Genetic characterization of the hydroxylating bacterial community based on *pcaH* nucleotide sequence analysis

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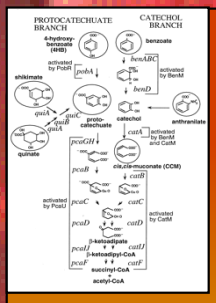
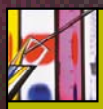


INTRODUCTION

In telluric environments the β-ketoadipate pathway is a major route of catabolism of natural aromatic compounds. This pathway occurs in phylogenetically diverse bacteria and fungi. It involves the mono- or di-hydroxylation of polycyclic and homocyclic aromatic compounds leading to the key intermediates, catechol or protocatechol which are cleaved between two adjacent hydroxyl groups by catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase (3,4-PCD), respectively.

The 3,4-PCD belongs to the dioxygenase C protein super-family and is involved in the degradation of several Polycyclic Aromatic Hydrocarbons (PAH) and other environmentally hazardous molecules (i.e. pesticides). Despite the obvious interest in *pca* microbial communities, the ecology of these functional communities remains obscure. Our work consisted in the development of a primer pair designed in the sequence coding the active site of the dioxygenase which permits the description of the hydroxylating bacterial community harbouring the protocatechuate 3,4-dioxygenase β-subunit (*pcaH*) gene.



MATERIALS – METHODS

DNA was extracted from soil samples according to Martin-Laurent et al. (2001). Three independent extractions were carried out for each soil (Table1).

The conserved region of *pcaH* gene was amplified using PCAHf and PCAHr designed using γ-Proteobacteria and Actinobacteria *pcaH* sequences. Purified *pcaH* PCR products were digested using *Hae*III restriction enzyme, yielding in fingerprints.

Purified *pcaH* PCR products were pooled then cloned and screened by PCR-RFLP (approximately 75 clones for each soil). At least one clone of each RFLP family was sequenced.

Table 1: Soil physico-chemical characteristics

Soil	Type	Clay	Sand	Silt	Organic C	Organic N	pH
Closeaux	**Eutric cambisol** Silty	**18 %**	**26 %**	**56 %**	**1.34 %**	**0.12 %**	**6.8**
Epoisses	**Eutric cambisol** Silty Clay	**43.2 %**	**6.5 %**	**50.3%**	**1.29 %**	**0.14 %**	**7.5**

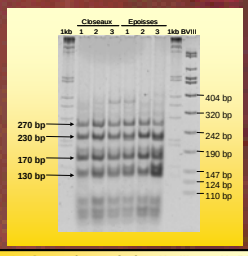


Fig. 1: Comparison of the *Hae*III *pcaH* RFLP fingerprints from Closeaux and Epoisses soils. Four dominant bands are disclosed at 270, 230, 170 and 130 bp.

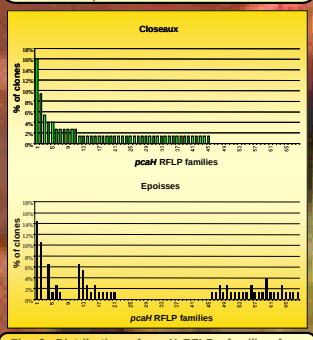


Fig. 2: Distribution of *pcaH* RFLP families from Closeaux ■ and Epoisses ■ soils.

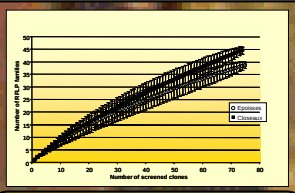


Fig. 3: Rarefaction curves of the observed diversity of *pcaH* RFLP families in Closeaux and Epoisses soils. The error bars are 95 % confidence intervals calculated from the variance of the number of phylotypes.

RESULTS – DISCUSSION

- Comparison of the *pcaH* RFLP fingerprints revealed no difference between these two soils as regards structure of the bacterial community harbouring the *pcaH* gene (Fig.1).

