicted by Glimmer/Critica	7.4	up	3.9	up
Cluster K	*Cf_1138*	vanillate O-demethylase oxygenase subunit	6.8	up	2.1	up
Cluster K	*Cf_1139*	fatty acid desaturase	7.7	up	1.8	up
Cluster K	*Cf_1140*	Protein fixC.	6.9	up	1.6	up
Cluster K	*Cf_1141*	3-oxoacyl-acyl carrier protein synthase II	7.8	up	2.3	up
Cluster K	*Cf_1142*	hypothetical protein predicted by Glimmer/Critica	7.4	up	1.1	down
Cluster L	*Cf_2276*	hypothetical protein	2.3	up	2.5	up
Cluster L	*Cf_2277*	Hypothetical protein MJ0779.	2.8	up	1.9	up
Cluster L	*Cf_2278*	Hypothetical protein MJ0900.	2.8	up	2.3	up
Cluster L	*Cf_2279*	hypothetical protein predicted by Glimmer/Critica	2.0	up	1.1	down
Cluster L	*Cf_2280*	General secretion pathway protein D precursor.	2.2	up	1.9	up
Cluster L	*Cf_2281*	hypothetical protein predicted by Glimmer/Critica	2.0	up	1.2	up
Cluster L	*Cf_2282*	hypothetical protein predicted by Glimmer/Critica	2.2	up	1.1	up
Cluster L	*Cf_2283*	hypothetical protein predicted by Glimmer/Critica	2.6	up	1.3	up
Cluster L	*Cf_2284*	hypothetical protein predicted by Glimmer/Critica	2.1	up	1.0	up
Cluster M	*Cf_986*	Flagellar protein flhE precursor.	3.3	up	15.5	up
Cluster M	*Cf_991*	CDS	2.5	up	8.5	up
Cluster M	*Cf_992*	Flagellar basal-body rod protein flgC.	2.4	up	7.9	up
Cluster M	*Cf_994*	CDS	2.9	up	9.5	up
Cluster M	*Cf_996*	CDS	3.3	up	11.8	up
Cluster M	*Cf_997*	Flagellar L-ring protein precursor	2.7	up	9.2	up
Cluster M	*Cf_998*	CDS	2.0	up	7.9	up
Cluster M	*Cf_1000*	CDS	2.2	up	10.4	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster M	*Cf_1003*	CDS	2.5	up	12.8	up
Cluster M	*Cf_1004*	CDS	3.4	up	18.4	up
Cluster M	*Cf_1005*	CDS	2.3	up	10.8	up
Cluster M	*Cf_1006*	CDS	2.1	up	8.1	up
Cluster M	*Cf_1007*	Flagellar fliL protein.	2.4	up	12.1	up
Cluster M	*Cf_1010*	hypothetical protein predicted by Glimmer/Critica	2.8	up	17.7	up
Cluster M	*Cf_1013*	CDS	2.4	up	10.3	up
Cluster M	*Cf_1019*	CDS	2.4	up	10.2	up
Cluster M	*Cf_1029*	Transcriptional activator flhD.	2.3	up	2.3	up
Cluster M	*Cf_1030*	CDS	2.0	up	1.3	up
Cluster M	*Cf_1031*	CDS	2.5	up	3.1	up
Cluster M	*Cf_1033*	Probable transcriptional regulator ycf27	2.1	up	4.8	up
Cluster M	*Cf_1034*	chemotaxis protein CheA	2.4	up	3.5	up
Cluster M	*Cf_1035*	Chemotaxis protein cheW.	2.7	up	3.7	up
Cluster M	*Cf_1036*	Hypothetical 14.4 kDa protein y4sN.	2.1	up	1.8	up
Cluster N	*Cf_1047*	Terminase, ATPase subunit (GpP).	2.3	up	4.2	up
Cluster N	*Cf_1048*	Presumed capsid scaffolding protein (GpO).	2.3	up	4.2	up
Cluster N	*Cf_1050*	Terminase, endonuclease subunit (GpM).	2.2	up	3.1	up
Cluster N	*Cf_1054*	hypothetical protein predicted by Glimmer/Critica	2.1	up	3.4	up
Cluster N	*Cf_1055*	Protein lysB.	2.5	up	5.2	up
Cluster N	*Cf_1058*	prophage PSPPH02, putative adenine modification methytransferase	2.1	up	4.6	up
Cluster N	*Cf_1062*	hypothetical protein	2.5	up	5.6	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster N	*Cf_1063*	Tail fiber assembly protein homolog from lambdoid prophage DLP12.	2.3	up	4.8	up
Cluster N	*Cf_1064*	Major tail sheath protein (Protein FI).	2.1	up	3.7	up
Cluster N	*Cf_1068*	Minor tail protein Gp26.	2.4	up	5.1	up
Cluster N	*Cf_1073*	hypothetical protein predicted by Glimmer/Critica	2.1	up	3.7	up
Cluster N	*Cf_1074*	hypothetical protein	2.3	up	3.9	up
Cluster O	*Cf_2102*	hypothetical protein	2.2	up	4.1	up
Cluster O	*Cf_2103*	hypothetical protein	2.2	up	5.7	up
Cluster O	*Cf_2110*	hypothetical protein predicted by Glimmer/Critica	2.5	up	5.6	up
Cluster O	*Cf_2111*	hypothetical protein predicted by Glimmer/Critica	2.9	up	11.1	up
Cluster O	*Cf_2112*	hypothetical protein predicted by Glimmer/Critica	2.2	up	5.2	up
Cluster O	*Cf_2115*	hypothetical protein	2.4	up	4.7	up
Cluster P	*Cf_2031*	Hypothetical protein	3.59	up	5.39	up
Cluster P	*Cf_2032*	IMP dehydrogenase/GMP reductase, DJ-1/PfpI family	4.45	up	12.02	up
Cluster P	*Cf_2033*	Hypothetical protein	3.66	up	10.14	up
Cluster P	*Cf_2034*	Hypothetical protein	2.54	up	5.44	up
Cluster P	*Cf_2035*	Prokaryotic type, Ku70/Ku80 beta-barrel domain; High confidence in function and specificity	3.52	up	6.9	up
Cluster P	*Cf_2036*	DNA ligase (EC 6.5.1.1) (Polydeoxyribonucleotide synthase [ATP]), ATP-dependent DNA ligase	2.89	up	4.89	up
Cluster P	*Cf_2037*	Haloacid dehalogenase-like hydrolase;Hypothetical protein	2.75	up	2.38	up
Cluster P	*Cf_2038*	Hypothetical protein	3.39	up	1.12	down
Cluster P	*Cf_2039*	CsbD-like;Conserved hypothetical protein	2.98	up	1.24	up
Cluster Q	*Cf_3498*	Response regulator receiver;Hypothetical protein	2.6	up	1.04	up

	GENE_NAME	FUNCTION	Fold Change T1		Fold Change T2	
Cluster Q	*Cf_3499*	CheY-like, Response regulator receiver domain; Conserved hypothetical protein	4.2	up	4.96	up
Cluster Q	*Cf_3500*	Transport-associated;Conserved hypothetical protein	7.07	up	15.3	up
Cluster Q	*Cf_3501*	Hypothetical protein	5.16	up	11.71	up
Cluster Q	*Cf_3502*	CsbD-like, CsbD-like;Conserved hypothetical protein	3.45	up	6.98	up
plasmid	*pTer331_2*	hypothetical protein	2.3	up	30.7	up
plasmid	*pTer331_3*	hypothetical protein predicted by Glimmer/Critica	2.2	up	21.6	up
plasmid	*pTer331_9*	Transcriptional repressor protein korB.	2.0	up	22.0	up
plasmid	*pTer331_10*	exported protein	2.5	up	27.6	up
plasmid	*pTer331_12*	T-DNA border endonuclease virD2	2.1	up	30.9	up
plasmid	*pTer331_15*	conjugation protein	2.1	up	22.9	up
plasmid	*pTer331_16*	12-oxophytodienoate reductase 3	2.4	up	12.4	up
plasmid	*pTer331_17*	Protein virD4.	2.4	up	27.1	up
plasmid	*pTer331_18*	VirB11 protein.	2.1	up	31.2	up
plasmid	*pTer331_20*	Protein virB9 precursor.	2.3	up	28.3	up
plasmid	*pTer331_21*	Protein virB8.	2.3	up	26.7	up
plasmid	*pTer331_22*	hypothetical protein predicted by Glimmer/Critica	2.9	up	25.3	up
plasmid	*pTer331_23*	type IV secretion system protein VirB6	2.1	up	20.5	up
plasmid	*pTer331_25*	type IV secretion system protein VirB5	2.3	up	22.4	up
plasmid	*pTer331_26*	Protein virB4 precursor.	2.1	up	23.0	up
plasmid	*pTer331_32*	hypothetical protein predicted by Glimmer/Critica	2.5	up	19.5	up
plasmid	*pTer331_34*	hypothetical protein predicted by Glimmer/Critica	2.2	up	21.5	up
plasmid	*pTer331_35*	hypothetical protein predicted by Glimmer/Critica	2.5	up	13.9	up

Table A2. Genes differentially expressed in *A. niger* at T1

ORF code	GI-number	BlastP results	Fold change T1		Funcat2 category
An12g07530	145247106	strong similarity to hypothetical protein B2J23.80 - *Neurospora crassa*	2.9	down	01 metabolism
An01g11390	145230485	strong similarity to Sequence 385 from patent WO0100842 - *Corynebacterium glutamicum*	3.4	down	02 energy
An01g08550	145229937	strong similarity to aminotriazole resistance protein ATR1 - *Saccharomyces cerevisiae*	4.5	down	20 cellular transport, transport facilitation and transport routes
An01g11380	145230483	strong similarity to Sequence 1 from patent EP0845532 - *Pseudomonas sp*.	5.6	down	99 unclassified protein
An15g05660	145251163	hypotetical protein	2.1	up	99 unclassified protein
An13g03910	145248545	strong similarity to dihydropyrimidinase PYD2 - *Saccharomyces kluyveri*	2.3	up	01 metabolism
An07g00370	145236966	strong similarity to allantoin transport protein DAL4 - *Saccharomyces cerevisiae*	2.3	up	20 cellular transport, transport facilitation and transport routes
An12g09920	145247586	weak similarity to PG123 patent WO9929870-A1 - *Porphorymonas gingivalis*	2.4	up	99 unclassified protein
An13g01520	145248085	hypothetical protein	2.6	up	99 unclassified protein
An07g08770	145238608	strong similarity to allantoate permease DAL5 - *Saccharomyces cerevisiae*	2.7	up	20 cellular transport, transport facilitation and transport routes
An02g11180	145233749	hypothetical protein	3.1	up	99 unclassified protein
An18g01740	145254465	strong similarity to aliphatic nitrilase - *Rhodococcus rhodochrous*	3.8	up	32 cell rescue, defense and virulence
An10g00730	145256235	strong similarity to aliphatic nitrilase - *Rhodococcus rhodochrous* K22	3.9	up	32 cell rescue, defense and virulence
An09g01140	145241572	similarity to patent WO200073470-A2 glucanase ZmGnsN3 - *Zea mays*	4.1	up	99 unclassified protein
An16g01850	145251970	similarity to blastomyces yeast phase-specific protein 1 bys1 - *Ajellomyces dermatitidis*	5.4	up	40 cell fate
An16g01400	145251880	similarity to cutinase transcription factor 1 CTF1 alpha - *Hematonectria haematococca*	8.7	up	11 transcription
An12g10200	145247636	weak similarity to ice nucleation gene inaX - *Xanthomonas campestris*	9.2	up	99 unclassified protein

ORF code	GI-number	BlastP results	Fold change T1		Funcat2 category
An01g14540	145231158	strong similarity to the hypothetical protein encoded by An13g01370 - *Aspergillus niger*	9.3	up	11 transcription
An02g01550	145231639	strong similarity to secreted serine protease 19 kDa CS antigen CS-Ag - *Coccidioides immitis*	9.6	up	32 cell rescue, defense and virulence
An01g06750	145229579	similarity to hypothetical acetyltransferase SCD72A.11 - *Streptomyces coelicolor*	11.6	up	99 unclassified protein
An01g14550	145231160	strong similarity to cyanide hydratase Cht - *Gloeocercospora sorghi*	101.8	up	32 cell rescue, defense and virulence

Table A3. Genes differentially expressed in *A. niger* at T2

ORF code	GI-number	BlastP results	Fold change T2		Funcat2 category
An12g07750	145247154	putative GPI anchored protein	2.1	down	99 unclassified protein
An08g09420	145240813	cell wall galactomannoprotein	2.1	down	99 unclassified protein
An01g11390	145230485	strong similarity to Sequence 385 from patent WO0100842 - *Corynebacterium glutamicum*	2.2	down	02 energy
An04g01690	145256851	similarity to glycoprotein X precursor - equine herpesvirus 1	2.3	down	99 unclassified protein
An16g06800	145252938	strong similarity to endoglucanase eglB - *Aspergillus niger*	2.6	down	01 metabolism
An16g05920	145252774	weak similarity to surface recognition protein PTH11 - *Magnaporthe grisea*	3.0	down	43 cell type differentiation
An01g12170	145230720	strong similarity to alcohol dehydrogenase B alcB - *Aspergillus nidulans*	3.1	down	01 metabolism
An09g06400	145242762	strong similarity to chitinase chiA - *Aspergillus nidulans*	3.1	down	01 metabolism
An06g00930	145236545	hypothetical protein	3.5	down	99 unclassified protein
An08g09680	145240863	strong similarity to 2,4 -dihydroxyacetophenone dioxygenase dad - *Alcaligenes* sp	3.6	down	99 unclassified protein
An16g05910	145252772	similarity to cholesterol 7alpha-hydroxylase CYP7 - *Sus scrofa*	4.2	down	01 metabolism
An01g11380	145230483	strong similarity to Sequence 1 from patent EP0845532 - *Pseudomonas* sp.	4.4	down	99 unclassified protein
An06g00900	145236541	hypothetical protein	5.8	down	99 unclassified protein
An06g00940	145236547	weak similarity to nucleobinding precursor NUCB1 - *Homo sapiens*	9.0	down	99 unclassified protein
An07g05900	145238042	strong similarity to fructosyl amino acid oxidase faoA - *Aspergillus terreus*	2.0	up	01 metabolism
An02g00560	145231426	strong similarity to uric acid-xanthine permease uapA - *Aspergillus nidulans*	2.1	up	20 cellular transpot, transport facilitation and transport routes
An03g05630	145235587	weak similarity to hypothetical protein An11g08750 - *Aspergillus niger*	2.1	up	99 unclassified protein

