

**Involvement of a laccase-like multicopper oxidase in humic substances degradation by
diverse polar soil bacteria**

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Soil humic substances (HS) are widely distributed in cold environments and comprise a significant fraction of soil organic carbon. Bacterial strains (n=281) were isolated at 15°C using a minimal medium containing humic acids (HA), a principal component of HS, from various polar soil samples: 217 strains from the Antarctic and 64 strains from Arctic soil. The 73 potential HA-degrading bacteria were affiliated with phyla *Proteobacteria* (73.9%), *Actinobacteria* (20.5%), and *Bacteroidetes* (5.5%). Most of them degraded HA at 10°C to 25°C, but not at temperatures over 30°C, indicating their cold-adapted degradative abilities. Thirty unique laccase-like multicopper oxidase (LMCO) gene fragments were PCR-amplified from 71% of the isolated HA-degrading bacteria, all of which included the conserved copper binding regions (CBR) I and II, both essential for laccase activity. The bacterial LMCO sequences differed from known fungal laccases; for example, a cysteine residue between CBR I and CBR II of fungal laccases was not detected in the bacteria LMCOs. In addition, computer-aided molecular modeling showed these LMCOs contain a highly conserved copper-dependent active site formed by three histidine residues between CBR I and CBR II. Phylogenetic- and modeling-based methods confirmed the wide occurrence of LMCO genes in HA-degrading polar soil bacteria and linked their putative gene functions with initial oxidative reaction for HA degradation.

Key words: Biodegradation, Bipolar, Cold-adapted, Humic acids, Laccase, Soil bacteria