# Assessment of the Fungal Diversity in Energy Transmission Towers by ITS-rDNA Clone Libraries

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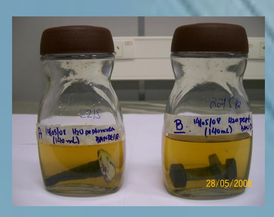
## Introduction

Many industrial sectors such as gas, oil, nuclear power, shipping, aircraft, chemical and civil engineering have substantial economical losses as a result of microbiologically-influenced corrosion (MIC). The costs associated with repairs due to corrosion and shutdown time in power plants reach billions of dollars annually (Koch et al. 2002) . Microorganisms implicated in corrosion of metals such as iron, copper and aluminum and their alloys are physiologically diverse and some may be also a threat to the stability of metals considered resistant to corrosion (Beech and Gaylarde 1999). The effect of bacteria in corrosion processes are well documented (Beech and Gaylarde 1999). However, although yeasts and filamentous can produce organics acids that are involved in MIC processes, fungi corrosion influenced by these microorganisms is still poorly investigated. In this sense, the aim of the present study was to profile the fungal diversity in metallic samples from transmission towers (São Paulo State, Brazil) presenting signs of corrosion or biofilm formation. In order to provide a detailed characterization of the fungal communities and to improve the understanding of MIC processes in such samples we evaluated the associated mycobiota by metagenomics.

## Materials and Methods



Samples were kept in peptone water in an orbital shaker (100 rpm) at 25°C for 10h

Centrifugation to recover material for DNA isolation

Extraction of metagenomic DNA

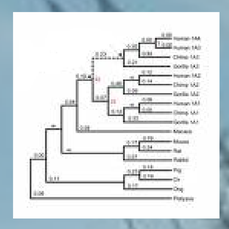
DNA amplification by PCR (primers ITS 1F and ITS 4)

Transformation in *E. coli* and selection of positive clones

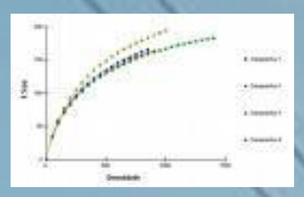
PCR of the selected clones by primer M13

Sequencing

Sequences were compared with sequence data available at NCBI-GenBank using BLAST N



Construction of Phylogenetic trees



Statistical analysis by Software DOTUR

## Results

A total of 288 clones were obtained (96 clones/library) for samples B, C and D). However, 160 out of 288 clones generated high-quality sequences were used in the diversity analysis (51, 58 and 51 sequences for libraries B, C and D, respectively). The most abundance of clones were related with order Capnodiales (33, 49, and 32 OTUs for libraries B, C and D, respectively), following the uncultured clones (6 OTUS for B, 1 OUT for C and 16 OTUs for D); Tremellales ( 8 and 6 OTUs for library B and C, respectively); Erythrobasidiales (4 OTUs for library B); Chaetothyriales (2 and 1 OTUs for libraries C end D, respectively) and Hypocreales with only 1 OTU for library D (Figure 1).

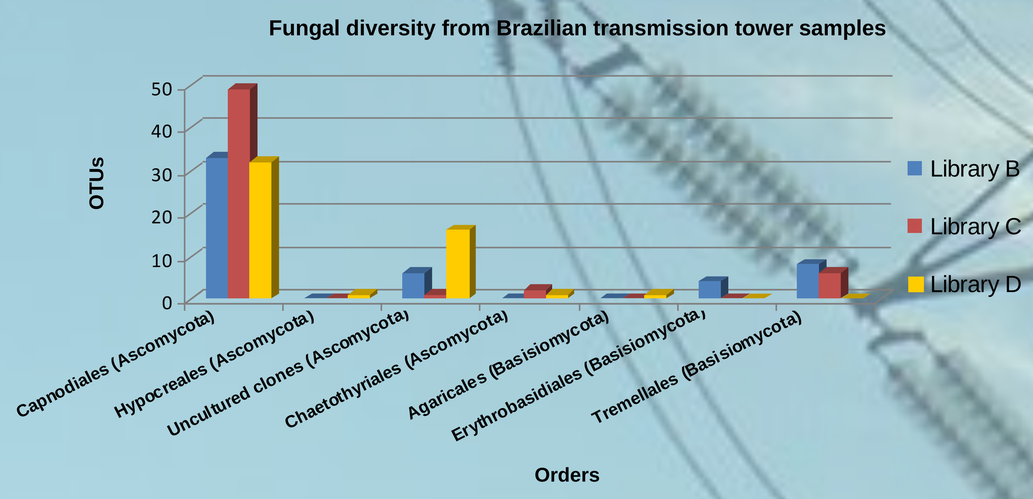



Figure 1. Numbers of OTUs recovered from transmission tower samples and their respective orders.