ORF code	GI-number	BlastP results	Fold change T2		Funcat2 category
An01g05820	145229395	strong similarity to homoprotocatechuate operon enzyme hpcE - *Escherichia coli*	2.1	up	01 metabolism
An08g08010	145240539	similarity to hypothetical protein MmcH - *Streptomyces lavendulae*	2.3	up	99 unclassified protein
An03g01220	145234751	weak similarity to apyrase of patent GB2261878-A - *Shigella flexneri*	2.3	up	99 unclassified protein
An12g02660	145246192	hypothetical protein	2.5	up	99 unclassified protein
An08e08270		trnaRacg	2.7	up	99 unclassified protein
An13g03910	145248545	strong similarity to dihydropyrimidinase PYD2 - *Saccharomyces kluyveri*	3.0	up	01 metabolism
An08g08000	145240537	strong similarity to maltose transport protein MAL31 - *Saccharomyces cerevisiae*	3.2	up	20 cellular transpot, transport facilitation and transport routes
An04g02760	145257196	strong similarity to starvation-sensing protein RspA - *Escherichia coli*	3.2	up	01 metabolism
An03g00580	145234627	strong similarity to the cytochrome P450 protein eln2 SEQ ID NO:1 of patent JP2000152788-A - *Coprinus cinereus*	3.4	up	01 metabolism
An08g03810	145239703	putative DNA-binding protein	3.5	up	99 unclassified protein
An07g04260	145237722	strong similarity to 3-hydroxy-3-methylglutaryl-coenzyme A synthase HMGS - *Saccharomyces cerevisiae*	3.9	up	01 metabolism
An01g00200	145228325	strong similarity to succinyl-CoA:3-ketoacid-CoA transferase SCOT - *Homo sapiens*	4.0	up	01 metabolism
An12g09940	145247590	strong similarity to stearoyl-CoA desaturase Ole1 - *Ajellomyces capsulata*	4.0	up	01 metabolism
An01g07940	145229821	weak similarity to protein Y38B5A.a - *Caenorhabditis elegans*	4.0	up	99 unclassified protein
An01g10790	145230375	strong similarity to hypothetical conidiation-specific protein con-10 - *Neurospora crassa*	5.5	up	43 cell type differentiation
An16g07700	145253112	similarity to dapA - *Corynebacterium glutamicum*	5.8	up	01 metabolism
An04g02220	145257019	strong similarity to L-serine dehydratase CHA1 - *Saccharomyces cerevisiae*	6.3	up	01 metabolism

ORF code	GI-number	BlastP results	Fold change T2		Funcat2 category
An11g01330	145243202	similarity to hypothetical protein encoded by SMc00431 - *Sinorhizobium meliloti*	7.7	up	99 unclassified protein
An16g01400	145251880	similarity to cutinase transcription factor 1 CTF1 alpha - *Hematonectria haematococca*	22.8	up	11 transcription
An01g06750	145229579	similarity to hypothetical acetyltransferase SCD72A.11 - *Streptomyces coelicolor*	112.3	up	99 unclassified protein

Table A 4. *Collimonas*-signature genes

Gene	D. E. *	Gene function
Colli20061116_71		hypothetical protein
Colli20061116_72		hypothetical protein
Colli20061116_74		hypothetical protein
Colli20061116_96		hypothetical protein
Colli20061116_103		Probable D-methionine-binding lipoprotein metQ precursor
Colli20061116_111		hypothetical protein
Colli20061116_142		hypothetical protein
Colli20061116_171		hypothetical protein
Colli20061116_172		hypothetical protein
Colli20061116_181		Putative ski2-type helicase (EC 3.6.1.-).
Colli20061116_183		putative aminotransferase
Colli20061116_186		protein phosphatase 2C
Colli20061116_192		2;4-dienoyl-CoA reductase
Colli20061116_197		Glutamate receptor 3.4 precursor
Colli20061116_199		Glutathione S-transferase D2
Colli20061116_204		hypothetical protein
Colli20061116_208		hypothetical protein predicted by Glimmer/Critica
Colli20061116_209		Transcriptional activator protein solR.
Colli20061116_211		hypothetical protein
Colli20061116_216		3-oxoadipate CoA-transferase subunit B
Colli20061116_232		peptidyl-dipeptidase
Colli20061116_234		Protein HI0882.
Colli20061116_237		hypothetical protein
Colli20061116_239	YES	Transcriptional activator protein czcR.
Colli20061116_238	YES	hypothetical protein
Colli20061116_252		SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5
Colli20061116_265		Protein PA3922 precursor.
Colli20061116_267		Niemann-Pick C1 protein precursor.
Colli20061116_268		Ycf48-like protein precursor.
Colli20061116_270		hypothetical protein
Colli20061116_275		Epoxide hydrolase 2
Colli20061116_276	YES	Pesticin receptor precursor (IRPC).

Gene	D. E. *	Gene function
Colli20061116_290		ketoglutarate semialdehyde dehydrogenase
Colli20061116_291		dihydrodipicolinate synthetase
Colli20061116_292		Uxu operon transcriptional regulator.
Colli20061116_294		galactarate dehydratase
Colli20061116_318		carboxymethylenebutenolidase
Colli20061116_462		50S ribosomal protein L3.
Colli20061116_466		30S ribosomal protein S19.
Colli20061116_470		50S ribosomal protein L29.
Colli20061116_498		putative haloacid dehalogenase-like hydrolase
Colli20061116_504		Protein cyaY.
Colli20061116_511		Cell division protein ftsA.
Colli20061116_513		fimbrial assembly membrane protein
Colli20061116_516		3-dehydroquinate synthase
Colli20061116_517		putative phosphohydrolase
Colli20061116_543		putative thiolase
Colli20061116_546		UPF0126 membrane protein HI1240.
Colli20061116_547		Vitamin B12 transporter btuB precursor
Colli20061116_550		AMP nucleosidase
Colli20061116_553		2-nitropropane dioxygenase
Colli20061116_557		glutamate synthase
Colli20061116_581		hypothetical protein
Colli20061116_597		Possible multiple antibiotic resistance protein
Colli20061116_602		enoyl-CoA hydratase
Colli20061116_606		cytochrome c oxidase; subunit II
Colli20061116_607		cytochrome c oxidase; subunit I
Colli20061116_620		RNA polymerase sigma factor rpoD (Sigma-42).
Colli20061116_630		Inner membrane protein ytfF.
Colli20061116_631		Arabinose operon regulatory protein.
Colli20061116_641		D-aminopeptidase (EC 3.4.11.-).
Colli20061116_646		putative peptide transport system ATP-binding protein
Colli20061116_653	YES	formate dehydrogenase A chain
Colli20061116_659		peptidyl-tRNA hydrolase
Colli20061116_661		ribose-phosphate pyrophosphokinase
Colli20061116_684		Ferric transport system permease protein fbpB.

Gene	D. E. *	Gene function
Colli20061116_689		phosphonoacetate hydrolase
Colli20061116_690		aldehyde dehydrogenase
Colli20061116_691		Uncharacterized protein L432.
Colli20061116_700		Spermidine/putrescine transport system permease protein potC.
Colli20061116_702		hypothetical protein
Colli20061116_703		protein of unknown function DUF502
Colli20061116_708		hypothetical protein
Colli20061116_709		RNA polymerase sigma-E factor (Sigma-24).
Colli20061116_736		Leu/Ile/Val-binding protein precursor (LIV-BP).
Colli20061116_742		C4-dicarboxylate transport protein 2.
Colli20061116_750		indole-3-glycerol phosphate synthase
Colli20061116_751		anthranilate synthase component II
Colli20061116_753		anthranilate synthase component I
Colli20061116_754		2-phosphoglycolate phosphatase
Colli20061116_757		putative membrane-bound transglycolase
Colli20061116_759		putative enoyl-CoA hydratase
Colli20061116_760		Holliday junction ATP-dependent DNA helicase ruvA
Colli20061116_761		Holliday junction ATP-dependent DNA helicase ruvB
Colli20061116_779		twin-arginine translocation pathway signal
Colli20061116_807		Twitching motility protein.
Colli20061116_812		Hydrogen peroxide-inducible genes activator
Colli20061116_813		ATP-dependent DNA helicase RecG
Colli20061116_814	YES	S-adenosylmethionine:tRNA ribosyltransferase-isomerase
Colli20061116_816		queuine tRNA-ribosyltransferase
Colli20061116_830		Biotin carboxyl carrier protein of acetyl-CoA carboxylase
Colli20061116_894		hypothetical protein
Colli20061116_899		Bacitracin resistance protein BacA
Colli20061116_902		rubredoxin reductase
Colli20061116_912		Hypothetical protein MJ0900.
Colli20061116_922		putative membrane protein
Colli20061116_933		Putative molybdopterin biosynthesis protein MJ0886.
Colli20061116_935		NAD-dependent formate dehydrogenase gamma subunit
Colli20061116_936		NAD-dependent formate dehydrogenase beta subunit
Colli20061116_983		GTP-binding signal recognition particle SRP54; G-domain

Gene	D. E. *	Gene function
Colli20061116_1076		RNA polymerase sigma factor rpoD (Sigma-70).
Colli20061116_1077		DNA primase
Colli20061116_1082		Carboxylate-amine ligase RSc3298 (EC 6.3.-.-).
Colli20061116_1085		Probable RNA polymerase sigma-D factor.
Colli20061116_1087		O-sialoglycoprotein endopeptidase
Colli20061116_1088		Putative ATP-dependent RNA helicase rhlE
Colli20061116_1089		tRNA synthetases class I domain protein
Colli20061116_1090		Putative HTH-type transcriptional regulator ydhB.
Colli20061116_1091		P25 protein (Brefeldin A resistance protein).
Colli20061116_1093		DNA polymerase III; alpha subunit
Colli20061116_1092		UPF0176 protein PSPTO_1734.
Colli20061116_1099		Maf-like protein NE0356.
Colli20061116_1105		UPF0082 protein MCA1220.
Colli20061116_1147		xylulokinase
Colli20061116_1149		maltose/maltodextrin transport ATP-binding protein
Colli20061116_1152		Maltose transport system permease protein malF.
Colli20061116_1153		Putative ABC transporter periplasmic-binding protein ycjN precursor.
Colli20061116_1156	YES	hypothetical protein predicted by Glimmer/Critica
Colli20061116_1164	YES	hypothetical protein
Colli20061116_1180		cadmium-translocating P-type ATPase
Colli20061116_1181		Sulfide:quinone oxidoreductase; mitochondrial precursor
Colli20061116_1182		Hydroxyacylglutathione hydrolase cytoplasmic
Colli20061116_1184		Hypothetical protein ybcJ.
Colli20061116_1190		spermidine N1-acetyltransferase
Colli20061116_1207		Hypothetical response regulatory protein VCA0850.
Colli20061116_1219		acyl-CoA dehydrogenase
Colli20061116_1221		30S ribosomal protein S16.
Colli20061116_1223		tRNA (guanine-N1)-methyltransferase
Colli20061116_1225		Probable nudix hydrolase C6G9.05 (EC 3.6.1.-).
Colli20061116_1233		chorismate binding enzyme
Colli20061116_1258		Maltose transport system permease protein malG.
Colli20061116_1261		acyl-CoA dehydrogenase
Colli20061116_1267		putative acyl-CoA thiolase
Colli20061116_1273		Protein tolQ.