- 68 *Hae*III RFLP families were identified. Analyses revealed (Fig. 2)

  (i) discriminating RFLP families attained 42 % in the Closeaux soil and 32 % in the Epoisses soil,

  (ii) the compositions of the hydroxylating community of the two soils diverged significantly.

- The rarefaction curves did not reach the asymptote indicating the high level of diversity of the hydroxylating community in the two soils (Fig. 3).

- 85 clones were sequenced (sequences were deposited in the GenBank database under the accession numbers DQ318038 to DQ318122) (Fig. 4).

  Comparison of the *pcaH* nucleotide sequences with known sequences did not reveal any significant similarities. The identity of the deduced amino-acid sequences with known PcaH sequences ranged from 38 % to 96 % with a mean of 70 % identity.

  These sequences are affiliated to different known sequences related to *Actinobacteria, Acidobacteria, Deinococcus-Thermus, α-, β- and γ-Proteobacteria* phyla.

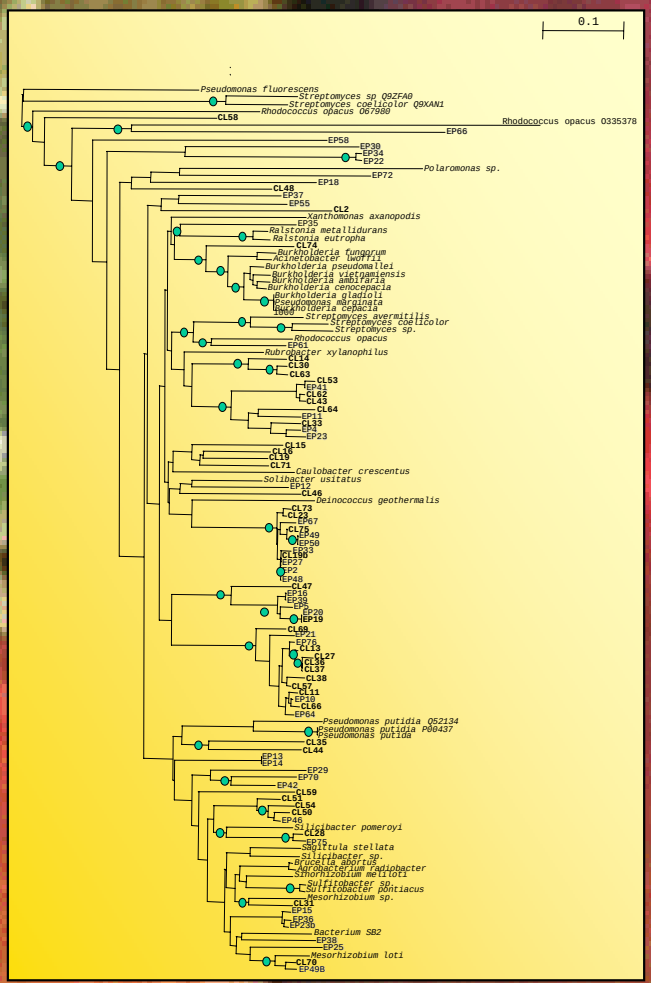


Fig. 4: Phylogenetic tree of PcaH sequences derived from Closeaux (CL) and Epoisses (**EP**) soils. The tree is based on the ClustalX multiple alignment of amino acid deduced sequences of PcaH. PcaH from *P. fluorescens*, *Streptomyces* sp. and *S. coelicolor* constitute the outgroup. Bootstrap values greater than 70 % are marked at branch nodes.

CONCLUSION

The degenerated primer set designed in this study permits the description of a wide diversity of *pcaH* sequences found in soil microorganisms able to degrade protocatechuate, providing a unique insight into the structure, diversity and composition of the hydroxylating community (El Azhari et al. (2006) J. Microbiol. Meth. (submitted)).

Reference:

Martin-Laurent et al., 2001. DNA extraction from soils: old bias for few microbial diversity analysis methods. Appl. Environ. Microbiol. 67: 2354-23-59.