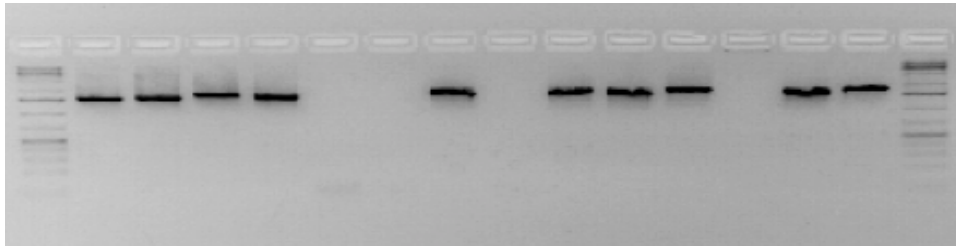
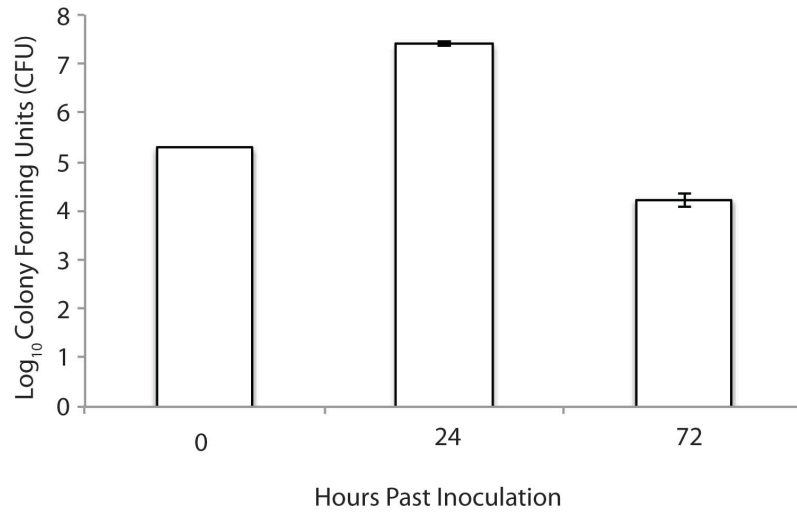


Supplementary Figure 1. PCR analysis confirms presence/absence of endohyphal bacteria in fungi. Gel shows PCR using bacterial primers 27F and 1492R with the following sources (left to right): genomic DNA from axenic *Luteibacter* sp. 9143; axenic *Luteibacter* sp. 9145; *Pestalotiopsis* sp. 9143 (naturally infected; outset of experiments); *Microdiplodia* sp. 9145 (naturally infected; outset of experiments); negative control (water); negative control for gel quality (blank); *Pestalotiopsis* sp. 9143 (naturally infected, at conclusion of experiments); *Pestalotiopsis* sp. 9143 (cured); *Pestalotiopsis* sp. 9143 (reinfected with *Luteibacter* sp. 9143); *Pestalotiopsis* sp. 9143 (cross-infected with *Luteibacter* sp. 9145); *Microdiplodia* sp. 9145 (naturally infected, at conclusion of experiments); *Microdiplodia* sp. 9145 (cured); *Microdiplodia* sp. 9145 (reinfected with *Luteibacter* sp. 9145); *Microdiplodia* sp. 9145 (cross-infected with *Luteibacter* sp. 9143); ladder.

Supplementary Fig. 1



Supplementary Figure 2. Log₁₀ colony-forming units calculated for four replicate cultures of *Luteibacter* sp. 9143 in LB media after 1:100 dilution from overnight culture at 0, 24, and 72 hours after inoculation. After 24 h the cultures are in stationary phase. After 72 hours they form dense mucilaginous clumps.



Supplementary Table 1. Columns indicate the parameters of the reintroduction protocol that were manipulated to evaluate factors influencing *in vitro* resynthesis of the endohyphal symbiosis of *Luteibacter* sp. 9143 in *Pestalotiopsis* sp. 9143. Rows define treatments. ‘Yes’ in the last column indicates the presence of EHB in the fungal cultures as defined in the methods above. A ‘-’ indicates bacteria were not viable after co-culturing. A ‘+’ indicates viable bacteria were present outside fungal cells. The treatment identical to resynthesis experiment 1 is underlined.