Gene	D. E. *	Gene function
Colli20061116_1274		Putative biopolymer transport exbD-like protein 1.
Colli20061116_1275		tetraacyldisaccharide 4'-kinase
Colli20061116_1278		adenylate kinase
Colli20061116_1280		patatin
Colli20061116_1288		50S ribosomal protein L28.
Colli20061116_1289		DNA repair protein radC homolog.
Colli20061116_1292		Galactoside transport system permease protein mglC.
Colli20061116_1293		High-affinity branched-chain amino acid transport system permease protein livM
Colli20061116_1326		Cation transport protein chaC.
Colli20061116_1341		succinyl-diaminopimelate desuccinylase
Colli20061116_1343		Twitching motility protein.
Colli20061116_1344		2;3;4;5-tetrahydropyridine-2;6-dicarboxylate N-succinyltransferase
Colli20061116_1349		putative membrane protein
Colli20061116_1350		DNA ligase
Colli20061116_1353		Glutathione-regulated potassium-efflux system protein kefB
Colli20061116_1355		Outer membrane pore protein E precursor.
Colli20061116_1363		ketoglutarate semialdehyde dehydrogenase
Colli20061116_1380		cyanophycin synthetase
Colli20061116_1384	YES	protein-P-II uridylyltransferase
Colli20061116_1390		undecaprenyl pyrophosphate synthetase
Colli20061116_1392		1-deoxy-D-xylulose 5-phosphate reductoisomerase
Colli20061116_1400		ribonuclease HII
Colli20061116_1431	YES	cytidine/deoxycytidylate deaminase; zinc-binding region
Colli20061116_1442		L-aspartate oxidase
Colli20061116_1444		Proline/betaine transporter
Colli20061116_1445		siderophore biosynthesis protein; putative
Colli20061116_1449		putative oxidoreductase
Colli20061116_1451		Putative HTH-type transcriptional regulator ywbI.
Colli20061116_1466		porphobilinogen deaminase
Colli20061116_1469	YES	Protein hemY.
Colli20061116_1480		ATP-dependent RNA helicase hrpA homolog
Colli20061116_1481		deoxycytidine triphosphate deaminase
Colli20061116_1487		Glucans biosynthesis glucosyltransferase H
Colli20061116_1490		Probable macrolide-specific efflux protein macA precursor.

Gene	D. E. *	Gene function
Colli20061116_1501		glucose-6-phosphate 1-dehydrogenase
Colli20061116_1503		Outer membrane pore protein E precursor.
Colli20061116_1509		glucose-6-phosphate isomerase
Colli20061116_1510		6-phosphogluconate dehydratase
Colli20061116_1521		putative membrane-bound lytic murein transglycosylase D precursor
Colli20061116_1522		hydroxyacylglutathione hydrolase
Colli20061116_1539		alpha;alpha-trehalose-phosphate synthase (UDP-forming)
Colli20061116_1558		Multidrug resistance outer membrane protein mdtQ precursor.
Colli20061116_1562		Translation initiation factor IF-3.
Colli20061116_1563	YES	50S ribosomal protein L20.
Colli20061116_1566		phenylalanyl-tRNA synthetase; beta subunit
Colli20061116_1567		Integration host factor beta-subunit (IHF-beta).
Colli20061116_1568		HTH-type transcriptional regulator mlrA
Colli20061116_1574		Nodulation protein D.
Colli20061116_1578		Serine/threonine-protein kinase pkn1
Colli20061116_1580		acyl carrier protein phosphodiesterase
Colli20061116_1581		2-isopropylmalate synthase
Colli20061116_1585		Putative tartrate transporter.
Colli20061116_1586		hypothetical protein predicted by Glimmer/Critica
Colli20061116_1598		cystathionine gamma-lyase-like protein
Colli20061116_1627		hypothetical protein
Colli20061116_1628		Protein HI0441 (ORFJ).
Colli20061116_1636		Chaperone protein fimC precursor.
Colli20061116_1642		probable pyridoxal phosphate aminotransferase protein
Colli20061116_1647		putative serine/threonine dehydratase
Colli20061116_1653		NADH dehydrogenase I; D subunit
Colli20061116_1662		NADH-ubiquinone oxidoreductase; chain M
Colli20061116_1663		proton-translocating NADH-quinone oxidoreductase; chain N
Colli20061116_1672		Lipase 1 precursor
Colli20061116_1703		putative epoxide hydrolase protein
Colli20061116_1704		hypothetical protein
Colli20061116_1705		Putative metal-dependent hydrolase DR_0841
Colli20061116_1706		Mechanosensory protein 2.
Colli20061116_1707		Egl nine homolog 3

Gene	D. E. *	Gene function
Colli20061116_1708		PKHD-type hydroxylase piuC
Colli20061116_1709		Probable tonB-dependent receptor bfrD precursor
Colli20061116_1721		Probable multidrug resistance protein
Colli20061116_1722	YES	probable multidrug resistance transmembrane protein
Colli20061116_1726		hypothetical protein
Colli20061116_1727		cytochrome c oxidase subunit II
Colli20061116_1729	YES	Probable cytochrome c oxidase subunit 3
Colli20061116_1730		cytochrome c oxidase subunit III
Colli20061116_1739		dihydrodipicolinate synthase
Colli20061116_1740		Lipoprotein 34 precursor.
Colli20061116_1741	YES	Coenzyme PQQ synthesis protein B
Colli20061116_1742		hypothetical protein predicted by Glimmer/Critica
Colli20061116_1746		DNA mismatch repair protein mutS.
Colli20061116_1759		hypothetical protein
Colli20061116_1788		gamma-glutamyltranspeptidase
Colli20061116_1811		UPF0350 protein YPTB3173.
Colli20061116_1815		dihydrolipoamide dehydrogenase
Colli20061116_1818		Hypothetical protein yhcM.
Colli20061116_1823		exopolyphosphatase
Colli20061116_1832		Competence lipoprotein comL precursor.
Colli20061116_1835		probable transmembrane protein
Colli20061116_1859		Plasma membrane iron permease.
Colli20061116_1869		branched-chain amino acid transport system ATP-binding protein
Colli20061116_1870		hypothetical protein predicted by Glimmer/Critica
Colli20061116_1884		HAM1 protein homolog.
Colli20061116_1885		putative oxygen-independent coproporphyrinogen III oxidase
Colli20061116_1902		Protein icc homolog.
Colli20061116_1916		hypothetical protein
Colli20061116_1922		HTH-type transcriptional regulator glxA.
Colli20061116_1929		3-isopropylmalate dehydratase small subunit
Colli20061116_1931		aspartate-semialdehyde dehydrogenase
Colli20061116_1932		Absent in melanoma 1 protein.
Colli20061116_1933		tRNA pseudouridine synthase A
Colli20061116_1935		tryptophan synthase beta chain

Gene	D. E. *	Gene function
Colli20061116_1936		tryptophan synthase; alpha chain
Colli20061116_1939		Transient receptor potential protein.
Colli20061116_1944		HTH-type protein slmA.
Colli20061116_1962		3-oxoacyl-(acyl-carrier-protein) reductase; putative
Colli20061116_1972		nucleoside-diphosphate kinase
Colli20061116_1978		UPF0070 protein 1057.
Colli20061116_1984		putative inner membrane-anchored protein
Colli20061116_1985		ATP phosphoribosyltransferase regulatory subunit
Colli20061116_1988		hypothetical protein Y4HM precursor
Colli20061116_1991		Ribose transport system permease protein rbsC
Colli20061116_2017		Hypothetical protein RA0937.
Colli20061116_2022		Probable chromate transport protein.
Colli20061116_2027		hypothetical protein
Colli20061116_2029		ABC-type transport system ipermease component
Colli20061116_2055	YES	hypothetical protein predicted by Glimmer/Critica
Colli20061116_2062		putative enoyl-CoA hydratase/isomerase family protein
Colli20061116_2064	YES	NAD-dependent formate dehydrogenase gamma subunit
Colli20061116_2065	YES	NAD-dependent formate dehydrogenase beta subunit
Colli20061116_2085		glycine dehydrogenase
Colli20061116_2086		glycine cleavage system T protein
Colli20061116_2088		serine O-acetyltransferase
Colli20061116_2122		hypothetical protein
Colli20061116_2129	YES	TetR-family transcriptional regulator
Colli20061116_2132		Putative thiosulfate sulfurtransferase
Colli20061116_2133		hypothetical protein
Colli20061116_2136		lipoprotein; putative
Colli20061116_2140		3-oxoadipate enol-lactone hydrolase / 4-carboxymuconolactone decarboxylase
Colli20061116_2141		3-carboxy-cis;cis-muconate cycloisomerase
Colli20061116_2143		protocatechuate 3;4-dioxygenase; beta subunit
Colli20061116_2144		Acetate operon repressor.
Colli20061116_2147		4-hydroxyphenylpyruvate dioxygenase
Colli20061116_2149		4-hydroxyphenylpyruvate dioxygenase
Colli20061116_2153		Shikimate transporter.
Colli20061116_2159		hypothetical protein predicted by Glimmer/Critica

Gene	D. E. *	Gene function
Colli20061116_2161		putative oxidoreductase
Colli20061116_2165		ABC transporter; nucleotide binding/ATPase protein
Colli20061116_2168		Proline-rich protein 6.
Colli20061116_2173		hypothetical protein
Colli20061116_2181		Inner membrane ABC transporter permease protein yehW.
Colli20061116_2184		Hypothetical protein yehZ precursor.
Colli20061116_2206		malate dehydrogenase (acceptor)
Colli20061116_2218		NADH dehydrogenase
Colli20061116_2223		probable transporter lipoprotein transmembrane
Colli20061116_2224		Antibiotic efflux pump periplasmic linker protein arpA precursor.
Colli20061116_2232		GntR family regulatory protein
Colli20061116_2238		Leu/Ile/Val-binding protein precursor (LIV-BP).
Colli20061116_2254		aldehyde dehydrogenase
Colli20061116_2261		sensor histidine kinase
Colli20061116_2265		cytochrome o ubiquinol oxidase; subunit I
Colli20061116_2274		short chain dehydrogenase
Colli20061116_2278	YES	Hypothetical protein MJ0900.
Colli20061116_2289		Hypothetical protein yaiT precursor.
Colli20061116_2301		UPF0194 membrane protein ybhG precursor.
Colli20061116_2309		Membrane transporter of Superfamily (MFS)
Colli20061116_2312		C4-dicarboxylate transport sensor protein
Colli20061116_2316		peptidase; M24 family protein
Colli20061116_2321		Inner membrane protein yebS.
Colli20061116_2322		Paraquat-inducible protein A.
Colli20061116_2332		aldehyde dehydrogenase
Colli20061116_2340		cardiolipin synthase
Colli20061116_2351		Protein gvpE 1.
Colli20061116_2357		L-ornithine 5-monooxygenase
Colli20061116_2372		Molybdopterin-converting factor subunit 1
Colli20061116_2374		threonine synthase
Colli20061116_2378		(p)ppGpp synthetase I (GTP pyrophosphokinase); SpoT/RelA
Colli20061116_2382		hypothetical protein predicted by Glimmer/Critica
Colli20061116_2392	YES	Galactoside transport system permease protein mglC homolog.
Colli20061116_2398		aspartokinase

Gene	D. E. *	Gene function
Colli20061116_2401		DNA-3-methyladenine glycosylase
Colli20061116_2402		cysteinyl-tRNA synthetase
Colli20061116_2404		peptidyl-prolyl cis-trans isomerase B
Colli20061116_2407		serine O-acetyltransferase
Colli20061116_2424		Hypothetical protein yieF.
Colli20061116_2425		putative redox protein
Colli20061116_2433		Chemotaxis protein cheW.
Colli20061116_2434		sensor histidine kinase/response regulator
Colli20061116_2435		protein-glutamate methylesterase
Colli20061116_2451		urocanate hydratase
Colli20061116_2456		High-affinity branched-chain amino acid transport ATP-binding protein livG
Colli20061116_2463		putative peptidyl-prolyl cis-trans isomerase
Colli20061116_2476		Outer membrane usher protein fimD precursor.
Colli20061116_2478		malate synthase A
Colli20061116_2479		Probable HTH-type transcriptional regulator ltrA.
Colli20061116_2484		DNA repair protein radA homolog
Colli20061116_2485		alanine racemase
Colli20061116_2488		hypothetical protein
Colli20061116_2496		acyl-CoA thioesterase I precursor
Colli20061116_2505		Multidrug resistance protein mdtC
Colli20061116_2508		putative phytoene synthase
Colli20061116_2509	YES	Phytoene dehydrogenase; chloroplast precursor
Colli20061116_2510		L-lactate dehydrogenase
Colli20061116_2511		Ankyrin-3 (ANK-3) (Ankyrin-G).
Colli20061116_2512		putative deoxyribonuclease
Colli20061116_2513		ribonuclease PH
Colli20061116_2521		glycosyl transferase; family 39
Colli20061116_2531		Taurine transport system permease protein tauC.
Colli20061116_2533	YES	Protein NMT1 homolog
Colli20061116_2538		Probable Multidrug resistance translocase
Colli20061116_2545		30S ribosomal protein S18.
Colli20061116_2554		sensory transduction histidine kinases
Colli20061116_2564		carbamoyl-phosphate synthase; small subunit
Colli20061116_2570		Phosphate transport system protein phoU.

