Clarification of Heavy-metal Tolerance Mechanisms of *Miscanthus sinensis* and *Artemisia indica* var. *maximowiczii* Naturally Growing at the Mine Site, Japan, Considering Effects of Root Endophytes

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Abstract

Phytoremediation has been paid attention due to its advantages of environmental-friendly and cost-effective. Phytostabilization, as one technology of phytoremediation, is a suitable method for using in mining areas, which uses green plants to accumulate heavy metals in roots or rhizosphere and do not cause heavy metals to run off from mining areas. It is important to figure out the heavy-metal tolerance mechanisms of plants growing naturally at mining areas. However, the studies of phytostabilization used in mine sites are still limited recently. In the present study, the soils of study sites in mine A and mine B, contained high concentrations of heavy metals, showing potential heavy metal tress for general plant growth. *Miscanthus sinensis* and *Artemisia indica* var. *maximowiczii* were found to grow naturally in mine A and B, respectively, indicating these two plants could be tolerant to heavy metals. *Miscanthus sinensis* and *A. indica* var. *maximowiczii* are common native and pioneer plants in Japan; however, it is unclear whether the heavy-metal tolerance mechanisms are common between these plants or not. In addition, root endophytes have been found to enhance heavy-metal tolerance of host plants. Therefore, the purpose of the present study was to clarify the heavy-metal tolerance mechanisms of *M. sinensis* and *A. indica* var. *maximowiczii* grown naturally in mine A and B, respectively, considering effects of root endophytes.

Comparing with general soil, higher concentrations of heavy metals, especially, cadmium (Cd), lead (Pb) and zinc (Zn) contained in the root-zone soil of *M. sinensis*. The soil pH was detected as 5.94 showing slightly acidity. *M. sinensis* growing naturally at study site in mine A accumulated heavy metals, such as Pb and Zn in underground parts. Translocation factors (TFs) and bioaccumulation factors (BCFs) of heavy metals, such as Al, Fe, Cu, Pb and Zn, were lower than 1 and it suggested that *M. sinensis* tolerated heavy metals by accumulating in the underground parts and reducing roots-to-shoots translocations. Additionally, comparing with other plant tissues, dead plant parts, including root barks and dead leaves, contained higher concentrations of heavy metals, suggesting that transportation of heavy metals from alive parts into dead parts would be one tolerance mechanism to exclude heavy metals. According to results of analysis of adventitious roots extracts, chlorogenic acid was mainly detected as a detoxicant, which could reduce toxicities of heavy metals. For root endophytes isolated from *M. sinensis*, *Phialocephala fortinii* showing high appearance rate and *Talaromyces verruculosus,* which showed high Zn-chelating compounds production, were used in inoculation test to verify the effect of heavy-metal tolerance on *M. sinensis*. For *M. sinensis* seedlings, whether inoculated with root endophytes or not, high concentrations of aluminum (Al) and iron (Fe) were accumulated in the underground parts, showing heavy-metal tolerance. Moreover, chlorogenic acid were detected in the roots to detoxify heavy metals. Comparing

with seedlings without root endophytes incubation, *P. fortinii* and *T. verruculosus* enhanced seedling growth by increasing K uptakes; furthermore, phytohormone indole-3-acetic acid (IAA) productions by *P. fortinii* and *T. verruculosus,* have been verified, which might contribute to growth enhancement. In conclusion, via inoculation test, root endophytes *P. fortinii* and *T. verruculosus* have been proved to enhance growth of *M. sinensis* by K uptake increasement and IAA production. The rapid growth enhanced by root endophytes would decrease heavy metal contents, which would contribute on heavy metal tolerance.

At study site in mine B, comparing with general soil, the root-zone soil contained higher concentrations of heavy metals, Cu, Pb and Zn. *Artemisia indica* var. *maximowiczii* growing naturally at the study site in mine B mainly accumulated high concentrations of Al, Fe and Cu in the roots. According to the results of TFs and BCFs, *A. indica* var. *maximowiczii absorbed* Zn highly from the soil and heavy metals, such as Cu and Pb, were mainly accumulated in the roots. The reduction of heavy-metal roots-to-shoots translocation would be one mechanism of heavy-metal tolerance. Furthermore, the productions of chlorogenic acid, 4-feruoeylqunic acid and 3,5-dicaffeoylqunic acid were detected as detoxicants in the roots, which would exhibit antioxidative and metal-chelating activities that would play a role in heavy metals tolerance in *A. indica* var. *maximowiczii*. For root endophytes, dark-septa endophytes (DSEs), including *Paraphoma*, *Phialocephala*, *Cadophora* and *Acephala, Mollisia* and unidentified red isolates were isolated from roots of *A. indica* var. *maximowiczii*. In addition, unidentified red fungi showed high siderophore production, which might reduce toxicities of heavy metals to host plant; therefore, inoculation test of using *A. indica* var. *maximowiczii* with root endophytes will be studied in the further work.

The present study clarified that *M. sinensis* and *A. indica* var. *maximowczii* growing naturally in mining sites tolerated heavy metals by highly accumulating in the underground parts, reducing the roots-to-shoots translocation and producing phenolic compounds. For root endophytes, the productions of heavy-metal chelating compounds by root endophytes, which would reduce toxicities of heavy metals to host plants, has been verified. In addition, root endophytic *P. fortinii* and *T. verruculosus* enhanced *M. sinensis* growth via the inoculation test. In conclusion, *M. sinensis* and *A. indica* var. *maximowczii* growing naturally in mine sites would be good candidates in phytostabilization and the present study will provide fundamental information of using both plants in phytostabilization at mine sites.