CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector
Current Biology 17, 773–777, May 1, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.03.039

Report

Endosymbiont-Dependent Host Reproduction Maintains Bacterial-Fungal Mutualism

Laila P. Partida-Martinez,[1] Shamci Monajembashi,[2] Karl-Otto Greulich,[2] and Christian Hertweck[1,3,*]
[1] Department of Biomolecular Chemistry
Leibniz Institute for Natural Product Research and Infection Biology
Hans Knöll Institute (HKI)
07745 Jena
Germany
[2] Leibniz Institute for Age Research
Fritz Lipmann Institute (FLI)
07745 Jena
Germany
[3] Friedrich-Schiller-University
07745 Jena
Germany

Summary

Bacterial endosymbionts play essential roles for many organisms, and thus specialized mechanisms have evolved during evolution that guarantee the persistence of the symbiosis during or after host reproduction [1, 2]. The rice seedling blight fungus *Rhizopus microsporus* represents a unique example of a mutualistic life form in which a fungus harbors endobacteria (*Burkholderia* sp.) for the production of a phytotoxin [3]. Here we report the unexpected observation that in the absence of endosymbionts, the host is not capable of vegetative reproduction. Formation of sporangia and spores is restored only upon reintroduction of endobacteria. To monitor this process, we succeeded in GFP labeling cultured endosymbionts. We also established a laserbeam transformation technique for the first controlled introduction of bacteria into fungi to observe their migration to the tips of the aseptate hyphae. The persistence of this fungal-bacterial mutualism through symbiont-dependent sporulation is intriguing from an evolutionary point of view and implies that the symbiont produces factors that are essential for the fungal life cycle. Reproduction of the host has become totally dependent on endofungal bacteria, which in return provide a highly potent toxin for defending the habitat and accessing nutrients from decaying plants. This scenario clearly highlights the significance for a controlled maintenance of this fungal-bacterial symbiotic relationship.

Results and Discussion

Stable symbiotic associations between endocellular bacteria and eukaryotes are well known in the animal and plant kingdoms, and numerous groundbreaking studies have contributed to a deeper insight into the hallmarks and mechanisms of living together [1, 2]. Notably, only very little is known about symbiotic interactions between fungi and endobacteria, and only a few examples restricted to arbuscular mycorrhizal (AM) fungi have been discovered within the last decade [4–6]. Only recently we found that such little explored symbioses also play a crucial role for the saprotrophic fungus *Rhizopus microsporus*, which is infamous for causing severe losses in rice nurseries. An antimitotic polyketide metabolite, rhizoxin, isolated from fungal cultures has been identified as the causative agent of the plant disease, known as rice seedling blight [7, 8]. We showed that this phytotoxin is not produced by the fungus, but by symbiotic bacteria of the genus *Burkholderia* that reside within the fungal cytosol [3]. This case represents an unparalleled example for a symbiosis, in which a fungus harbors bacteria for the production of a virulence factor. Our findings were corroborated by curing the fungus with an antibiotic, which resulted in a symbiont-free, rhizoxin-negative phenotype [3]. Furthermore, we succeeded in isolating the endosymbiont in pure culture and in proving its capability for rhizoxin production [9]. Cloning, sequencing, and mutagenesis of the entire gene locus encoding rhizoxin biosynthesis within the symbiont genome revealed the molecular basis for toxin production [10]. Finally, reinfection of the cured fungal strain with the isolated symbiont reestablished a rhizoxin-producing fungal-bacterial symbiosis [3]. More recently we found that also the "mycotoxin" rhizonin is actually produced by bacterial endosymbionts [11]. These findings were in full agreement with Koch's laws and implied that the symbionts can be transmitted horizontally. Such a scenario is typical in cyclical endosymbioses, which require regular reassociation events, and is exemplified by the *Geosiphon pyriforme*-*Nostoc punctiforme* symbiosis [12, 13]. However, as for the *Burkholderia*-*Rhizopus* symbiosis, the low frequency of infection and single-spore germination experiments pointed toward a permanent rather than a cyclical association type.

