



Rhizosphere and endophytic bacteria promote *Silene paradoxa* growth and tolerance to heavy metals and are transferred to the next generation of plants as seed endophytes

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It is well known that bacteria are commonly associated to the plants, either on the outer surfaces (epiphytes) that inside the plant tissues (endophytes). These bacteria mainly derived from soil and reach the various organs of the plant throughout the root system. Despite recent works have shown that endophytic bacteria can have an important role in the physiology of the plant, little is known of their possible involvement in the plant resistance and tolerance to heavy metals. Furthermore, until now only limited research has been conducted to unravel the exact role and possible applications of seed endophytes.

The aim of this work was to characterize the plant-associated bacterial communities present in the rhizosphere and inside the seeds, roots and aerial parts of plants of *Silene paradoxa*, a plant highly well-adapted to extreme environments, such as metal-contaminated soils.

Thus, soil samples and plants of *S. paradoxa* were collected from i) the landfill of a Cu mine at Fenice Capanne (Grosseto, Italy); ii) a serpentine soil (with a high Ni content) at Pieve Santo Stefano (Arezzo, Italy); iii) a limestone uncontaminated soil in Colle Val d'Elsa (Siena, Italy). Bacterial communities associated with the three different plant organs have been then characterized by high-throughput sequencing of the 16S rRNA genes (microbiome). Bacteria were also isolated from seeds and soils and the colony forming units (CFU) was determined on plates containing different concentrations of Ni and Cu (5, 10 and 15 mM).

The results showed a greater bacterial diversity among the three soils compared to plants. In particular, even though some phyla occurred in all the soils (Actinobacteria, Proteobacteria, Chlorflexi and Acidobacteria), in general the bacterial community structure of the three soils was quite different from each other. Interestingly, the endophytic composition within each plant compartment was observed to be strongly affected by the soil of origin. Furthermore, CFU values revealed that bacteria isolated from seeds of plants growing on soils contaminated with Cu and Ni had a greater capacity to grow on Cu- and Ni-enriched media, respectively, compared to the control. Furthermore, plants inoculated with a selected Cu-tolerant seed endophyte showed a better tolerance to copper toxicity.

In conclusion, based on the data obtained it is plausible to assume that some of the plant-associated bacteria for *S. paradoxa* can be directly selected from soil by the plants for their beneficial characteristics (i.e. metal resistance) and could be transferred via the seed to benefit the next generation. As they might possess several plant growth-promoting and biocontrol properties, the study of endophytes application in diverse processes such as biofertilization, bioenergy production and bioremediation should be encouraged.