| Age of bacterial culture | MgCl₂ wash | Concentration of PDB | Mycelium: bacteria ratio | Medium in co-culture transfers | Successful resynthesis |
|---------------------------------|------------------------------|-----------------------------|---------------------------------|---------------------------------------|-------------------------------|
| 3 d | yes | 2.40% | 5:1 | 1X PDA | no |
| <u>3 d</u> | <u>yes</u> | <u>2.40%</u> | <u>5:1</u> | <u>water agar</u> | <u>yes</u> |
| 3 d | yes | 2.40% | 7:1 | 1X PDA | no |
| 3 d | yes | 2.40% | 7:1 | water agar | yes |
| 3 d | yes | 2.40% | 10:1 | 1X PDA | no |
| 3 d | yes | 2.40% | 10:1 | water agar | no |
| 3 d | yes | 0.1X | 5:1 | 1X PDA | no |
| 3 d | yes | 0.1X | 5:1 | water agar | yes |
| 3 d | yes | 0.1X | 7:1 | 1X PDA | no |
| 3 d | yes | 0.1X | 7:1 | water agar | yes |
| 3 d | yes | 0.1X | 10:1 | 1X PDA | no |
| 3 d | yes | 0.1X | 10:1 | water agar | no |
| 3 d | yes | 0.01X | 5:1 | 1X PDA | no |
| 3 d | yes | 0.01X | 5:1 | water agar | yes |
| 3 d | yes | 0.01X | 7:1 | 1X PDA | yes |
| 3 d | yes | 0.01X | 7:1 | water agar | yes |
| 3 d | yes | 0.01X | 10:1 | 1X PDA | no |
| 3 d | yes | 0.01X | 10:1 | water agar | yes |
| 3 d | yes | 0.001X | 5:1 | 1X PDA | no |

| | | | | | |
|-----|-----|---------|------|------------|------------|
| 3 d | yes | 0.001X | 5:1 | water agar | yes |
| 3 d | yes | 0.001X | 7:1 | 1X PDA | no |
| 3 d | yes | 0.001X | 7:1 | water agar | yes |
| 3 d | yes | 0.001X | 10:1 | 1X PDA | no |
| 3 d | yes | 0.001X | 10:1 | water agar | yes |
| 3 d | yes | 0.0001X | 5:1 | 1X PDA | no |
| 3 d | yes | 0.0001X | 5:1 | water agar | no |
| 3 d | yes | 0.0001X | 7:1 | 1X PDA | no |
| 3 d | yes | 0.0001X | 7:1 | water agar | no |
| 3 d | yes | 0.0001X | 10:1 | 1X PDA | no |
| 3 d | yes | 0.0001X | 10:1 | water agar | no |
| 3 d | no | 2.40% | 5:1 | 1X PDA | - |
| 3 d | no | 2.40% | 5:1 | water agar | - |
| 3 d | no | 2.40% | 7:1 | 1X PDA | - |
| 3 d | no | 2.40% | 7:1 | water agar | - |
| 3 d | no | 2.40% | 10:1 | 1X PDA | - |
| 3 d | no | 2.40% | 10:1 | water agar | - |
| 3 d | no | 0.1X | 5:1 | 1X PDA | - |
| 3 d | no | 0.1X | 5:1 | water agar | - |
| 3 d | no | 0.1X | 7:1 | 1X PDA | - |
| 3 d | no | 0.1X | 7:1 | water agar | - |
| 3 d | no | 0.1X | 10:1 | 1X PDA | - |
| 3 d | no | 0.1X | 10:1 | water agar | - |
| 3 d | no | 0.01X | 5:1 | 1X PDA | - |
| 3 d | no | 0.01X | 5:1 | water agar | - |
| 3 d | no | 0.01X | 7:1 | 1X PDA | - |
| 3 d | no | 0.01X | 7:1 | water agar | - |
| 3 d | no | 0.01X | 10:1 | 1X PDA | - |