Cured Fungi Are Incapable of Sporulation

In the course of the studies on the true producer of the phytotoxin, we found that no spores could be harvested from the cured strain. A microscopic investigation revealed that the symbiont-free mycelium forms neither sporangia nor columellae, which is in stark contrast to the wild-type (Figure 1). Apparently, the fungus produces only hyphae, but does not differentiate in the absence of the endosymbionts. In an alternative approach, wild-type spores were immersed in ciprofloxacin-containing media to eliminate endobacteria and were then incubated to yield symbiont-free clones. Again, cured fungi proved to be incapable of vegetative reproduction through spores. Cocultivation of the symbiont-free strains with isolated endosymbionts resulted in an intact symbiosis, in which asexual reproduction was re-established. To exclude any secondary effects that were potentially caused by the antibiotic, all cured strains were cultured in the absence of any additives.

*Correspondence: christian.hertweck@hki-jena.de

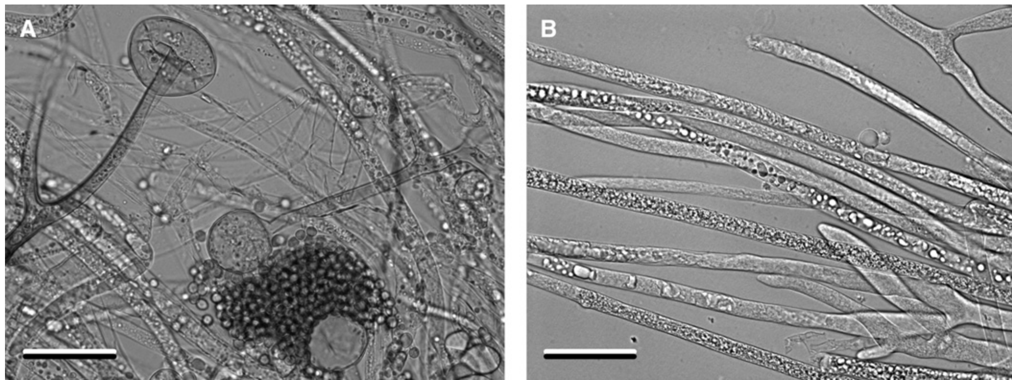

Figure 1. Micrographs of *R. microsporus* Cultures

(A) Typical appearance of the wild-type showing hyphae, columellae, and globose sporangium with spores.

(B) The cured strain does not form sporangia.

Scale bars represent 30 μm.

Restitution of Symbiosis by Laser-Mediated Microinjection of GFP-Labeled Endobacteria

The most surprising and unprecedented observation in this study is that the cured host forms only bulk hyphae and is strictly incapable of vegetative reproduction through spores under laboratory conditions. The formation of sporangia and sporulation is exclusively observed in the wild-type and after restoring the fungus-bacterium association with living bacteria. This scenario implies a highly specialized symbiotic interaction. To unequivocally prove the transmission mode and monitor the fate of bacteria in the cytosol, we needed to establish techniques for labeling and introducing the cultured endobacteria.