Gene	D. E. *	Gene function
Colli20061116_2575		polyphosphate kinase
Colli20061116_2576		2;3-bisphosphoglycerate-dependent phosphoglycerate mutase
Colli20061116_2590		hypothetical protein
Colli20061116_2613		Putative HTH-type transcriptional regulator ydcN.
Colli20061116_2644		putative carboxy-terminal processing protease precursor
Colli20061116_2652		Probable multidrug resistance protein mdtA precursor
Colli20061116_2654		Toluene efflux pump outer membrane protein ttgF precursor.
Colli20061116_2655		transporter; putative
Colli20061116_2662		Hydrogen peroxide-inducible genes activator.
Colli20061116_2670		ABC-type sugar transport system; ATPase component
Colli20061116_2671		Predicted N-acetylglucosamine kinase
Colli20061116_2675		Hypothetical protein yddH.
Colli20061116_2676		3-oxoacyl-
Colli20061116_2680		hypothetical protein
Colli20061116_2682		DNA polymerase III alpha subunit
Colli20061116_2685		hypothetical protein; INTERPRO suggestion: probable haloacid dehalogenase-like hydrolase
Colli20061116_2713		hypothetical protein predicted by Glimmer/Critica
Colli20061116_2747		seryl-tRNA synthetase
Colli20061116_2762		oxidoreductase; FAD-binding
Colli20061116_2771		Protein fecR.
Colli20061116_2798	YES	lipopolysaccharide biosynthesis
Colli20061116_2808		Probable NADPH:quinone oxidoreductase 2
Colli20061116_2812		Nitrilotriacetate monooxygenase component A
Colli20061116_2817		hypothetical protein
Colli20061116_2827		cytochrome d terminal oxidase; polypeptide subunit I
Colli20061116_2838		putative ABC transport protein; substrate-binding component
Colli20061116_2845		Multidrug resistance protein B.
Colli20061116_2847		ABC transporter ATP-binding protein
Colli20061116_2851		Hypothetical protein yraQ.
Colli20061116_2866		xanthine dehydrogenase
Colli20061116_2874		Hypothetical ABC transporter ATP-binding protein MJ0412.
Colli20061116_2875		Pyrimidine precursor biosynthesis enzyme THI13.
Colli20061116_2884		nitrilotriacetate monooxygenase
Colli20061116_2888		putative amidase

Gene	D. E. *	Gene function
Colli20061116_2913	YES	High-affinity branched-chain amino acid transport system permease protein livH
Colli20061116_2914	YES	High-affinity branched-chain amino acid transport system permease protein livM
Colli20061116_2915	YES	High-affinity branched-chain amino acid transport ATP-binding protein livG
Colli20061116_2917		Urease accessory protein ureD.
Colli20061116_2918		urease; gamma subunit
Colli20061116_2931		putative holo-
Colli20061116_2933		DNA repair protein recO (Recombination protein O).
Colli20061116_2935		ribonuclease III
Colli20061116_2944		3-oxoacyl-
Colli20061116_2946		3-oxoacyl-
Colli20061116_2968		chorismate synthase
Colli20061116_2996		argininosuccinate lyase
Colli20061116_2999		High-affinity branched-chain amino acid transport system permease protein livM
Colli20061116_3004		putative lipoprotein
Colli20061116_3007		hypothetical protein predicted by Glimmer/Critica
Colli20061116_3011	YES	HTH-type transcriptional repressor purR
Colli20061116_3013		putative ATP-binding protein of ribose ABC transport system
Colli20061116_3015		Protein pmbA.
Colli20061116_3019		probable carboxylesterase
Colli20061116_3022		copper-translocating P-type ATPase
Colli20061116_3033		amino-acid N-acetyltransferase (ArgA)
Colli20061116_3034		Probable Fe(2+)-trafficking protein.
Colli20061116_3035		putative zinc protease
Colli20061116_3044		N-acetylglucosamine-6-phosphate deacetylase
Colli20061116_3084		hypothetical protein
Colli20061116_3090		histidine ammonia-lyase
Colli20061116_3094		Transcriptional regulator modE.
Colli20061116_3095		aliphatic sulfonates ABC transporter; ATP-binding protein
Colli20061116_3096		Putative aliphatic sulfonates transport permease protein ssuC.
Colli20061116_3100		NADH-dependent FMN reductase
Colli20061116_3101		Thiosulfate-binding protein precursor.
Colli20061116_3103		Membrane protein mosC.
Colli20061116_3111		Histone deacetylase 1 (HD1).
Colli20061116_3112		hypothetical protein

Gene	D. E. *	Gene function
Colli20061116_3118		Protein nirF.
Colli20061116_3119		peptidylprolyl isomerase
Colli20061116_3122		2-hydroxyacid dehydrogenase
Colli20061116_3132		putative lipoprotein
Colli20061116_3133		tRNA-processing ribonuclease BN
Colli20061116_3179		RecJ exonuclease
Colli20061116_3190		Heat shock 70 kDa protein (HSP70).
Colli20061116_3193		Co-chaperone protein hscB homolog.
Colli20061116_3195		Iron sulfur cluster assembly protein 1; mitochondrial precursor
Colli20061116_3201		UvrABC system protein B
Colli20061116_3202		aromatic-amino-acid transaminase
Colli20061116_3222		xylose isomerase
Colli20061116_3230		Probable HTH-type transcriptional regulator ltrA.
Colli20061116_3247		Inner membrane ABC transporter permease protein yddR.
Colli20061116_3253		HTH-type transcriptional regulator pecT
Colli20061116_3265		Putative HTH-type transcriptional regulator PH1519.
Colli20061116_3266	YES	hypothetical protein
Colli20061116_3267		serine protease
Colli20061116_3271		Putative HTH-type transcriptional regulator ygiP.
Colli20061116_3272		Multidrug resistance protein mdtM.
Colli20061116_3275		hypothetical protein
Colli20061116_3285		homogentisate 1;2-dioxygenase
Colli20061116_3286		4-hydroxybenzoate transporter.
Colli20061116_3288		Protein emrE
Colli20061116_3291		ATP-dependent DNA ligase
Colli20061116_3293		exonuclease
Colli20061116_3294		STE24 endopeptidase
Colli20061116_3297		argininosuccinate synthase
Colli20061116_3298		UPF0345 protein MCA1207.
Colli20061116_3314		Protein tldD homolog.
Colli20061116_3316		Hypothetical protein yhdP precursor.
Colli20061116_3324		riboflavin biosynthesis protein
Colli20061116_3326		lipoprotein signal peptidase
Colli20061116_3333		Cold shock-like protein cspA.

Gene	D. E. *	Gene function
Colli20061116_3335		pseudouridine synthase; Rsu
Colli20061116_3340		Virulence factor mviN homolog.
Colli20061116_3343		ABC transporter related
Colli20061116_3344		Hypothetical signaling protein CC0091.
Colli20061116_3367		Activator of 90 kDa heat shock protein ATPase homolog 1
Colli20061116_3379		thermolabile hemolysin
Colli20061116_3421		Hypothetical transport protein yetK.
Colli20061116_3423		fumarate hydratase; class I
Colli20061116_3464		glucose-methanol-choline oxidoreductase
Colli20061116_3472		Fimbrial protein precursor
Colli20061116_3473	YES	protein of unknown function DUF193
Colli20061116_3475	YES	Pesticin receptor precursor (IRPC) (IPR65).
Colli20061116_3476		putative oxidoreductase
Colli20061116_3478		Protein tolQ.
Colli20061116_3479		Biopolymer transport exbD1 protein.
Colli20061116_3482		Peptidoglycan-associated lipoprotein precursor.
Colli20061116_3483		Aspartyl/asparaginyl beta-hydroxylase
Colli20061116_3484		Hypothetical protein sll1773.
Colli20061116_3487		hypothetical protein
Colli20061116_3488		Adenine deaminase
Colli20061116_3489		Hypothetical protein yaiC.
Colli20061116_3497		Virulence sensor protein bvgS precursor
Colli20061116_3503		putative glutamate synthase
Colli20061116_3504		maleylacetoacetate isomerase
Colli20061116_3505		hypothetical protein
Colli20061116_3506		probable aminotransferase
Colli20061116_3507		N utilization substance protein B homolog
Colli20061116_3508		6;7-dimethyl-8-ribityllumazine synthase
Colli20061116_3509		3;4-dihydroxy-2-butanone 4-phosphate synthase
Colli20061116_3510		tRNA-dihydrouridine synthase A
Colli20061116_3513		omega-amino acid-pyruvate aminotransferase
Colli20061116_3514		GTP-binding protein typA/BipA
Colli20061116_3515		tRNA pseudouridine synthase B
Colli20061116_3516		Ribosome-binding factor A.

Gene	D. E. *	Gene function
Colli20061116_3517		Translation initiation factor IF-2.
Colli20061116_3518		Transcription elongation protein nusA.
Colli20061116_3519		UPF0090 protein Bcep18194_A4639.
Colli20061116_3520		putative ribosomal large subunit pseudouridine synthase B
Colli20061116_3528		hypothetical protein
Colli20061116_3529		Nodulation protein D 1.
Colli20061116_3531		N-carbamyl-L-amino acid amidohydrolase
Colli20061116_3532		chlorohydrolase family protein
Colli20061116_3538		pyruvate kinase
Colli20061116_3540	YES	2-hydroxy-3-oxopropionate reductase
Colli20061116_3557		Segregation and condensation protein A.
Colli20061116_3576		DnaJ domain protein
Colli20061116_3580		hypothetical protein
Colli20061116_3598		cytosol aminopeptidase
Colli20061116_3605		hypothetical protein
Colli20061116_3623		Leucine-specific-binding protein precursor
Colli20061116_3635		dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex
Colli20061116_3636		pyruvate dehydrogenase E1 component
Colli20061116_3644		Putative auxin efflux carrier component 8
Colli20061116_3645		Putative electron transport protein yjeS.
Colli20061116_3647		N-acetylmuramoyl-L-alanine amidase
Colli20061116_3688		CDK5RAP1-like protein.
Colli20061116_3689	YES	PhoH-like protein.
Colli20061116_3691		hypothetical protein
Colli20061116_3694		Magnesium and cobalt efflux protein corC.
Colli20061116_3700		hypothetical protein
Colli20061116_3707		aminoglycoside phosphotransferase
Colli20061116_3714		aspartate 1-decarboxylase precursor
Colli20061116_3720		putative antibiotic resistance protein
Colli20061116_3722		p-hydroxybenzoic acid efflux pump subunit aaeA
Colli20061116_3723		High affinity sulfate transporter 2.
Colli20061116_3728		phosphodiesterase/alkaline phosphatase D
Colli20061116_3732		putative membrane protein
Colli20061116_3737		Leucine-; isoleucine-; valine-; threonine-; and alanine-binding protein precursor

Gene	D. E. *	Gene function
Colli20061116_3738		D-isomer specific 2-hydroxyacid dehydrogenase family protein
Colli20061116_3742		conserved hypothetical protein similar to putative glucosaminyltransferase
Colli20061116_3744		hypothetical protein
Colli20061116_3745		putative outer membrane protein
Colli20061116_3779		phosphatidylglycerophosphatase A
Colli20061116_3804		10 kDa chaperonin (Protein Cpn10) (groES protein).
Colli20061116_3807		Probable D-methionine transport system permease protein metI.
Colli20061116_3813		Nucleolar GTP-binding protein 2.
Colli20061116_3814		Histone deacetylase 1 (HD1).
Colli20061116_3822		hypothetical protein predicted by Glimmer/Critica
Colli20061116_3823		cysteine synthase B
Colli20061116_3832		Probable global transcription activator SNF2L1
Colli20061116_3836		chorismate mutase/prephenate dehydratase
Colli20061116_3837		phosphoserine aminotransferase
Colli20061116_3841		ubiquinone biosynthesis O-methyltransferase
Colli20061116_3853		Spermidine/putrescine transport system permease protein potC.
Colli20061116_3864		Outer membrane protein W precursor.
Colli20061116_3865		Hypothetical protein ywlC.
Colli20061116_3866		phosphoribosylaminoimidazole carboxylase; ATPase subunit
Colli20061116_3867		phosphoribosylaminoimidazole carboxylase catalytic subunit
Colli20061116_3868		phosphoribosylaminoimidazole-succinocarboxamide synthase
Colli20061116_3874		hypothetical protein
Colli20061116_3884		succinyl-CoA ligase; alpha subunit
Colli20061116_3892		HTH-type transcriptional activator ampR.
Colli20061116_3898		acyl-CoA dehydrogenase family protein
Colli20061116_3910		hypothetical protein
Colli20061116_3920		3-methyl-2-oxobutanoate hydroxymethyltransferase
Colli20061116_3924		Phosphoserine phosphatase
Colli20061116_3940		Outer membrane lipoprotein omlA precursor.
Colli20061116_3951	YES	hypothetical protein predicted by Glimmer/Critica
Colli20061116_3959		Protein piccolo
Colli20061116_3965		Nif-specific regulatory protein.
Colli20061116_3970		signal recognition particle protein
Colli20061116_3973		alkaline phosphatase precursor

Gene	D. E. *	Gene function
Colli20061116_3976		prolyl-tRNA synthetase
Colli20061116_3981		50S ribosomal protein L27.
Colli20061116_3991		Cell division topological specificity factor.
Colli20061116_4011		pyrophosphatase; NUDIX family
Colli20061116_4012		hypothetical protein
Colli20061116_4018		Hybrid peroxiredoxin hyPrx5
Colli20061116_4020		Cell division protein ftsA.
Colli20061116_4024		UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
Colli20061116_4038		Phytochrome-like protein cph2
Colli20061116_4052		putative ubiquinone biosynthesis protein
Colli20061116_4053		hypothetical protein
Colli20061116_4054	YES	uroporphyrin-III C-methyltransferase
Colli20061116_4066		Hydrogen peroxide-inducible genes activator
Colli20061116_4079		Oligopeptide transport ATP-binding protein appF.
Colli20061116_4082		Inner membrane ABC transporter permease protein yddR.
Colli20061116_4087		Biotin synthesis protein bioC.
Colli20061116_4100		phenylalanine-4-hydroxylase
Colli20061116_4138		Putative general secretion pathway protein H precursor
Colli20061116_4154		Leucine-specific-binding protein precursor
Colli20061116_4156		Hypothetical protein TP0675.
Colli20061116_4166		probable UDP-sugar diphosphatase
Colli20061116_4171		hypothetical protein
Colli20061116_4184		aspartate aminotransferase
Colli20061116_4185		topB2; DNA topoisomerase III [EC:5.99.1.2]
Colli20061116_4188		hypothetical protein
Colli20061116_4201		Rod shape-determining protein mreB.
Colli20061116_4219		Leucine-responsive regulatory protein.
Colli20061116_4220	YES	ornithine aminotransferase
Colli20061116_4221	YES	Arginine deiminase
Colli20061116_4222		5-methyltetrahydrofolate--homocysteine methyltransferase
Colli20061116_4225		Inner membrane protein yicO.
Colli20061116_4236		phosphoenolpyruvate-protein phosphotransferase
Colli20061116_4255		UPF0102 protein PSPTO_4420.