| | | | | | |
|-----|-----|---------|------|------------|------------|
| 3 d | no | 0.01X | 10:1 | water agar | - |
| 3 d | no | 0.001X | 5:1 | 1X PDA | - |
| 3 d | no | 0.001X | 5:1 | water agar | - |
| 3 d | no | 0.001X | 7:1 | 1X PDA | - |
| 3 d | no | 0.001X | 7:1 | water agar | - |
| 3 d | no | 0.001X | 10:1 | 1X PDA | - |
| 3 d | no | 0.001X | 10:1 | water agar | - |
| 3 d | no | 0.0001X | 5:1 | 1X PDA | - |
| 3 d | no | 0.0001X | 5:1 | water agar | - |
| 3 d | no | 0.0001X | 7:1 | 1X PDA | - |
| 3 d | no | 0.0001X | 7:1 | water agar | - |
| 3 d | no | 0.0001X | 10:1 | 1X PDA | - |
| 3 d | no | 0.0001X | 10:1 | water agar | - |
| 1 d | yes | 2.40% | 5:1 | 1X PDA | + |
| 1 d | yes | 2.40% | 5:1 | water agar | no |
| 1 d | yes | 2.40% | 7:1 | 1X PDA | + |
| 1 d | yes | 2.40% | 7:1 | water agar | no |
| 1 d | yes | 2.40% | 10:1 | 1X PDA | + |
| 1 d | yes | 2.40% | 10:1 | water agar | yes |
| 1 d | yes | 0.1X | 5:1 | 1X PDA | + |
| 1 d | yes | 0.1X | 5:1 | water agar | no |
| 1 d | yes | 0.1X | 7:1 | 1X PDA | no |
| 1 d | yes | 0.1X | 7:1 | water agar | yes |
| 1 d | yes | 0.1X | 10:1 | 1X PDA | no |
| 1 d | yes | 0.1X | 10:1 | water agar | yes |
| 1 d | yes | 0.01X | 5:1 | 1X PDA | no |
| 1 d | yes | 0.01X | 5:1 | water agar | yes |
| 1 d | yes | 0.01X | 7:1 | 1X PDA | no |

| | | | | | |
|-----|-----|---------|------|------------|------------|
| 1 d | yes | 0.01X | 7:1 | water agar | yes |
| 1 d | yes | 0.01X | 10:1 | 1X PDA | no |
| 1 d | yes | 0.01X | 10:1 | water agar | yes |
| 1 d | yes | 0.001X | 5:1 | 1X PDA | no |
| 1 d | yes | 0.001X | 5:1 | water agar | no |
| 1 d | yes | 0.001X | 7:1 | 1X PDA | no |
| 1 d | yes | 0.001X | 7:1 | water agar | yes |
| 1 d | yes | 0.001X | 10:1 | 1X PDA | no |
| 1 d | yes | 0.001X | 10:1 | water agar | yes |
| 1 d | yes | 0.0001X | 5:1 | 1X PDA | no |
| 1 d | yes | 0.0001X | 5:1 | water agar | no |
| 1 d | yes | 0.0001X | 7:1 | 1X PDA | no |
| 1 d | yes | 0.0001X | 7:1 | water agar | no |
| 1 d | yes | 0.0001X | 10:1 | 1X PDA | no |
| 1 d | yes | 0.0001X | 10:1 | water agar | no |
| 1 d | no | 2.40% | 5:1 | 1X PDA | - |
| 1 d | no | 2.40% | 5:1 | water agar | - |
| 1 d | no | 2.40% | 7:1 | 1X PDA | - |
| 1 d | no | 2.40% | 7:1 | water agar | - |
| 1 d | no | 2.40% | 10:1 | 1X PDA | - |
| 1 d | no | 2.40% | 10:1 | water agar | - |
| 1 d | no | 0.1X | 5:1 | 1X PDA | - |
| 1 d | no | 0.1X | 5:1 | water agar | - |
| 1 d | no | 0.1X | 7:1 | 1X PDA | - |
| 1 d | no | 0.1X | 7:1 | water agar | - |
| 1 d | no | 0.1X | 10:1 | 1X PDA | - |
| 1 d | no | 0.1X | 10:1 | water agar | - |
| 1 d | no | 0.01X | 5:1 | 1X PDA | - |

| | | | | | |
|-----|----|---------|------|------------|---|
| 1 d | no | 0.01X | 5:1 | water agar | - |
| 1 d | no | 0.01X | 7:1 | 1X PDA | - |
| 1 d | no | 0.01X | 7:1 | water agar | - |
| 1 d | no | 0.01X | 10:1 | 1X PDA | - |
| 1 d | no | 0.01X | 10:1 | water agar | - |
| 1 d | no | 0.001X | 5:1 | 1X PDA | - |
| 1 d | no | 0.001X | 5:1 | water agar | - |
| 1 d | no | 0.001X | 7:1 | 1X PDA | - |
| 1 d | no | 0.001X | 7:1 | water agar | - |
| 1 d | no | 0.001X | 10:1 | 1X PDA | - |
| 1 d | no | 0.001X | 10:1 | water agar | - |
| 1 d | no | 0.0001X | 5:1 | 1X PDA | - |
| 1 d | no | 0.0001X | 5:1 | water agar | - |
| 1 d | no | 0.0001X | 7:1 | 1X PDA | - |
| 1 d | no | 0.0001X | 7:1 | water agar | - |
| 1 d | no | 0.0001X | 10:1 | 1X PDA | - |
| 1 d | no | 0.0001X | 10:1 | water agar | - |