The *Burkholderia* symbionts are capable of invading the fungal cell by a yet undiscovered mechanism. This process is unpredictable in time and location, so we sought to mechanically introduce labeled bacteria into the cured fungi. For this purpose, we first established an electroporation procedure for transformation of electrocompetent cells of isolated endobacteria. With this protocol, we successfully introduced a plasmid (pHTK2) harboring a gene encoding green fluorescent protein (GFP), which has been previously employed in biofilm studies of the *Burkholderia cepacia* complex [14]. Positive transformants selected for trimethoprim resistance showed green fluorescence under blue light (GFP filter). Subsequently, a technique for the directed introduction of the GFP-labeled endosymbionts into the fungal mycelium was required. To our knowledge, the targeted infection of fungi by bacteria has not yet been reported. To achieve this goal, we adopted a method for the transformation of plants with DNA, which involves a laser microbeam coupled to a microscope [15, 16]. It has been shown that laser microinjection allows natural barriers to be overcome, i.e., enables transfection in cases where other techniques fail [17]. The beam was focused on the fungal cytoplasm (Figure 2A), and several laser pulses proved to be sufficient for microinjection at a specific site. The process was monitored by CLS microscopy, which showed that the labeled bacteria were rapidly taken up by the fungus through osmotic pressure (Figures 2B–2E). The motion of bacteria within the cytosol was clearly visible. As displayed in Figures 2 and 3A, the motile bacteria are likely prone to chemotaxis and migrate toward the tips of the hyphae. Notably, this is the region with the best supply of nutrients and where sporangia are formed. In this context, it should also be highlighted that *Rhizopus* is an aseptate fungus and thus movement of the bacteria is not physically restricted.

Endosymbiont-Dependent Sporulation

While the symbiont-free host strains were strictly incapable of developing sporangia in the cultivation medium, the reinfected strain behaved like the wild-type. 4 days after microinjection, the formation of sporangia was clearly visible. Furthermore, GFP labeling of the symbionts allowed their detection in the sporangia as well as within the spores (Figures 3B–3D). In all investigated individual spores, GFP-labeled, rod-shaped endobacteria were clearly visible. The transformation experiment for restoration of symbiosis has been successfully repeated in two other *R. microsporus* strains. Taken together, these experiments provide clear evidence that sporulation is triggered only by the presence of endobacteria.

Ecological and Evolutionary Implications

A critical issue for the survival of all mutualistic life forms is to ensure the transmission of the symbionts during or after host reproduction. Symbionts can be heritable through a vertical mode, i.e., directly from parent to offspring, which is characteristic for obligate symbiotic relationships. Alternatively, partners may reproduce independently and reassociate through horizontal symbiont transfer at a later stage [1]. Irrespective of the mode of transmission, specific mechanisms must have evolved for maintenance of the symbiosis as a long-term strategy for survival of the mutualistic partners.

Species of the genus *Burkholderia* are known for their ability to access and inhabit unusual ecological niches [18]. The fungus *R. microsporus* represents an ideal vector for rapid bacterial dispersal in new roots [19] and a most efficient spreading of the symbiosis through spores. In this context, it is remarkable that endobacteria isolated from fungi of geographically distant collection sites all over the globe are very closely related [3]. The fungus, on the other hand, lost control over autonomous

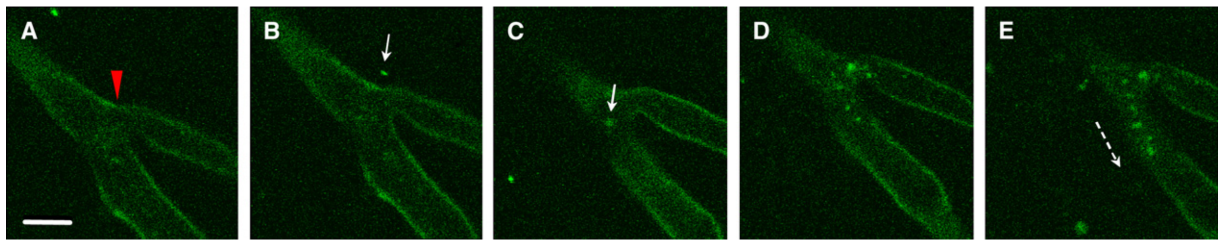

Figure 2. Monitoring Microinjection of GFP-Labeled Endosymbionts into the Fungus and Migration within the Hyphae

Micrographs (A)–(E) are screen captures taken at 0.5 min intervals. Scale bar represents 10 μm. Red arrow indicates site of laser injection; white arrows highlight GFP-labeled bacteria and direction of their migration.

reproduction but has gained the antimitotic agent rhizoxin, which is produced by the endosymbionts, as a chemical weapon.