Gene	D. E. *	Gene function
Colli20061116_4265		putative membrane protein
Colli20061116_4266		putative membrane protein
Colli20061116_4267		hypothetical protein
Colli20061116_4278		UPF0250 protein RSc0326.
Colli20061116_4279		probable class IV aminotransferase
Colli20061116_4303		hypothetical protein
Colli20061116_4304		Heat shock 70 kDa protein (HSP70).
Colli20061116_4305		Chloride channel protein; skeletal muscle
Colli20061116_4307		Inner membrane protein yphA.
Colli20061116_4310		Leucine-responsive regulatory protein.
Colli20061116_4313		succinyl-diaminopimelate desuccinylase
Colli20061116_4320		L-asparaginase
Colli20061116_4329		Transcriptional regulatory protein srrA
Colli20061116_4336		biotin carboxylase subunit of acetyl-CoA carboxylase
Colli20061116_4349		acetyl-CoA acetyltransferase
Colli20061116_4350		isocitrate dehydrogenase kinase/phosphatase
Colli20061116_4351		serine dehydratase-like
Colli20061116_4352		isovaleryl-CoA dehydrogenase
Colli20061116_4353		HTH-type transcriptional regulator cueR
Colli20061116_4354	YES	Hypothetical protein yqjP.
Colli20061116_4355		Arginine exporter protein argO.
Colli20061116_4357		thiol:disulfide interchange protein
Colli20061116_4369		Hypothetical protein ybdD.
Colli20061116_4370		Carbon starvation protein A.
Colli20061116_4385		Cytochrome c6 precursor
Colli20061116_4392		Antigen 43 precursor
Colli20061116_4460		Exu regulon transcriptional regulator.
Colli20061116_4461		Primosomal protein N'
Colli20061116_4463		HTH-type transcriptional regulator metR.
Colli20061116_4464		agmatinase
Colli20061116_4467		Probable sugar efflux transporter.
Colli20061116_4468		succinate-semialdehyde dehydrogenase
Colli20061116_4481	YES	ATP synthase F1; beta subunit
Colli20061116_4482		ATP synthase F1; gamma subunit

Gene	D. E. *	Gene function
Colli20061116_4484		ATP synthase delta chain
Colli20061116_4485		ATP synthase B chain
Colli20061116_4486		ATP synthase c chain
Colli20061116_4488		putative ATP synthase protein I
Colli20061116_4490		Probable chromosome partitioning protein parB.
Colli20061116_4491		Chromosome partitioning protein parA.
Colli20061116_4494		tRNA uridine 5-carboxymethylaminomethyl modification enzyme gidA
Colli20061116_4496		High-affinity branched-chain amino acid transport system permease protein livM
Colli20061116_4497		High-affinity branched-chain amino acid transport system permease protein livM
Colli20061116_4498		putative exported protein
Colli20061116_4499		ABC transporter ATP-binding protein
Colli20061116_4500		ABC transporter related
Colli20061116_4501		oxidoreductase; GMC family
Colli20061116_4505		Hypothetical protein yhjG.
Colli20061116_4507		proline dehydrogenase
Colli20061116_4511		gluconate kinase
Colli20061116_4512		Nodulation protein D 1.
Colli20061116_4515		phosphoenolpyruvate carboxykinase (GTP)
Colli20061116_4517		hypothetical protein predicted by Glimmer/Critica
Colli20061116_4519		glucose-methanol-choline oxidoreductase
Colli20061116_4520		cytochrome c; class I
Colli20061116_4521		asparaginase
Colli20061116_4522		peptidyl-dipeptidase
Colli20061116_4523	YES	Major outer membrane protein P.IB precursor
Colli20061116_4526	YES	Protein HI0131 precursor.
Colli20061116_4529		putative two-component system sensor kinase
Colli20061116_4538		tRNA modification GTPase trmE.
Colli20061116_4541		Ribonuclease P protein component

* Genes differentially expressed when *C. fungivorans* Ter331 was confronted with the fungus *A. niger*

Table A5. Gene clusters differentially expressed in *C. fungivorans* Ter331 during in vitro confrontation with *A. niger*. Present, x; absent, -

