

H-NS is one of the bacterial nucleoid-associated proteins (NAPs), which influences DNA compaction and transcription. IncP-7 carbazole degradative plasmid pCAR1 has a *pmr* gene encoding an H-NS like protein, which is important for expression of many genes of both pCAR1 itself and host chromosome. In order to clarify the function of Pmr, we used *Pseudomonas putida* KT2440 as a model host. KT2440 have five H-NS like proteins, TurA to E, thus Pmr and TurA-E could function cooperatively when KT2440 receives pCAR1 through conjugation. Previous study had shown that Pmr could physically interact with TurA, TurB, TurE, and Pmr itself *in vitro*, and Pmr, TurA, TurB are mainly transcribed in KT2440 (pCAR1), so the main functional H-NS like proteins in host cell are Pmr, TurA, and TurB. As a result of transcriptome comparison, disruption of each of these genes influenced the transcription of hundreds of genes both on pCAR1 and host chromosome. Modified CHIP-chip analysis with Pmr, TurA, TurB showed that their binding sites are almost identical. It was possible that oligomerization among these proteins masked the real results, so more detailed investigation is necessary.

Under these considerations, we constructed the expression system of whole or partial proteins of Pmr, TurA, and TurB in *E. coli*, and analyzed them *in vitro*. Chemical cross-linking showed that Pmr, TurA, TurB form homodimers and homooligomers. It also showed that the first 61 residues of Pmr are enough to form homodimer and homooligomer. Further functional comparison between Pmr and chromosomal H-NS like proteins will be presented and discussed.

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#### [P-E.131]

### Characterization of bacterial communities associated to plant species from the Sardinian mining district for heavy metal phytoremediation

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Keywords: phytoremediation; heavy metals; plant-associated microorganisms

**Introduction:** Heavy metal pollution of soil is a significant environmental problem having negative impact on human health and agriculture. Particularly, abandoned mining areas represent an important problem all over Europe. The Sardinian mining district (SW Sardinia, Italy) has been a major source of Pb and Zn in Europe since its closure in the mid-19th century. Then, the whole mining complex was abandoned resulting in contamination of the area. Phytoremediation is an emerging technology that uses plants and their associated microorganisms for soil remediation of both organic and inorganic contaminants. Plant-associated microorganisms play an important role in the phytoremediation process by affecting heavy metal mobility and availability to the plant and increasing heavy metal tolerance and plant growth. Bioaugmentation-assisted phytoremediation is a promising method for the cleaning-up of soils contaminated by metals.

**Objectives:** Actually, there is a need to improve our understanding of the mechanisms involved in plant promotion and to select plant growth-promoting bacteria for specific restoration programmes. The relevance of natural resources of the Sar-

dinian mining district and its touristic importance require the preservation of the local biodiversity. Thus, the objective of this work is the characterization of bacterial community associated to autochthonous plant species.

**Results:** In a previous study, *Scrophularia canina* subsp. *bicolor* and *Pistacia lentiscus* were identified as autochthonous plant species suitable for phytoremediation of the Sardinian mining district. In a field trial at Campo Pisano mine (Iglesias, Sardinia), root and rhizosphere samples were obtained from plants grown in Pb and Zn contaminated soil. More than 280 bacterial strains were selected for tolerance to Pb or Zn. Bacteria were identified by 16S rRNA sequence analysis. Characterization of the isolates regarding characteristics that may be relevant for a beneficial plant-microbe interaction (metal tolerance, indol-acetic acid and siderophore production) is currently in progress.

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#### [P-E.132]

### Culture-independent analysis of diversity in microbial communities: pros and cons of the partial sequence-based profiling

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Keywords: DGGE/TGGE; identification; 16S rRNA sequence analysis

Molecular biology has revolutionized the study of microorganisms in complex environments and improved as well our understanding of the diversity within microbial communities in many habitats. Among the culture-independent techniques developed so far, DGGE/TGGE analysis of amplified 16S rRNA fragments is currently used to a great extent for the characterization of microbial consortia, due to its fast response and highly descriptive power. This approach relies on the separations of partial 16S rRNA gene amplicons targeting two specific hypervariable regions, namely V3 and V6-V8. Separation is followed by sequencing and identification of different bands based on BLAST comparisons.

Not to mention the prominent aspects of selectivity of the PCR technique, selection of specific gene regions to be amplified and the sequence analysis thereafter represent the key steps for the taxonomic identification of microbial species. The attribution of names of taxonomic relevance to the different sequences is often based on an approximate procedure, as the identity thresholds used to delineate species are extrapolated from partial gene lengths. Comparison with gene sequences of the type strains is then not frequently performed.

Some case studies are presented here concerning the identification of bacterial species and genera - belonging to different phyla such as *Actinobacteria* (*Bifidobacterium*, *Rhodococcus*), *Firmicutes* (*Bacillus*, *Enterococcus*, and *Lactobacillus*), *Alphaproteobacteria* (*Sinorhizobium*), *Betaproteobacteria* (*Burkholderia*), and *Gammaproteobacteria* (*Pseudomonas*) - all relevant to environmental and industrial biotechnology. Similarity values of partial gene sequences of the two widely used hypervariable regions were compared with the whole ones.

Results indicate that identity values of gene fragments were useful for the affiliation to a phylogenetic group, but could not be always considered reliable for identification at the species level. Sequence-based studies are of fundamental importance for the analyses of the microbial communities but the information they deliver should be critically evaluated.

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