Endosymbiont-dependent host reproduction provides a viable control over symbiont transmission and prevents the loss of any mutualistic partner. Yet effects of endosymbionts upon growth and reproduction of the host have been described in only a few animal symbioses, where microorganisms often provide essential nutrients [20, 21]. In extreme cases, their lack can even lead to sterility. For example, adult *Xyleborus ferrugineus* beetles that developed from symbiont-free eggs reproduced only when a mutualistic fungus was inoculated into the diet [22]. Oogenesis in the date stone beetle, *Coccotrypes dectyliperda*, depends on symbiotic bacteria [23], and in the *Buchnera*-aphid symbiosis, the hosts suffer sterility when deprived of the symbionts resulting from lacking nutrients [24–26].

The control over fungal spore formation and reproduction by endobacteria is unprecedented. Although fungal development has long been the subject of vast research endeavor, surprisingly little is known about the factors mediating sporulation, and as of yet, no specific triggers have been reported. Here we report the first example of a bacterium required for sporulation in a fungus. Low molecular weight molecules play pivotal roles in the communication of bacteria and eukaryotes [2, 27–30], so we postulate that the induction of sporulation has a chemical basis. Irrespective of the nature of the trigger or essential factors, the symbiont must bear genes

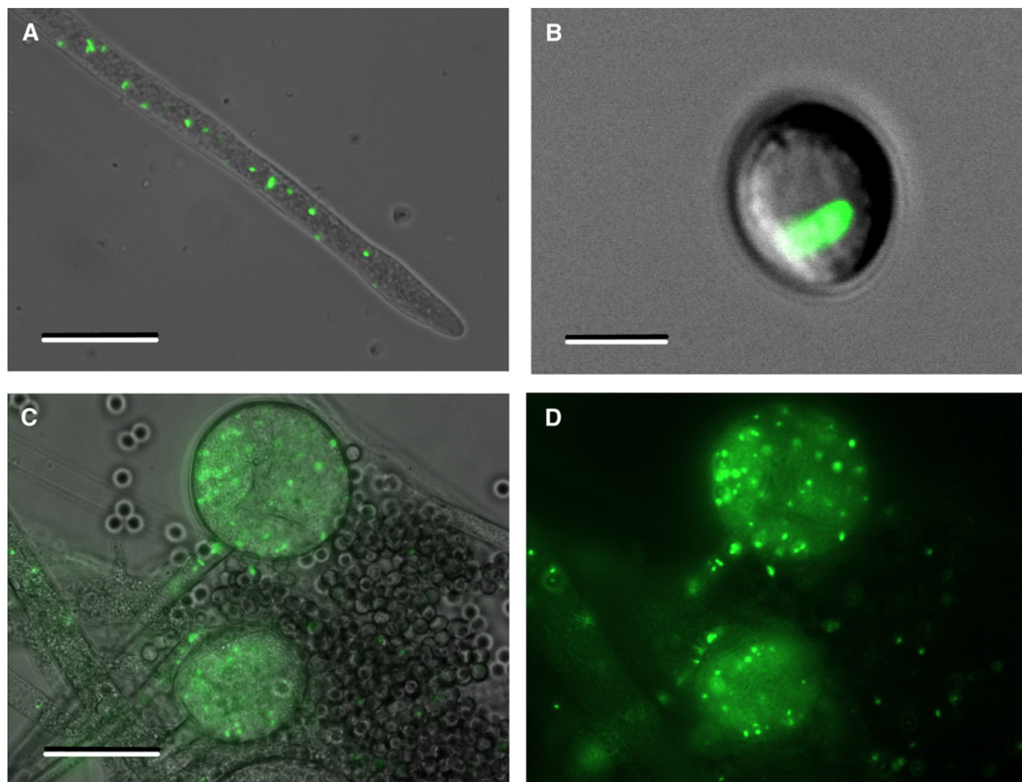

Figure 3. Confocal Laser Scanning Micrographs of Fungi after Microinjection with GFP-Labeled Bacteria

(A) Hypha showing bacteria migrating to the tip (scale bar represents 25 μm).

