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Are Bacteria Omnipresent on *Phanerochaete chrysosporium* Burdsall?

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Cultures of *Phanerochaete chrysosporium* were examined for the presence of bacteria as previously described (F. Seigle-Murandi, P. Guiraud, C. Falsen, and K.-E. Eriksson, Appl. Environ. Microbiol. 62:2477-2481, 1996). Under no conditions could bacteria be isolated from cultures of *P. chrysosporium.* With PCR primers corresponding to small-subunit rRNA genes, no bacterium-like product could be amplified from cultures of the widely used *P. chrysosporium* strain BKM-F-1767. Thus, we could find no evidence of bacteria in association with *P. chrysosporium* BKM-F-1767.

The white rot fungus *Phanerochaete chrysosporium* Burdsall has become the model system for studies of microbial degradation of lignin (for reviews, see references 3, 4, 5, and 7). In nature, *P. chrysosporium* can be isolated along with bacteria and other fungi, and it has been suggested that this association could be responsible for efficient degradation of lignocellulose (2).

Recently, Seigle-Murandi et al. (9) reported the consistent isolation of bacteria from *P. chrysosporium* cultures and reported that the various bacterial species could not be eliminated from the fungal cultures. Bacterial strains reportedly associated with *P. chysosporium* included *Agrobacterium radiobacter.* The physiological significance of this obligate coexistence was not established, although a potential symbiotic role in lignin degradation was suggested (9).

We reinvestigated the issue by selective plating of culture filtrates. Strains included *P. chrysosporium* BKM-F-1767 and ME446 (Center for Forest Mycology Research, Madison, Wis.), INA 12 (Chinese University of Hong Kong), and BG24 and BG10 (Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa). Bacterial strains CCUG 32543 *(Sphingomonas* sp.) and CCUG 32544 *(A. radiobacter),* isolated from *P. chrysosporium* cultures (9), were provided by F. Seigle-Murandi (Universite Joseph Fourier, Meylan, France).

Fungal strains were grown for 2 days at 37°C on malt extract agar (MEA), and attempts were made to isolate bacteria as described previously (9). Specifically, 5 ml of sterile water was added to the plates, and after swirling, the suspension was passed through a 3-μm-pore-size filter (Millipore). The filtrate was spread onto MEA supplemented with benomyl (10 mg liter^{-1}) and incubated at room temperature. No bacterial colonies were observed. Experiments with *P. chrysosporiurn* BKM-F-1767 were repeated in separate laboratories (Forest Products Laboratory, Madison, Wis., and The Pennsylvania State University, University Park), and in no instance were any bacteria isolated from this widely used strain.

To confirm that viable bacterial strains could be successfully recovered with the procedure, CCUG 32544 cells were harvested from MEA in sterile water and filtered through 3-μm-pore-size filters, and the filtrate was spread on MEA with benomyl. Bacterial colonies appeared after 2 days of incubation at 24°C. We then coincubated *P. chrysosporium* BKM-F-1767 with strain CCUG 32544. Bacterial colonies were visible for several days following coinoculation, but a typical mycelial culture morphology was observed by day 5. After 1 week of incubation at 37°C, filtrates were spread on MEA with benomyl. No bacteria were recovered from the coinoculated cultures or from BKM-F-1767 alone. Coinoculation experiments were repeated at 24°C, a nonoptimal temperature for *P. chrysosporium* but possibly favoring bacterial growth (9). Again, no viable bacteria were recovered.

In the event that bacteria might be associated with fungal cultures but tightly bound to hyphae, PCR amplification of ribosomal RNA genes was performed. Bacterial and fungal DNAs were isolated as described previously (6). The universal primers specific for small-subunit rRNA genes were 5'-GTG CCAGC(A/C)GCCGCGGTAA-3' and 5'-GGTTACCTTGT TACGACTT-3' (1). The PCRs and thermocycler program were as described previously (8). Amplification of CCUG 32544 and CCUG 32543 DNA yielded typical prokaryotic products of approximately 1,000 bp (Fig. 1, lanes 1 and 2) (1, 8). In contrast, a single product of 1,254 bp (Fig. 1, lane 3) was amplified from the *P. chysosporium* DNA, strongly arguing against the presence of any bacterial contamination. When DNA was isolated from cultures coinoculated with bacteria and fungi, both PCR gene products could be detected (Fig. 1, lanes 5 and 6).

In conclusion, selective plating and PCR amplifications failed to detect any bacteria in cultures of *P. chrysosporium,* including strain BKM-F-1767.

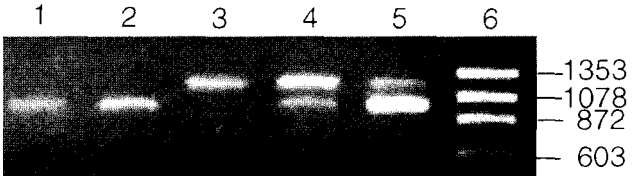

FIG. 1. PCR amplification of small-subunit rRNA genes using universal primers. Bacterial and *P. chrysosporium* products are approximately 1,000 bp and 1,254 bp, respectively. Lanes: 1, bacterial strain CCUG 32543; 2, bacterial strain CCUG 32544; 3, *P. chysosporium* BKM-F-1767; 4, mixed culture of CCUG; 32543 and BKM-F-1767; 5, mixed culture of CCUG 32544 and BKM-F-1767: and 6, *Hae* III-digested ϕ X174 as a molecular size marker.

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