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter10	Ter14	Ter91	Ter331
Cluster A	*Cf_2063*	acetolactate synthase large subunit	9.8	up	6.9	up	x	-	x	x	x
Cluster A	*Cf_2064*	NAD-dependent formate dehydrogenase gamma subunit	3.4	up	2.2	up	x	x	x	x	x
Cluster A	*Cf_2065*	NAD-dependent formate dehydrogenase beta subunit	2.5	up	2.3	up	x	x	x	x	x
Cluster A	*Cf_2066*	uncharacterized anaerobic dehydrogenase	5.3	up	3.9	up	x	x	x	-	x
Cluster A	*Cf_2067*	NAD-dependent formate dehydrogenase delta subunit	3.7	up	1.4	up	x	x	x	-	x
Cluster A	*Cf_2070*	Protein cbbY, plasmid	35.2	up	3.9	up	x	-	x	-	x
Cluster A	*Cf_2071*	hypothetical protein predicted by Glimmer/Critica	23.5	up	2.8	up	NA	NA	NA	NA	NA
Cluster A	*Cf_2072*	Oxalate:formate antiporter	9.9	up	3.3	up	x	-	x	-	x
Cluster A	*Cf_2073*	fumarylacetoacetate hydrolase family protein	6.8	up	6.3	up	x	-	x	x	x
Cluster A	*Cf_2074*	2-dehydropantoate 2-reductase	7.2	up	3.5	up	x	-	x	x	x
Cluster A	*Cf_2075*	Monocarboxylate transporter 2	9.4	up	4.5	up	x	-	x	x	x
Cluster A	*Cf_2076*	Putative quinone oxidoreductase	2.9	up	1.4	up	-	-	x	-	x
Cluster A	*Cf_2079*	acetolactate synthase large subunit	14	up	8.5	up	x	-	x	x	x
Cluster A	*Cf_2080*	putative enzyme	13.4	up	6.1	up	x	-	x	x	x
Cluster A	*Cf_2081*	PAS	4.3	up	2.7	up	x	x	x	x	x
Cluster A	*Cf_2082*	putative enzyme	2.4	up	2.2	up	x	-	x	x	x
Cluster A	*Cf_2083*	Putative HTH-type transcriptional regulator yeaM	2.2	up	1.9	up	x	x	x	x	x
Cluster B	*Cf_3540*	2-hydroxy-3-oxopropionate reductase	4.9	up	5.4	up	x	x	x	x	x
Cluster B	*Cf_3541*	hydroxypyruvate isomerase	3.8	up	4.2	up	x	x	x	x	x
Cluster B	*Cf_3542*	glyoxylate carboligase	5.3	up	6.3	up	x	x	x	x	x
Cluster C	*Cf_2982*	hypothetical protein	2.6	up	18.3	up	x	x	x	x	x
Cluster C	*Cf_2983*	hypothetical protein	2.7	up	6.6	up	x	x	x	x	x
Cluster C	*Cf_2984*	hypothetical protein predicted by Glimmer/Critica	13.1	up	4.7	up	x	-	x	-	x
Cluster C	*Cf_2985*	formamidase	32.8	up	4.8	up	x	-	x	-	x
Cluster C	*Cf_2986*	aliphatic amidase	55.8	up	9.3	up	x	-	x	-	x
Cluster C	*Cf_2987*	ABC-type urea permease	17	up	2	up	x	x	x	-	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter10	Ter14	Ter91	Ter331
Cluster C	*Cf_2988*	ABC-type urea permease	22.4	up	4.1	up	x	-	x	x	x
Cluster C	*Cf_2989*	ABC-type urea permease	48.5	up	10.7	up	x	-	x	-	x
Cluster C	*Cf_2990*	ABC-type urea permease	22.6	up	3.2	up	x	-	x	-	x
Cluster C	*Cf_2991*	ABC-type urea permease	70.9	up	6.8	up	x	-	x	-	x
Cluster C	*Cf_2992*	two-component hybrid sensor and regulator	2.3	up	3.4	up	x	-	x	-	x
Cluster C	*Cf_2993*	putative two-component response regulator protein	2.3	up	2	up	x	x	x	-	x
Cluster D	*Cf_4054*	uroporphyrin-III C-methyltransferase	5.1	up	3.1	up	x	x	x	x	x
Cluster D	*Cf_4055*	Nitrate transport protein nasD.	9.5	up	3.5	up	x	x	x	x	x
Cluster D	*Cf_4056*	Nitrate transport permease protein nrtB.	16.3	up	6.1	up	x	x	x	x	x
Cluster D	*Cf_4057*	Nitrate transport protein nrtA.	20.8	up	5.5	up	x	x	x	x	x
Cluster E	*Cf_223*	probable high affinity nitrate transporter transmembrane protein	7.2	up	7	up	-	x	x	x	x
Cluster E	*Cf_224*	nitrite reductase	17.1	up	5.9	up	x	x	x	x	x
Cluster E	*Cf_225*	nitrite reductase	12.6	up	5.2	up	x	x	x	x	x
Cluster E	*Cf_226*	nitrite reductase	4.6	up	2.1	up	x	x	x	x	x
Cluster E	*Cf_227*	nitrate reductase large subunit	6.1	up	4.5	up	x	x	x	x	x
Cluster F	*Cf_556*	glutamate synthase	2.2	up	1.6	down	x	x	x	x	x
Cluster F	*Cf_2775*	Allantoin permease (Allantoin transport protein).	5.9	up	7	up	x	x	x	x	x
Cluster G	*Cf_4241*	Acetyltransferase	2.8	up	1.8	up	x	x	x	x	x
Cluster G	*Cf_4242*	ammonium transporter	9.8	up	1.9	up	x	x	x	x	x
Cluster G	*Cf_4243*	Nitrogen regulatory protein P-II.	5.7	up	2.5	up	x	-	x	x	x
Cluster H	*Cf_2912*	ABC-type urea permease	11.6	up	6.8	up	x	x	x	x	x
Cluster H	*Cf_2913*	ABC-type urea permease	6.1	up	8	up	x	x	x	x	x
Cluster H	*Cf_2914*	ABC-type urea permease	6.8	up	6.3	up	x	x	x	x	x
Cluster H	*Cf_2915*	ABC-type urea permease	6.8	up	6.3	up	x	x	x	x	x
Cluster H	*Cf_2916*	ABC-type urea permease	9.2	up	7	up	x	x	x	-	x
Cluster I	*Cf_2786*	GumF protein	3.7	up	15.8	up	-	-	x	-	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter10	Ter14	Ter91	Ter331
Cluster I	*Cf_2787*	phosphomannomutase	3.3	up	12.3	up	-	x	x	-	x
Cluster I	*Cf_2788*	mannose-6-phosphate isomerase / mannose-1-phosphate guanylyltransferase	5.5	up	54.3	up	x	x	x	x	x
Cluster I	*Cf_2789*	mannose-1-phosphate guanyltransferase	8.3	up	74.6	up	x	x	x	x	x
Cluster I	*Cf_2790*	GumM protein	7.7	up	50.2	up	x	-	x	x	x
Cluster I	*Cf_2791*	Hypothetical 55.0 kDa protein in cps region	3.8	up	24.4	up	x	x	x	-	x
Cluster I	*Cf_2792*	Polypeptide N-acetylgalactosaminyltransferase 1	5.4	up	34.8	up	x	-	x	x	x
Cluster I	*Cf_2793*	cellulase	2.5	up	15.1	up	x	x	x	x	x
Cluster I	*Cf_2794*	O-acetyltransferase oatA (EC 2.3.1.-).	5.3	up	28.7	up	x	-	x	-	x
Cluster I	*Cf_2795*	GumH protein	4.8	up	26	up	x	x	x	x	x
Cluster I	*Cf_2796*	hypothetical protein predicted by Glimmer/Critica	4.3	up	27.6	up	x	-	x	x	x
Cluster I	*Cf_2797*	hypothetical protein	4.5	up	33.8	up	x	x	x	x	x
Cluster I	*Cf_2798*	lipopolysaccharide biosynthesis	7.4	up	76.2	up	x	x	x	x	x
Cluster I	*Cf_2799*	EPS I polysaccharide export outer membrane protein epsA precursor.	5.1	up	56.5	up	x	x	x	x	x
Cluster I	*Cf_2800*	hypothetical protein	9.6	up	100.4	up	x	-	x	x	x
Cluster I	*Cf_2801*	hypothetical protein predicted by Glimmer/Critica	5.2	up	53.4	up	x	-	x	x	x
Cluster I	*Cf_2802*	Putative colanic biosynthesis UDP-glucose lipid carrier transferase.	3.9	up	16.7	up	x	x	x	x	x
Cluster I	*Cf_2803*	hypothetical protein	3.8	up	30.1	up	x	-	x	x	x
Cluster J	*Cf_2051*	Putative polysaccharide export protein wza precursor.	11	up	2.9	up	x	-	x	-	x
Cluster J	*Cf_2052*	Probable low molecular weight protein-tyrosine-phosphatase amsI	9.3	up	2.1	up	x	-	x	-	x
Cluster J	*Cf_2053*	exopolysaccharide transport protein	9.1	up	3.5	up	x	-	x	-	x
Cluster J	*Cf_2054*	hypothetical protein predicted by Glimmer/Critica	7.7	up	4.2	up	x	-	x	x	x
Cluster J	*Cf_2055*	hypothetical protein predicted by Glimmer/Critica	6.9	up	1.9	up	x	x	x	x	x
Cluster J	*Cf_2056*	hypothetical protein	4.6	up	1.7	up	x	-	x	-	x
Cluster J	*Cf_2057*	Hypothetical 41.2 kDa protein in cps region	5	up	1.2	up	x	-	x	-	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter10	Ter14	Ter91	Ter331
Cluster J	*Cf_2058*	hypothetical protein	4.6	up	1.9	up	x	-	x	-	x
Cluster J	*Cf_2059*	UTP--glucose-1-phosphate uridylyltransferase	24	up	2.7	up	x	-	x	-	x
Cluster J	*Cf_2060*	sugar transferase	22.1	up	2.9	up	x	-	x	-	x
Cluster K	*Cf_1128*	hypothetical protein predicted by Glimmer/Critica	2.4	up	1.8	up	NA	NA	NA	NA	NA
Cluster K	*Cf_1129*	High molecular weight rubredoxin	6.5	up	3.4	up	NA	NA	NA	NA	NA
Cluster K	*Cf_1130*	Abhydrolase domain-containing protein 12.	6.7	up	3.9	up	x	x	x	x	x
Cluster K	*Cf_1131*	fatty acid desaturase	9	up	2.9	up	x	-	x	-	x
Cluster K	*Cf_1132*	Putative polyketide synthase pksM.	8.5	up	3.6	up	x	-	x	-	x
Cluster K	*Cf_1133*	Acyl-CoA desaturase 1	4.7	up	2.1	up	x	-	x	-	x
Cluster K	*Cf_1134*	Delta-9 acyl-lipid desaturase 2 (EC 1.14.19.-).	3.4	up	2	up	NA	NA	NA	NA	NA
Cluster K	*Cf_1135*	probable peptide synthetase protein	4.7	up	2.9	up	x	-	x	-	x
Cluster K	*Cf_1136*	Possible Multidrug resistance protein B.	4.5	up	3.5	up	-	-	x	-	x
Cluster K	*Cf_1137*	hypothetical protein predicted by Glimmer/Critica	7.4	up	3.9	up	-	-	x	-	x
Cluster K	*Cf_1138*	vanillate O-demethylase oxygenase subunit	6.8	up	2.1	up	-	-	x	-	x
Cluster K	*Cf_1139*	fatty acid desaturase	7.7	up	1.8	up	-	-	x	-	x
Cluster K	*Cf_1140*	monooxygenase	6.9	up	1.6	up	-	-	x	-	x
Cluster K	*Cf_1141*	3-oxoacyl-acyl carrier protein synthase II	7.8	up	2.3	up	-	-	x	-	x
Cluster K	*Cf_1142*	hypothetical protein predicted by Glimmer/Critica	7.4	up	1.1	down	NA	NA	NA	NA	NA
Cluster L	*Cf_2276*	hypothetical protein	2.3	up	2.5	up	x	-	x	x	x
Cluster L	*Cf_2277*	Hypothetical protein MJ0779.	2.8	up	1.9	up	x	-	x	x	x
Cluster L	*Cf_2278*	Hypothetical protein MJ0900.	2.8	up	2.3	up	x	x	x	x	x
Cluster L	*Cf_2279*	hypothetical protein predicted by Glimmer/Critica	2	up	1.1	down	x	-	x	x	x
Cluster L	*Cf_2280*	General secretion pathway protein D precursor.	2.2	up	1.9	up	x	x	x	x	x
Cluster L	*Cf_2281*	hypothetical protein predicted by Glimmer/Critica	2	up	1.2	up	x	-	x	x	x
Cluster L	*Cf_2282*	hypothetical protein predicted by Glimmer/Critica	2.2	up	1.1	up	x	-	x	x	x
Cluster L	*Cf_2283*	hypothetical protein predicted by Glimmer/Critica	2.6	up	1.3	up	x	-	x	x	x
Cluster L	*Cf_2284*	hypothetical protein predicted by Glimmer/Critica	2.1	up	1	up	x	-	x	x	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter1 0	Ter1 4	Ter9 1	Ter33 1
Cluster M	*Cf_986*	Flagellar protein flhE precursor.	3.3	up	15.5	up	-	-	x	-	x
Cluster M	*Cf_991*	CDS	2.5	up	8.5	up	x	x	x	-	x
Cluster M	*Cf_992*	Flagellar basal-body rod protein flgC.	2.4	up	7.9	up	x	x	x	-	x
Cluster M	*Cf_994*	CDS	2.9	up	9.5	up	x	x	x	-	x
Cluster M	*Cf_996*	CDS	3.3	up	11.8	up	x	x	x	-	x
Cluster M	*Cf_997*	Flagellar L-ring protein precursor	2.7	up	9.2	up	x	x	x	-	x
Cluster M	*Cf_998*	CDS	2	up	7.9	up	x	x	x	-	x
Cluster M	*Cf_1000*	CDS	2.2	up	10.4	up	x	x	x	-	x
Cluster M	*Cf_1003*	CDS	2.5	up	12.8	up	x	x	x	-	x
Cluster M	*Cf_1004*	CDS	3.4	up	18.4	up	x	x	x	-	x
Cluster M	*Cf_1005*	CDS	2.3	up	10.8	up	x	x	x	-	x
Cluster M	*Cf_1006*	CDS	2.1	up	8.1	up	x	x	x	-	x
Cluster M	*Cf_1007*	Flagellar fliL protein	2.4	up	12.1	up	x	-	x	-	x
Cluster M	*Cf_1010*	hypothetical protein predicted by Glimmer/Critica	2.8	up	17.7	up	NA	NA	NA	NA	NA
Cluster M	*Cf_1013*	CDS	2.4	up	10.3	up	x	x	x	-	x
Cluster M	*Cf_1019*	CDS	2.4	up	10.2	up	x	-	x	-	x
Cluster M	*Cf_1029*	Transcriptional activator flhD.	2.3	up	2.3	up	x	-	x	-	x
Cluster M	*Cf_1030*	CDS	2	up	1.3	up	x	x	x	-	x
Cluster M	*Cf_1031*	CDS	2.5	up	3.1	up	x	x	x	-	x
Cluster M	*Cf_1033*	Probable transcriptional regulator ycf27	2.1	up	4.8	up	-	x	x	-	x
Cluster M	*Cf_1034*	chemotaxis protein CheA	2.4	up	3.5	up	x	x	x	-	x
Cluster M	*Cf_1035*	Chemotaxis protein cheW.	2.7	up	3.7	up	x	-	x	-	x
Cluster M	*Cf_1036*	Hypothetical 14.4 kDa protein y4sN.	2.1	up	1.8	up	x	x	x	-	x
Cluster N	*Cf_1047*	Terminase, ATPase subunit (GpP).	2.3	up	4.2	up	-	-	-	-	x
Cluster N	*Cf_1048*	Presumed capsid scaffolding protein (GpO).	2.3	up	4.2	up	-	-	-	-	x
Cluster N	*Cf_1050*	Terminase, endonuclease subunit (GpM).	2.2	up	3.1	up	-	-	-	-	x
Cluster N	*Cf_1054*	hypothetical protein predicted by Glimmer/Critica	2.1	up	3.4	up	-	-	-	-	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter10	Ter14	Ter91	Ter331
Cluster N	*Cf_1055*	Protein lysB.	2.5	up	5.2	up	-	-	-	-	x
Cluster N	*Cf_1058*	prophage PSPPH02, putative adenine modification methytransferase	2.1	up	4.6	up	-	-	-	-	x
Cluster N	*Cf_1062*	hypothetical protein	2.5	up	5.6	up	-	-	-	-	x
Cluster N	*Cf_1063*	Tail fiber assembly protein homolog from lambdoid prophage DLP12.	2.3	up	4.8	up	-	-	-	-	x
Cluster N	*Cf_1064*	Major tail sheath protein (Protein FI).	2.1	up	3.7	up	-	-	-	-	x
Cluster N	*Cf_1068*	Minor tail protein Gp26.	2.4	up	5.1	up	-	-	-	-	x
Cluster N	*Cf_1073*	hypothetical protein predicted by Glimmer/Critica	2.1	up	3.7	up	NA	NA	NA	NA	NA
Cluster N	*Cf_1074*	hypothetical protein	2.3	up	3.9	up	-	-	-	-	x
Cluster O	*Cf_2102*	hypothetical protein	2.2	up	4.1	up	x	-	x	-	x
Cluster O	*Cf_2103*	hypothetical protein	2.2	up	5.7	up	x	-	x	-	x
Cluster O	*Cf_2110*	hypothetical protein predicted by Glimmer/Critica	2.5	up	5.6	up	x	-	x	-	x
Cluster O	*Cf_2111*	hypothetical protein predicted by Glimmer/Critica	2.9	up	11.1	up	x	-	x	-	x
Cluster O	*Cf_2112*	hypothetical protein predicted by Glimmer/Critica	2.2	up	5.2	up	x	-	x	-	x
Cluster O	*Cf_2115*	hypothetical protein	2.4	up	4.7	up	x	-	x	-	x
Cluster P	*Cf_2031*	Hypothetical protein	3.59	up	5.39	up	-	-	x	-	x
Cluster P	*Cf_2032*	IMP dehydrogenase/GMP reductase, DJ-1/PfpI family	4.45	up	12.02	up	-	-	x	-	x
Cluster P	*Cf_2033*	Hypothetical protein	3.66	up	10.14	up	-	-	x	-	x
Cluster P	*Cf_2034*	Hypothetical protein	2.54	up	5.44	up	-	-	x	-	x
Cluster P	*Cf_2035*	Prokaryotic type, Ku70/Ku80 beta-barrel domain;High confidence in function and specificity	3.52	up	6.9	up	-	-	-	-	x
Cluster P	*Cf_2036*	DNA ligase (EC 6.5.1.1) (Polydeoxyribonucleotide synthase [ATP]), ATP-dependent DNA ligase	2.89	up	4.89	up	-	-	x	-	x
Cluster P	*Cf_2037*	Haloacid dehalogenase-like hydrolase;Hypothetical protein	2.75	up	2.38	up	-	-	x	-	x
Cluster P	*Cf_2038*	Hypothetical protein	3.39	up	1.12	down	-	-	x	-	x
Cluster P	*Cf_2039*	CsbD-like;Conserved hypothetical protein	2.98	up	1.24	up	NA	NA	NA	NA	NA
Cluster Q	*Cf_3498*	Response regulator receiver;Hypothetical protein	2.6	up	1.04	up	-	-	x	-	x

	GENE NAME	FUNCTION	Fold change T1		Fold change T2		Ter 6	Ter1 0	Ter1 4	Ter9 1	Ter33 1
Cluster Q	*Cf_3499*	CheY-like, Response regulator receiver domain;Conserved hypothetical protein	4.2	up	4.96	up	-	-	x	-	x
Cluster Q	*Cf_3500*	Transport-associated;Conserved hypothetical protein	7.07	up	15.3	up	x	-	x	-	x
Cluster Q	*Cf_3501*	Hypothetical protein	5.16	up	11.71	up	x	-	x	-	x
Cluster Q	*Cf_3502*	CsbD-like, CsbD-like;Conserved hypothetical protein	3.45	up	6.98	up	NA	NA	NA	NA	NA
plasmid	*pTer331_2*	hypothetical protein	2.3	up	30.7	up	-	-	-	-	x
plasmid	*pTer331_3*	hypothetical protein predicted by Glimmer/Critica	2.2	up	21.6	up	-	-	-	-	x
plasmid	*pTer331_9*	Transcriptional repressor protein korB.	2	up	22	up	-	-	-	-	x
plasmid	*pTer331_10*	exported protein	2.5	up	27.6	up	-	-	-	-	x
plasmid	*pTer331_12*	T-DNA border endonuclease virD2	2.1	up	30.9	up	-	-	-	-	x
plasmid	*pTer331_15*	conjugation protein	2.1	up	22.9	up	-	-	-	-	x
plasmid	*pTer331_16*	12-oxophytodienoate reductase 3	2.4	up	12.4	up	-	-	-	-	x
plasmid	*pTer331_17*	Protein virD4.	2.4	up	27.1	up	-	-	-	-	x
plasmid	*pTer331_18*	VirB11 protein.	2.1	up	31.2	up	-	-	-	-	x
plasmid	*pTer331_20*	Protein virB9 precursor.	2.3	up	28.3	up	-	-	-	-	x
plasmid	*pTer331_21*	Protein virB8.	2.3	up	26.7	up	-	-	-	-	x
plasmid	*pTer331_22*	hypothetical protein predicted by Glimmer/Critica	2.9	up	25.3	up	-	-	-	-	x
plasmid	*pTer331_23*	type IV secretion system protein VirB6	2.1	up	20.5	up	-	-	-	-	x
plasmid	*pTer331_25*	type IV secretion system protein VirB5	2.3	up	22.4	up	-	-	-	-	x
plasmid	*pTer331_26*	Protein virB4 precursor.	2.1	up	23	up	-	-	-	-	x
plasmid	*pTer331_32*	hypothetical protein predicted by Glimmer/Critica	2.5	up	19.5	up	-	-	-	-	x
plasmid	*pTer331_34*	hypothetical protein predicted by Glimmer/Critica	2.2	up	21.5	up	-	-	-	-	x
plasmid	*pTer331_35*	hypothetical protein predicted by Glimmer/Critica	2.5	up	13.9	up	-	-	-	-	x

Summary

It has been recognized that studying bacterial-fungal interactions is essential to obtain a better understanding of terrestrial microbial ecology and that studies on bacterial-fungal interactions may lie at the basis of novel applications in agriculture, food industry and human health. Nevertheless, the incentives, the genetic determinants and the mechanisms that underlie bacterial-fungal interactions are still poorly understood. Bacterial mycophagy is a trophic behaviour that takes place when bacteria obtain nutrients from living fungal hyphae, allowing the conversion of living fungal biomass into bacterial biomass (29). This trophic behavior was demonstrated for the first time for bacteria of the genus *Collimonas,* based on their ability to grow at the expenses of living fungal hyphae in a soil-like microcosm (28, 30). In this thesis I addressed the following research questions: (1) Which of the mechanisms putatively involved in *Collimonas* mycophagy are actually activated when *Collimonas* interact with a fungus (2) What is the fungal response to the presence of *Collimonas* bacteria? (3) What is the role played by plasmid pTer331, detected in the genome of the mycophagous bacterium *C. fungivorans* Ter331, in the ecology of this bacterium? Are the genes encoded on plasmid pTer331 involved in mycophagy? (4) Are the putative determinants of mycophagy uniformly distributed among *Collimonas* species?