(B) A single vegetative spore containing a rod-shaped GFP-labeled endobacterium (scale bar represents 2 μm).

(C and D) Sporangiophores, sporangia, and spores formed after restitution of the symbiosis (white light and fluorescence mode, scale bar represents 30 μm).

required for fungal development that have been functionally redundant in an early stage of the symbiosis. In the course of evolution, an ancestral fungal host strain that was associated with bacteria producing the sporulation factor must have lost the ability to produce the signal or essential components on its own. This loss of function might be rationalized by either gene deletion or lack of gene expression. It should be noted that adding crude extracts of a symbiont fermentation did not induce sporulation in the cured fungus.

The *Rhizopus-Burkholderia* association represents an intriguing example for the evolution of a symbiosis. It is generally conceivable that the bacteria exploit its host in a less than mutualistic way. However, considering the benefit of the antimitotic agent produced by the symbiont, all observations imply an evolutionary parasitism-mutualism shift, and eventually a transition from facultative to an obligatory association [31]. Despite the evidence for a highly specialized interaction, which points toward an old symbiosis, the bacterial endosymbionts can still be isolated and grown in pure culture. In insect symbioses, substantial gene loss resulted in an integration of host and symbiont metabolic functions [32]. Obviously, genome reduction of the *Burkholderia* symbiont has not led to the loss of vital functions. This is reflected by the still relatively large size of the genome of the *Rhizopus* endosymbiont (3.8 Mb; C.H. and L.P.P.-M., unpublished results) compared to the yet unculturable symbiont of *Gigaspora* (1.2 Mb) [6].

In conclusion, we have demonstrated that the phytopathogenic alliance of *Rhizopus* and *Burkholderia* employs a new mechanism for the maintenance of the symbiosis. Two lines of evidence prove that the endosymbionts are vertically transmitted through spores, which are formed only when symbionts are present. Consequently, vegetative reproduction of the fungal host is strictly dependent of the endosymbionts. To monitor the process of bacterial migration in the fungal cytosol and the mode of vertical transmission, we succeeded in GFP labeling cultured endobacteria and established a laserbeam transformation technique for the first controlled introduction of endosymbionts into fungi. Both the *Rhizopus* host and the *Burkholderia* endosymbionts were cultured independently and could be reassembled by the laser technique. The persistence of this mutalism through controlled sporulation is of high significance from an evolutionary point of view. Our results support the hypothesis that the mutualism results from an ancient infection followed by the evolution of a concerted mode of vertical transmission and imply an intermediary functional redundancy in both partners. During evolution, the fungus lost its ability to produce endogenous sporulation factors and became dependent on endobacteria for reproduction, which impressively demonstrates the impact of the symbiosis: the fungal host gained rhizoxin as an efficient chemical means for defending the habitat against competitors and a supply of nutrients from dead plant matter.

Experimental Procedures

Strains

Rhizopus microsporus CBS112285 (HKI 0383) harboring endobacteria *Burkholderia* sp. B5 (HKI 0456) was used in this study. Endobacteria were isolated from their fungal host as reported.

Elimination of Endobacteria in Fungal Strains

Endobacteria from *Rhizopus microsporus* CBS112285 (HKI 0383) were eliminated by continuous antibiotic treatment. Plates were prepared with PDA (Potato-Dextrose-Agar) and supplemented with 0.02 mg mL^{-1} ciprofloxacin (Bayer AG, Germany). Growing mycelium (3 days old) was then transferred to a submerged culture of TSB (Tripton-Soya-Broth) with ciprofloxacin (0.02 mg mL^{-1}) and incubated overnight at 30°C and 115 rpm orbital shaking. The culture was centrifuged for 5 min at 4000 rpm, and a slant from the supernatant was plated again onto PDA-ciprofloxacin. This procedure was repeated three times, and the complete elimination of bacteria was analyzed by PCR and monitoring the metabolic profile (absence of rhizoxin as an indicator) of the cured fungi in production media (1% corn starch, 0.5% glycerol, 1% gluten meal, 1% dried yeast, 1% corn steep liquor, and 1% $CaCO_3$ at pH 6.5) without addition of ciprofloxacin. The sterile fungal strain was named CBS112285/S.