Statistical comparisons from the pooled data generated by DOTUR at 3% distance level resulted in 30 different OTUs. One clone representative of each OUT was selected for BLAST$_N$ and phylogenetic analyses (Figure 2). Overall, were obtained 10 OTU related to yeasts and 20 related to filamentous fungi comprising representatives of the phyla Ascomycota (141 clones), including the genera *Capnobotryella, Devriesia*, *Fusarium, Strelitziana* and *Teratosphaeria*; and Basidiomycota (19 clones), which were related to the genera *Cryptococcus, Fellomyces, Kockovaella, Panaeolus, Rhodotorula*, *Sirobasidium, Sporobolomyces* and *Tremella* (Figure 2). The fungal communities from samples B and D exhibited similar relative species richness according with rarefaction curves (Figure 3). In contrast, sample C showed significant higher OTUs richness when compared with other samples and more clones would be necessary for the curve reach a plateau.

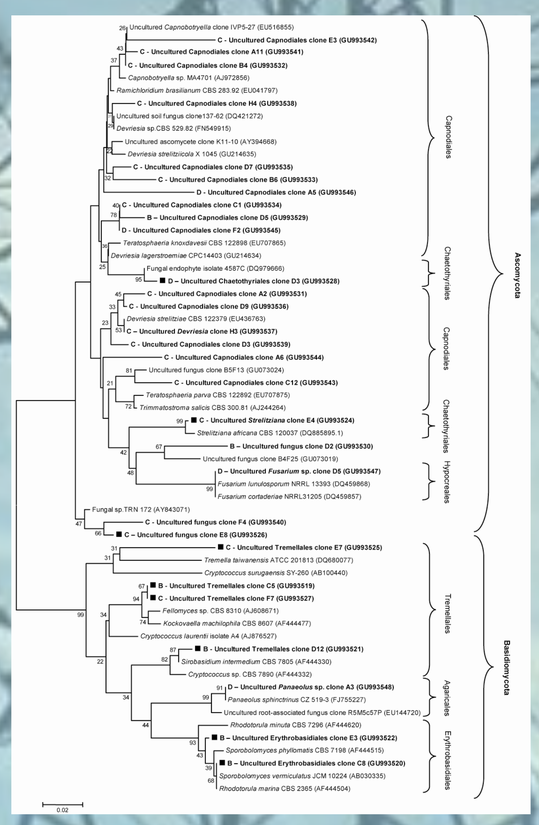



Figure 2. Unrooted neighbor-joining tree ITS-DNA gene of selected clones obtained from transmission tower samples. Sequences of closest relative taxa are also shown in the figure. Numbers on branches are bootstrap support values obtained from 1,000 pseudoreplicates.

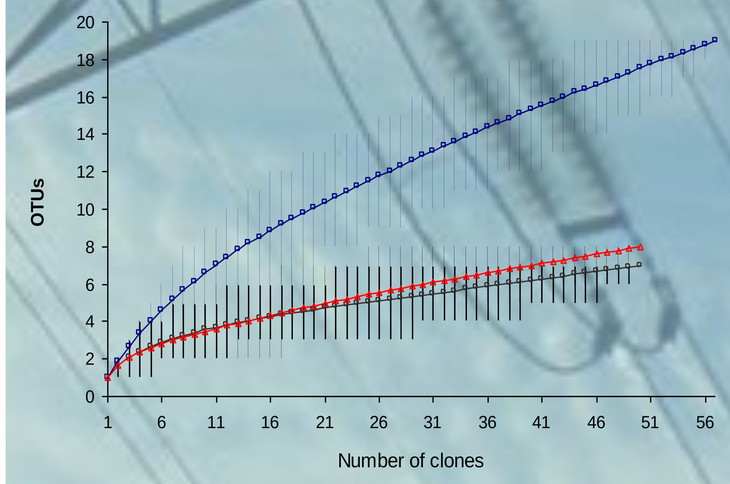



Figure 3. Rarefaction curves using ITS-DNA sequences from samples of transmission towers. Curves were generated by DOTUR analysis using 3% genetic distance level for clone libraries. Error bars represent 95% CI

## Conclusion

- Clones obtained from transmission tower samples using culture-independent methods belonged to two major fungal phyla: Ascomycota and Basidiomycota, indicating a broad microbial diversity in these metallic samples;
- Members of fungal community from transmission tower samples are ubiquitous fungi commonly found in other environments and some have been previously reported in microbiologically-influenced corrosion;
- This study presents an emerging view of the fungal community diversity of Brazilian energy transmission towers, allowing insights into the possible roles of microorganisms in this unusual environment.

## Support

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## References

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