The model organism *C. fungivorans* Ter331 shows an antagonistic interaction towards the fungus *Aspergillus niger*. When the two organisms are confronted *in vitro* the fungal growth is inhibited and accumulation of bacterial biomass, in the form of slime, can be observed on the plate. In order to gain a better mechanistic understanding of the antagonism of *Collimonas* bacteria towards fungi, the involvement of the mycophagous phenotype, and the response of the fungus to the presence of *Collimonas*, bacterial and fungal RNA were isolated at two time points during the interaction and analyzed by microarray analysis. The experiment yielded a list of genetic determinants activated in both organisms as a consequence of their interaction. The presence of the fungus stimulated the expression of several bacterial genes, including genes involved in motility, synthesis of

exopolysaccharides and of a putative antimicrobial agent. In addition the presence of the fungus activated genes involved in the consumption of fungal derived substrates, suggesting that production of bacterial slime observed on plate may originate from a conversion of fungal biomass into bacterial biomass. Even though the relationship between mycophagy and antifungal activity has not been clarified yet, these findings support the existence of a common denominator between antifungal activity and mycophagy. In the fungus, transcriptional changes were observed for genes involved in lipid and cell wall metabolism and in cell defense, a result that correlates well with the hyphal deformations that were microscopically observed. Transcriptional profiles revealed signs of distress in both partners, indicating that the interaction between *Collimonas* and *Aspergillus* is characterized by a complex interplay between trophism, antibiosis, and competition for nutrients. This finding hint at the possibility that the specific fungal-bacterial combination used in this experiment does not allow *Collimonas* to express its mycophagous determinants at their full potential. Future experiments confronting *Collimonas* with other fungal species will expand our understanding of the genetic determinants of mycophagy (Chapter 2).

Plasmid pTer331 was isolated from its natural host *C. fungivorans* Ter331. The role played by this plasmid in the ecology of the bacterium and, in particular, in its mycophagous phenotype, was investigated studying the coding capacity and the distribution of the plasmid among the *Collimonas* strains belonging to our collection. Sequence annotation of pTer331 yielded 44 putative genes, mostly involved in replication, partitioning and transfer of the plasmid itself, suggesting that pTer331 is a cryptic plasmid that does not confer any evident phenotypic trait to its host. The failure to detect pTer331 in strains other than *C. fungivorans* Ter331 indicated that the plasmid does not play a role in traits that are common to all *Collimonas* strains, including antifungal activity, mycophagy, weathering and chitinolysis. The hypothesis that pTer331 could confer a selective advantage for the colonization of the plant rhizosphere was assessed by obtaining a plasmid-free strain and comparing the performance of this strain and the

wild type in colonizing the rhizosphere of tomato plants. The plasmid had no significant contribution in the rhizosphere competence of *C. fungivorans* Ter331. The presence on the plasmid of a hot-spot for insertion of additional genetic modules, suggests that this cryptic plasmid may incidentally acquire genes useful for the host survival, enhance its survival and spread in the bacterial population. The existence of pTer33-related plasmids carrying accessory genes beneficial to their host, supports the hypothesis that pTer331 might constitute a minimal plasmid form, which, in certain instances, acquires accessory genes (Chapter 3).

Collimonas is a genus of soil bacteria which comprises three recognized species: *C. fungivorans*, *C. pratensis* and *C. arenae*. The bacteria belonging to this genus share the ability to lyse chitin (chitinolysis) and feed on living fungal hyphae (mycophagy), but they differ in colony morphology, physiological properties and antifungal activity. Microarray based comparative genomic hybridization was used to gain insight into the genetic background underlying the phenotypic variability of collimonads. With the aid of microarray technology the genomic content of the reference strain *C. fungivorans* Ter331 was compared to the genomic content of four strains, representatives for the three *Collimonas* species. A set of highly conserved genes as well as a set of variable genes was identified, providing a list of candidate genes underlying the common and variable features of *Collimonas* bacteria. Even though mycophagy is a trait characterizing all *Collimonas* strains, several genetic determinants putatively involved in bacterial mycophagy presented a patchy distribution among the analyzed strains. These determinants included possession of motility, secretion of bioactive compounds and ability to grow on fungal derived substrates. This finding suggests that some genetic determinants putatively underlying mycophagy in *C. fungivorans* Ter331 might be absent in other strains, but these strains probably possess different mycophagous determinants. An increasing body of evidence indicates that several genes and gene functions additively contribute to the mycophagous behavior and that none of the genetic determinants is strictly necessary for mycophagy. We hypothesize that the possession of a different collection of these genetic determinants

might be at the base of specialization of *Collimonas* strains towards different fungal hosts (Chapter 4).

The results obtained in this research constitute a contribution to our understanding of the interactions between bacteria and fungi, a topic that, despite its potential applications in ecology, agriculture and human health, is relatively neglected.

Samenvatting

De bestudering van bacterie-schimmel interacties is essentieel voor een beter begrip van de terretrische microbiële ecologie, en kan aan de basis liggen van nieuwe toepassingen voor de landbouw, de voedingindustrie en geneeskunde. Desalnietemin zijn zowel de stimuli, de genetische determinanten als de onderliggende mechanismen van bacterie-schimmel interacties nog steeds nauwelijks gekend. Bacteriële mycofagie is een trofisch gedrag dat plaatsvindt als bacteriën voedingsstoffen onttrekken aan levende schimmelhyfen, waarbij biomassa van de levende schimmel wordt omgezet in bacteriële biomassa (29). Dit trofisch gedrag is voor het eerst aangetoond bij het bacteriële genus *Collimonas*, waarbij de bacteriën in een bodem microcosmexperiment in staat bleken te kunnen groeien ten koste van levende schimmels (28, 30). In deze thesis worden de volgende onderzoeksvragen gesteld: (1) Welke van de mechansismen mogelijks betrokken bij *Collimonas* mycofagie worden geactiveerd tijdens de interactie van *Collimonas* met een schimmel? (2) Wat is de reactie van de schimmel op de aanwezigheid van de *Collimonas* bacteriën? (3) Welke rol speelt het pTer331 plasmide, aangetoond in het genoom van de mycofage bacterie *C. fungivorans* Ter331, in de ecologie van deze bacterie en zijn de genen van het pTer331 plasmide betrokken bij mycofagie? (4) Zijn de potentiële determinanten voor mycofagie uniform verspreid over alle *Collimonas* soorten?

Het modelorganisme *C. fungivorus* Ter331 vertoont een antagonistische interactie tegenover de schimmel *Aspergillus niger*. Wanneer beide organsimen *in vitro* met elkaar geconfronteerd worden is de schimmelgroei geremd, en wordt groei van de bacteriële biomassa in de vorm van slijm op de petrischaal waargenomen. Voor een beter mechanistisch begrip van het antagonisme van de *Collimonas* bacteriën tegenover schimmels, de betrokkenheid van het mycofage fenotype en de reactie van de schimmel op de aanwezigheid van *Collimonas*, werd bacterie- en schimmel-RNA op twee tijdspunten gedurende de interactie geïsoleerd en vervolgens analyseerd met behulp van microarrays. Het experiment leverde een lijst op van genetische determinanten die geactiveerd werden in beide organismen ten gevolge van

hun interactie. De aanwezigheid van de schimmel stimuleerde de expressie van verscheidene bacteriële genen, waaronder genen betrokken bij beweeglijkheid, synthese van exopolysacchariden en potentieel antimicrobiële agentia. Bovendien activeerde de aanwezigheid van de schimmel genen betrokken bij de consumptie van substraten afkomstig van schimmels, wat er op duidt dat de productie van het bacteriële slijm, dat wordt waargenomen op de petrischalen, gevormd is door een conversie van schimmelbiomassa in bacteriële biomassa. Hoewel het verband tussen mycofagie en antischimmelactiviteit nog niet is opgehelderd, ondersteunen deze bevindingen het bestaan van een gemeenschappelijke factor tussen antischimmelactiviteit en mycofagie. In de schimmel werden transcriptionele veranderingen waargenomen bij genen betrokken bij vet- en celwandmetabolisme en celdefensie. Dit resultaat correleert goed met de microscopisch waargenomen misvormingen van de hyfes. De transcriptionele profielen vertonen bij beide partners signalen van stress, wat erop wijst dat de interactie tussen *Collimonas* en *Aspergillus* gekarakteriseerd wordt door een complexe wisselwerking van trofie, antibiose en competitie voor nutriënten. Deze bevinding zou kunnen wijzen op de mogelijkheid dat de specifiek in dit experiment gebruikte bacterie-schimmel interactie *Collimonas* niet toelaat om zijn mycofage determinanten ten volle tot expressie te brengen. Toekomstige experimenten waarbij *Collimonas* met andere schimmelsoorten wordt geconfronteerd zal ons inzicht in de genetische determinanten van mycofagie vergroten (Hoofdstuk 2).

Plasmide pTer331 werd geïsoleerd uit zijn natuurlijke gastheer *C. fungivorans* Ter331. De functie van dit plasmide voor de ecologie van de bacterie en in het bijzonder zijn mycofage fenotype werd onderzocht door het bestuderen van de coderende capaciteit en verspreiding van het plasmide onder de *Collimonas* stammen, die aanwezig zijn in onze collectie. Sequentie annotatie van pTer331 leverde 44 potentiële genen op die hoofdzakelijk betrokken zijn bij de replicatie, verdeling en transfer van het plasmide. Dit suggereert dat pTer331 een cryptisch plasmide is dat geen duidelijke fenotypische kenmerken aan zijn gastheer verleent. De

onmogelijkheid tot het detecteren van het pTer331 plasmide bij andere stammen dan *C. fungivorans* Ter331 wees erop dat het plasmide geen rol speelt bij gemeenschappelijke kenmerken van alle *Collimonas* stammen, waaronder antischimmelactiviteit en mycofagie, verwering en chitine afbraak. De hypothese dat pTer331 een selectief voordeel voor de kolonisatie van de plantenrhizosfeer kan opleveren werd onderzocht door het verkrijgen van een plasmidevrije stam en het vergelijken van de prestaties van deze stam met die van het wild type bij het koloniseren van de rhizosfeer van tomatenplanten. Het plasmide leverde geen significante bijdrage aan de competentie van *C. fungivorans* Ter331 tot het groeien in de rhizosfeer. De aanwezigheid op het plasmide van een hot-spot voor insertie van bijkomende genetische modules suggereert dat dit cryptisch plasmide mogelijk incidenteel genen verwerft die nuttig zijn voor de gastheeroverleving, en daardoor zijn eigen voortbestaan en verspreiding binnen de bacteriële populatie versterkt. Het bestaan van pTer331-verwante plasmiden die bijkomende genen bevatten die voordelig zijn voor de gastheer ondersteunt de hypothese dat het pTer331-plasmide mogelijks een minimale plasmidevorm is dat in sommige omstandigheden bijkomende genen verwerft (Hoofdstuk 3).

Collimonas is een genus van bodembacteriën dat drie erkende soorten bevat: *C. fungivorans*, *C. pratensis* en *C. arenae*. De bacteriën behorend tot dit genus hebben allen de mogelijkheid tot het afbreken van chitine en het zich voeden op levende schimmelhyfae (mycofagie), maar ze verschillen in hun koloniemorfologie, fysiologische eigenschappen en antischimmelactiviteit. Vergelijkende genomische hybridisatie op microarrays werd gebruikt om inzicht te verwerven in de genetische achtergrond van de fenotypische variabiliteit van de collimonads. Met behulp van microarray technologie werd de genomische inhoud van de referentiestam *C. fungivorans* Ter331 vergeleken met die van 4 stammen representatief voor de drie *Collimonas* soorten. Zowel een set van sterk geconserveerde als sterk variabele genen werd geïdentificeerd, wat een lijst opleverde van kandidaat genen onderliggend aan de gemeenschappelijke en variabele kenmerken van de *Collimonas* bacteriën. Hoewel mycofagie een karakteristiek kenmerk is van

alle *Collimonas* stammen, vertoonden verscheidene genetische determinanten, die betrokken zijn bij bacteriële mycofagie een onregelmatige verdeling onder de geannalyseerde stammen. Deze determinanten betroffen het bezit van beweeglijkheid, secretie van bioactieve componenten en de mogelijkheid om op van schimmels afkomstige substraten te groeien. Deze bevinding suggereert dat sommige genetische determinanten, die potentieel betrokken zijn bij mycofagy in *C. fungivorans* Ter331, mogelijk afwezig zijn in andere stammen, maar dat deze stammen waarschijnlijk andere determinanten voor mycofagie bezitten. Een steeds groter wordende reeks argumenten wijst erop dat verscheidene genen en genfuncties additioneel bijdragen aan het mycofaag gedrag, en dat geen enkele van deze genetische determinanten alleen strict noodzakelijk is voor mycofagie. Onze hypothese is dat het bezit van een verschillende collectie van deze genetische determinanten aan de basis ligt van de specialisatie van de *Collimonas* stammen voor bepaalde schimmelgastheren (Hoofdstuk 4).