Transformation of Endobacterial Strains with a Green Fluorescent Protein Vector

Endobacterial strain *Burkholderia* sp. B5 was cultivated in TSB, and cells were made competent as described by Choi et al. [33]. For electroporation, 750 ng of vector pHTK2 [14], kindly provided by K. Tomlin at the University of Calgary, were first denaturated for 5 min at 95°C and then mixed with 100 μl *Burkholderia* sp. B5 electrocompetent cells. The mixture was transferred to a sterile 2 mm gap width electroporation cuvette, and a pulse with the following settings was applied: 25 μF, 200 Ω, 2.5 KV on a Bio-Rad Gene-PulserXCell (Bio-Rad, Germany). 1 ml of TSB was then added to the cells and the contents transferred into a glass tube for overnight cultivation at 30°C at 115 rpm. The cells were then harvested in a microcentrifuge tube and concentrated to 50 μL. Cells were plated in nutrient agar supplemented with 0.1 mg mL^{-1} trimethoprim (Fluka, Netherlands). Transformed colonies, designated B5 GFP, appeared after 3–4 days and were analyzed with a Leica DM4500 B light microscope (Leica Microsystems, Germany) equipped with a blue light, BP 470/40 excitation filter.

Microinjection of Bacterial Symbionts into the Fungal Cytoplasm

For cell preparation, fungal strains were plated onto PDA agar and allowed to grow at 30°C for at least 4 days. Transformed *Burkholderia* sp. B5 cells were picked from the NA-trimethoprim plate and cultivated in 1 ml TSB with trimethoprim (0.1 mg mL^{-1}) at 30°C, 115 rpm for 2 days. In a borosilicate cover glass chamber (Lab-Tek Chambered Coverglass System, Nalge Nunc International, USA), 700 μl of TSB with 4% glycerol were mixed with a small mycelial pellet and 200 μl of a culture of green fluorescent bacterial cells. With a laser microbeam coupled to a microscope, it was possible to inject the green fluorescent bacterial cells into the fungal cytoplasm. The setup used here is based on an inverted confocal laser scanning microscope (LSM 510, Carl Zeiss, Jena, Germany). The output laser beam of a pulsed laser (diode-pumped, Q-Switched Frequency-Tripled Laser System: Triton; TEM00, 349 nm; maximum power 1 W, pulsed; repetition rate: single shot, 1 KHz; pulse width: <15 ns; pulse energy: up to 250 μJ; Spectra Physics, Darmstadt, Germany) is expanded by a telescope system and is focused into the object plane of the microscope by a Zeiss Plan-Neofluar 100/1.30 oil objective (spot diameter < 500 nm) after reflection by a dielectric mirror (Laser Optik, Germany). The dielectric mirror is placed on the empty laser scanning position of the fluorescence reflector slider and transmits the scanning lasers as well as the emitted fluorescence and reflects the pulsed laser beam of Triton. Thus the imaging functions of the LSM are not reduced. LSM is additionally equipped with a cell culture cultivation system (Zeiss, Germany) that consists of an incubation chamber, heating microscope stage, temperature and CO_2 controller units, objective heater, and a humidifying system. This allows long-time live-cell imaging. To uptake the bacteria into the fungal cytoplasm, the beam was focused on the fungal cytoplasm. The bacteria were incorporated into the fungal cytoplasm by several laser pulses (at repetition rate of 20 Hz for a few seconds; at ~0.5 μJ in object plane), and the injection procedure was recorded/controlled by time series function of LSM (stack size: 512 × 512; slice time: 985 ms).

