GENETIC AND PHYSIOLOGICAL DIVERSITY OF THE NITROGEN-FIXING BACTERIA NODULATING A METALLIFEROUS PLANT ECOTYPE OF *ANTHYLLIS VULNERARIA*

Clémence Chaintreuil*, Lucette Maure*, Brigitte Brunel*, Carine Boutet*, Willy Suzanne*, Xavier Bailly*, Lionel Moulin*, Antoine Galiana*, José Escarré, Bernard Dreyfus*, Jean-Claude Cleyet-Marel***

**LSTM : UMR 113 IRD/CIRAD/INRA/AGRO-M/UMII, Campus International de Baillarguet, TA10/J Montpellier Cedex 05 France*
***CEFE-CNRS, UMR 5175, 1919 Route de Mende, 34293 Montpellier Cedex 05 France*

Heavy metal contamination of soils originating from industrial activities (e.g., metal mining and smelting) is a major environmental problem in many parts of the world. In addition to the high heavy metal levels, plants and microorganisms have to face to very low content in nitrogen and to limited water supply due to porous substrates (Requena et al., 2001 ; Escarré et al., 2000). Isolation and selection of both metal-resistant and symbiotic nitrogen-fixing plant-bacterium system will be a crucial aspect to overcome the main constraint of N input in mine tailings. We have identified in an ancient Zn-Pb mine in the South of France, a metalliferous *Anthyllis vulneraria* ecotype able to fix nitrogen in the presence of heavy metals. Our objective was therefore to isolate and to characterize rhizobium strains from nodules directly collected from root plant systems growing in metal-contaminated substrates.

According to the 16S sequencing data, the bacterial isolates obtained belong to *Mesorhizobium* genus, with an unexpected diversity. Metal-resistant phenotypes of *Mesorhizobium* isolates were characterised by culture in media supplemented with Zn or Pb. Isolates exhibited variable tolerance degree to Zn and at least 4 different phenotypes were recorded. Pb resistance was 5-10 times lower and few phenotypes were observed. The presence of the resistance genes like czcD gene was confirmed by PCR and sequencing in some strains.

The selection and the practical use of metalliferous efficient nitrogen-fixing *Anthyllis vulneraria-Mesorhizobium* associations will help to improve plant cover of heavy-metal-enriched spoils heaps and therefore could be proposed as a new tool for bioremediation of area affected by heavy-metal contamination.

References

Escarré J., Lefèbvre C., Gruber W., Leblanc M., Lepart J., Rivière Y., Delay B. 2000. Zinc and cadmium hyperaccumulation by *Thlaspi cearulescens* from metalliferous and non-metalliferous sites in the Meditarranean are: implications for phytoremediation. New Phytologist 145, 429-437.

Requena N., Perez-Solis E., Azcon-Aguilar C., Jeffries P., Barea J.M. 2001. Management of indigenous plant-microbe symbiosis aids restoration of desertified ecosystems. Applied and Environmental Microbiology 67, 495-498.