De resultaten verkregen in dit onderzoek leveren een bijdrage aan ons inzicht betreffende de interacties tussen bacteriën en schimmels; een onderwerp dat ondanks zijn potentieel belangrijke toepassingen in natuurbeheer, landbouw en geneeskunde relatief verwaarloosd is.

Riepilogo

È ormai riconosciuto che lo studio delle interazioni tra batteri e funghi è importante per migliorare la nostra comprensione dell'ecologia dei microrganismi del suolo ed ha, inoltre, potenziali applicazioni nel campo dell'agricoltura, dell'industria alimentare e della medicina. Nonostante questo c'è una scarsa conoscenza dei meccanismi che sono coinvolti nelle interazioni tra batteri e funghi e dei fattori ambientali e genetici che le influenzano. La micofagia batterica è stata definita come l'interazione trofica che si verifica quando un batterio utilizza un micelio fungino vivo per ottenerne nutrimento. Questo tipo di interazione è caratterizzato dalla trasformazione di biomassa fungina in biomassa batterica (29). La micofagia batterica è stata documentata per la prima volta per batteri del genere *Collimonas* i quali, quando sono co-inoculati in un microcosmo con un micelio fungino, sono capaci di crescere a sue spese (28, 30). Nella presente tesi ho sviluppato i seguenti temi di ricerca: (1) Quali, tra i meccanismi che si sospetta siano alla base della micofagia batterica, sono attivati quando i batteri del genere *Collimonas* interagiscono con il fungo *Aspergillus niger*? (2) Come risponde *A. niger* alla presenza dei batteri? (3) Qual'è il ruolo svolto dal plasmide pTer331, isolato dal genoma del batterio *C. fungivorans* Ter331, nell'ecologia di questo batterio? I geni codificati sul plasmide hanno un ruolo nella micofagia di questo batterio? (4) I fattori genetici che si presume siano alla base della micofagia hanno una distribuzione uniforme nelle diverse specie batteriche che fanno parte del genere *Collimonas*?

Quando il ceppo batterico *C. fungivorans* Ter331 interagisce *in vitro* con il fungo *A. niger* la crescita del fungo è inibita (antagonismo) e si osserva l'accumulo di biomassa batterica sotto forma di secrezione extracellulare. Per chiarire la relazione tra questo fenomeno di antagonismo e la micofagia batterica e per definire i meccanismi coinvolti in questa interazione, ho isolato l'RNA del fungo e del baterio in due momenti successivi durante l'interazione e li ho analizzati mediante la tecnica del microarray. L'esperimento ha prodotto un elenco dei geni attivati in entrambi gli organismi come conseguenza della loro interazione. Anche se la relazione

tra micofagia e attività antifungina non è ancora del tutto chiara, i risultati dell'esperimento confermano l'esistenza di un denominatore comune tra i due fenomeni. La presenza del fungo ha stimolato nel batterio l'espressione di geni coinvolti nella mobilità, nella sintesi di esopolisaccaridi e di un presunto antibiotico. Inoltre il batterio ha attivato geni coinvolti nel metabolismo di sostanze di origine fungina e questo risultato sostiene l'ipotesi che la secrezione batterica osservata in questo contesto possa essere prodotta a partire dalla biomassa fungina. I geni la cui espressione è cambiata nel fungo, sono geni coinvolti nella difesa della cellula e nel metabolismo di lipidi e parete cellulare; un risultato che sembra connesso con le deformazioni delle ife che sono state osservate al microscopio. L'analisi dei profili di trascrizione dei due organismi ha rivelato segni di sofferenza in entrambi, suggerendo che l'interazione tra *C. fungivorans* e *A. niger* è complessa e comprende trofismo, antibiosi, e competizione per i nutrienti. I risultati suggeriscono che la combinazione *C. fungivorans* / *A. niger* usata in questo esperimento, potrebbe non essere ottimale per consentire a *Collimonas* di esprimere pienamente il suo potenziale micofago. Esperimenti che mettano a confronto *Collimonas* con altre specie fungine sono fondamentali per ampliare la nostra comprensione dei fattori genetici che stanno alla base della micofagia (capitolo 2).

C. fungivorans Ter331 costituisce uno degli ospiti in cui il plasmide pTer331 può trovarsi in natura. Il ruolo svolto da questo plasmide nell'ecologia di *Collimonas* e, in particolare, nel fenotipo della micofagia è stato studiato analizzando l'informazione genetica codificata sul plasmide e la distribuzione dello stesso tra i ceppi di *Collimonas* che fanno parte della nostra collezione. L'annotazione della sequenza del plasmide pTer331 ha rivelato la presenza di 44 geni, i quali sembrano per lo più coinvolti nella replica, nel trasferimento orizzontale del plasmide e nella sua equa distribuzione alle cellule figlie. L'analisi della sequenza genetica suggerisce quindi che pTer331 è un plasmide criptico, il quale non attribuisce alcun fenotipo evidente al suo ospite. L'assenza di pTer331 in ceppi di *Collimonas* che siano diversi da *C. fungivorans* Ter331 dimostra che il plasmide non codifica nessuno dei tratti fenotipici che sono comuni a tutti i

ceppi di *Collimonas*, compresa l'attività antifungina, la micofagia, la capacità di intaccare i minerali e l'abilità di lisare la chitina. L'ipotesi che pTer331 possa avere un ruolo nella colonizzazione batterica della rizosfera, è stata verificata sperimentalmente confrontando la prestazione di *Collimonas* Ter331 e quella dello stesso ceppo batterico, precedentemente privato del plasmide, in una prova di colonizzazione della rizosfera di piante di pomodoro. I risultati di questo esperimento hanno stabilito che il plasmide non apporta alcun contributo significativo alla colonizzazione. La presenza sul plasmide di un sito suscettibile all'inserimento di ulteriori moduli genetici ha fatto nascere l'ipotesi che questo plasmide possa incidentalmente acquisire dall'esterno geni che favoriscono la sopravvivenza della cellula batterca ospitante, favorendo così anche la sopravvivenza e la diffusione nella popolazione batterica del plasmide stesso. L'esistenza in natura di plasmidi simili a pTer331 i quali codificano geni utili al loro ospite supporta l'ipotesi che pTer331 possa essere una versione "minima" del plasmide che, in alcuni casi, acquisisce geni "accessori" (capitolo 3).

Il genere *Collimonas* comprende tre specie riconosciute: *C. fungivorans*, *C. pratensis* e *C. arenae*. I batteri appartenenti a questo genere hanno tutti la capacità di lisare chitina (chitinolisi) e la capacità di derivare nutrienti dalle ife fungine (micofagia), ma differiscono per caratteristiche quali: la morfologia delle colonie, le proprietà fisiologiche e l'attività antifungina. Al fine di identificare i geni che stanno alla base della variabilità fenotipica di *Collimonas*, ho effettuato una comparazione del genoma di diverse specie. Con l'aiuto della tecnica dell'ibridazione su microarray, ho confrontato il contenuto genomico del ceppo di riferimento, *C. fungivorans* Ter331, con il contenuto genomico di altri quattro ceppi rappresentanti delle tre specie che fanno parte del genere *Collimonas*. Questo esperimento ha portato all'identificazione di un gruppo di geni conservati in tutti i ceppi, e di un secondo gruppo di geni presenti in alcuni dei ceppi ed assenti in altri; fornendo così una lista dei geni candidati a spiegare le caratteristiche comuni e variabili di *Collimonas*. Anche se è la micofagia è una proprietà di tutti i ceppi di *Collimonas*, alcuni determinanti genetici che sono sospettati

di essere coinvolti nella micofagia mostrano una distribuzione variabile tra i ceppi analizzati. Questi determinanti includono la mobilità, la capacità di secernere composti bioattivi e la capacità di crescere su alcuni substrati tipicamente derivanti dalle ife fungine. Questo risultato ha portato all'ipotesi che alcuni dei determinanti genetici alla base della micofagia in *C. fungivorans* Ter331 siano assenti negli altri ceppi di *Collimonas* i quali, però, possiedono altri determinanti con una funzione equivalente. Un numero crescente di risultati sembra indicare che la micofagia non dipende dal possesso di un solo derminante genetico ma che, al contrario, molteplici determinanti, nessuno dei quali è indispensabile, contribuiscono con una azione additiva a determinare questa proprietà. Il possesso di una diversa combinazione di determinanti potrebbe essere alla base della specifica interazione dei ceppi di *Collimonas* con diverse specie fungine (capitolo 4).
I risultati ottenuti con questa ricerca costituiscono un contributo alla nostra comprensione delle interazioni tra i batteri e funghi, un tema che è stato finora poco esplorato.

Aknowledgements

I would like to thank all the people that offered me support, advice and friendship during my PhD period and played a crucial role in making possible this work.

Thanks to Dr. Wietse de Boer and Dr. Johan Leveau for providing supervision and guidance. During the years I spent working on my PhD you gave me a good example of analytical reasoning, creative thinking and goal oriented work. I would like to thank Dr. Kathrin Fritsche for the crucial support that she offered during my PhD work. Many thanks for making your expertise available for this project and devoting to this work much of your time and efforts. I am thankful to Marlies van den Berg who helped in the laboratory to realize many of the experiments that are part of this thesis.

The TME department is an excellent working group, where everyone is available and easy to talk to. I would like to thank all the people that are part of the group.

During the past years I enjoyed the friendly and collaborative atmosphere of the NIOO in Heteren. Many people contribute to make it the way it is, many thanks to all. Special thanks to Dr. Koen Verhoeven, for his availability to discuss statistical issues.

Thanks to the technicians who did a great job taking care that in the lab everything runs smoothly, the reception and administration that helped me through bureaucracy, many thanks to the technical department, Ab and Gilles, who are very kind and helped me to solve problems even when it was not part of their duties, bedankt!

It was a pleasure to meet at the NIOO people with whom I shared breaks, lunches, birthday dinners, and week-end events. Super thanks to past and present employees and friends (my apologies if, by mistake, I forget someone!): Tom, Roxi, Marcos, Sonja, Bibi, Sachie, Joris, Jeroen, Gustavo,

Luisa, Anja and Patrick, Eiko and Jop, Eva, Radek, Katrin, Stefan, Hannes, Anna, Ziemek, Gera, Olga, Mirka, Annemieke, Paul, Emilia, Remy, Sara, Mitja, Tanja, Taja, Annelies, Tess, Elly, Tim, Ciska, Sabrina, Alexandra, Almudena and Ronald. I especially would like to thank Anja with whom I shared the experience of doing a PhD and cycled more than 17000 km on the way between Wageningen and Heteren. I am happy that my good luck gave me the opportunity to become friends.

Finally I would like to thank for the support I got from my family.
Nei ringraziamenti della mia tesi di dottorato occupano un posto di primo piano le persone che con il loro amore ed il loro supporto incondizionato hanno reso possiblile la mia educazione e che rappresentano una parte così importante della mia vita. Un bacio, con infinito amore, alla mia famiglia (mà Rosella, pà Domenico, Fede, Fabri, Giagio, Tommi, Beucci, zio Gianni, zia Franca, Dani, Marco, Mate, Eri, Nico, Tino, Gegio, Luchino e Fernando).

Curriculum vitae

Francesca Mela was born on 12 September 1978 in Imperia, Italy. She received her secondary education at the Liceo classico E. de Amicis in Imperia. Afterwards she started her university education at the Università degli studi di Firenze, where on 21 March 2005 she obtained her master degree. In 2003, she received an Erasmus scholarship to spend five months at the Staatliches Weinbauinstitut Freiburg, Germany, and carry out experiments necessary for the completion of her master thesis, which aimed at understanding the distribution of the phytopathogenic fungus *Fomitiporia mediterranea* using molecular tools. In 2004 she started an internship at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, where she worked in the group of Evolutionary Phytopathology headed by Prof. Dr. P. W. Crous. During her stay she collaborated in the project "Phylogeny and taxonomy of *Phaeoacremonium* and its relatives", aimed at obtaining morphology and molecular tools for the classification of fungal pathogens of the genera *Phaeoacremonium* and *Paeomoniella*. In 2005 she started her PhD project at the Centre for terrestrial Ecology of the Netherlands Institute of Ecology NIOO-KNAW, under the supervision of Dr. W. de Boer, Dr. J. H. J. Leveau and Prof. Dr. J. A. van Veen. This project was part of the BSIK project entitled "Ecogenomics: the living soil" and resulted in the thesis presented here. In June 2010 she started working as post-doc in the group of Prof. Dr. P. Heutink in the section of clinical genetics of the Vrije Universiteit Medical Center, in Amsterdam.