Injected fungal cells were then plated on PDA-trimethoprim plates. After 6 days at 30°C, a mycelium pellet was inoculated in 100 ml production media and cultivated for 6 days, and mycelial cake was then harvested and extracted with ethyl acetate (grown culture: ethyl acetate 1:1 *v/v*).

Infection of Fungi by Cocultivation of Fungi and Bacteria
Fluorescent bacterial cells were first grown in liquid media (TSB with trimethoprim) and then plated in PDA-trimethroprim. Bacteria were allowed to grow for 2 days before inoculating a small pellet of mycelial fungus. Cocultivation in the PDA-trimethoprim petri dish continued for 1 week. Fungal extracts were prepared in the same manner as for injected fungal strains. Microscopic preparations were observed under a Leica DM4500 B light microscope attached to a digital camera (Leica DFC480, Leica Microsystems, Germany).

Acknowledgments

We are grateful to M.-G. Schwinger for fungal strain preservation and K. Scherlach for support in HPLC analyses. We thank K.L. Tomlin and H. Ceri at the University of Calgary for kindly providing vector pHKT2 and I. Schmitt and A.A. Brakhage (HKI) for helpful comments. Financial support by the DFG is gratefully acknowledged. The authors declare no conflict of interest.

Received: February 8, 2007
Revised: March 1, 2007
Accepted: March 7, 2007
Published online: April 5, 2007

References

1. Moran, N.A. (2006). Symbiosis. Curr. Biol. *16*, R866–R871.
2. Dale, C., and Moran, N.A. (2006). Molecular interactions between bacterial symbionts and their hosts. Cell *126*, 453–465.
3. Partida-Martinez, L.P., and Hertweck, C. (2005). Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature *437*, 884–888.
4. deBoer, W., Folman, L.B., Summerbell, R.C., and Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Lett. *29*, 795–811.
5. Artursson, V., Finlay, R.D., and Jansson, J.K. (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. *8*, 1–10.
6. Minerdi, D., Bianciotto, V., and Bonfante, P. (2002). Endosymbiotic bacteria in mycorrhizal fungi: from their morphology to genomic sequences. Plant Soil *244*, 211–219.
7. Sato, Z., Noda, T., Matsuda, I., Iwasaki, S., Kobayashi, H., Furukawa, J., and Okuda, S. (1983). Studies on rhizoxin, a phytotoxin produced by *Rhizopus chinensis* causing rice seedling blight. Annu. Phytopathol. Soc. Japan *49*, 128.
8. Iwasaki, S., Kobayashi, H., Furukawa, J., Namikoshi, M., Okuda, S., Sato, Z., Matsuda, I., and Noda, T. (1984). Studies on macrocyclic lactone antibiotics. VII. Structure of a phytotoxin "rhizoxin" produced by *Rhizopus chinensis*. J. Antibiot. (Tokyo) *37*, 354–362.
9. Scherlach, K., Partida-Martinez, L.P., Dahse, H.-M., and Hertweck, C. (2006). Antimitotic rhizoxin derivatives from cultured symbionts of the rice pathogenic fungus *Rhizopus microsporus*. J. Am. Chem. Soc. *128*, 11529–11536.
10. Partida-Martinez, L.P., and Hertweck, C. (2007). A gene cluster encoding rhizoxin biosynthesis in *Burkholderia rhizoxina*, the bacterial endosymbiont of the fungus *Rhizopus microsporus*. ChemBioChem *8*, 41–45.
11. Partida-Martinez, L.P., Looß, C., Ishida, K., Ishida, M., and Hertweck, C. (2007). Rhizonin, the first mycotoxin isolated from lower fungi, is not a fungal metabolite, but produced by bacterial endosymbionts. Appl. Environ. Microbiol. *73*, 793–797.
12. Schüßler, A., Mollenhauer, D., Schnepf, E., and Kluge, M. (1994). *Geosiphon pyriforme*, an endosymbiotic association of fungus and cyanobacteria: the spore structure resembles that of arbuscular mycorrhizal (AM) fungi. Bot. Acta *107*, 36–45.
13. Schüßler, A., and Kluge, M. (2000). Geosiphon pyriforme, an endocytosymbiosis between fungus and cyanobacteria, and its meaning as a model system for AM research. In The Mycota IX, B. Hock, ed. (Berlin: Springer Verlag), pp. 151–161.
14. Tomlin, K.L., Clark, S.R., and Ceri, H. (2004). Green and red fluorescent protein vectors for use in biofilm studies of the intrinsically resistant *Burkholderia cepacia* complex. J. Microbiol. Methods *57*, 95–106.
15. Weber, G., Monajembashi, S., Greulich, K.-O., and Wolfrum, J. (1989). Uptake of DNA in chlorplasts of *Brassica napus* (L.) facilitated by a UV-laser microbeam. Eur. J. Cell Biol. *49*, 73–79.
16. Weber, G., Monajembashi, S., Wolfrum, J., and Greulich, K.-O. (1990). Genetic changes induced in higher plant cells by a laser microbeam. Physiol. Plant. *79*, 190–193.
17. Greulich, K.-O. (1999). Micromanipulation by light in biology and medicine: the laser microbeam and optical tweezers (Basel: Birkhäuser).
18. Coenye, T., and Vandamme, P. (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ. Microbiol. *5*, 719–729.
19. Sanders, I.R. (2005). Conspirators in blight. Nature *437*, 823–824.
20. Moran, N.A., and Baumann, P. (2000). Bacterial endosymbionts in animals. Curr. Opin. Microbiol. *3*, 270–275.
21. Zientz, E., Dandekar, T., and Gross, R. (2004). Metabolic interdependence of obligate intracellular bacteria and their insect hosts. Microbiol. Mol. Biol. Rev. *68*, 745–770.
22. Norris, D.M., and Baker, J.K. (1967). Symbiosis: effects of a mutualistic fungus upon growth and reproduction of *Xyleborus ferrugineus*. Science *156*, 1120–1122.
23. Zchori-Fein, E., Borad, C., and Harari, A.R. (2006). Oogenesis in the date stone beetle, *Coccotrypes dectyliperda*, depends on symbiotic bacteria. Physiol. Entomol. *31*, 164–169.
24. Douglas, A.E. (1998). Host benefit and the evolution of specialization in symbiosis. Heredity *81*, 599–603.
25. Houk, E.J., and Griffiths, G.W. (1980). Intracellular symbiotes of Homoptera. Annu. Rev. Entomol. *25*, 161–187.
26. Moran, N., and Dunbar, H. (2006). Sexual acquisition of beneficial symbionts in aphids. Proc. Natl. Acad. Sci. USA *103*, 12803–12806.
27. Rosenblueth, M., and Martinez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. Mol. Plant Microbe Interact. *19*, 827–837.
28. Dudler, R., and Eberl, L. (2006). Interactions between bacteria and eukaryotes via small molecules. Curr. Opin. Biotechnol. *17*, 268–273.
29. Piel, J. (2004). Metabolites from symbiotic bacteria. Nat. Prod. Rep. *21*, 519–538.
30. Koropatnick, T.A., Engle, J.T., Apicella, M.A., Stabb, E.V., Goldman, W.E., and McFall-Ngal, M.J. (2004). Microbial factor-mediated development in a host-bacterial mutualism. Science *306*, 1186–1188.
31. Ochman, H., and Moran, N.A. (2001). Genes lost and found: evolution of bacterial pathogenesis and symbiosis. Science *292*, 1096–1099.
32. Wernegreen, J.J. (2002). Genome evolution in bacterial endosymbionts. Nat. Rev. Genet. *3*, 850–861.
33. Choi, K.H., Kumar, A., and Schweizer, H.P. (2006). A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J. Microbiol. Methods *64*, 391–397.