Advances in monitoring microbial diversity in man-made environments: general guidelines for a multi-step methodology

REFERENCE S4/P23

Carvalho, G.[1,3], Tenreiro, T.[2], Dourado, A.C.[3], Silva, A.F.[3], Jesus, B.M.[4], Tenreiro, R.[2] and Barreto Crespo, M.T.[3]

1. Chemistry Department, REQUIMTE/CQFB, FCT/UNL, Caparica
2. ICAT, FCUL, Lisboa
3. IBET/ITQB, Oeiras
4. Instituto de Oceanografia, FCUL, Lisboa

gildacarvalho@dq.fct.unl.pt

Monitoring microbial diversity in man-made environments is challenging different fields of microbiology, from microbial ecology to environmental biotechnology. Molecular approaches, as community fingerprinting by denaturing gel electrophoresis, allowed the assessment of microbial diversity and microbial populations shifts, beyond culture-dependent strategies. However, selection of optimal settings for this multi-step methodology is often difficult and few comparative studies are available. In this study, the steps necessary for microbial diversity monitoring, using either denaturing gradient (DGGE) or temperature gradient (TGGE) gel electrophoresis, were optimised for a selection of samples collected in man-made environments (sediments from polluted estuarine mudflat, activated sludge from wastewater treatment systems, biomass from lab-scale reactors, and reservoir waters). DNA extraction efficiency and biodiversity profiles were compared using step-by-step extraction methods and commercially available kits. Different sets of primers targeting bacterial or eukaryotic rDNA were also evaluated. Finally, DGGE and TGGE were compared for their sensitivity and discriminating power, with different gel staining techniques. This comparative study aims at providing guidelines for environmental biodiversity studies, from DNA extraction to denaturing gel electrophoresis.

Acknowledgements: FCT for post-doc grants G. Carvalho (BPD/30800/2006) and B. Jesus (BPD/20993/2004).

Assessment of a winery effluent microbial community dynamics by LH-PCR/DGGE analysis

REFERENCE S4/P24

Eusébio, A.[1], Tacão, M.[1], Chaves, S.[2], Gadanho, M.[2], Duarte, J.C.[1], Vara, E.[1] and Tenreiro, R.[2]

1. Departamento de Biotecnologia/UME, Instituto Nacional de Engenharia, Tecnologia e Inovação, Lisboa
2. Instituto de Ciência Aplicada e Tecnologia, Faculdade de Ciências da Universidade de Lisboa, Lisboa

ana.eusebio@ineti.pt

A biological treatment process of a winery effluent was performed in an aerobic 20 L-JACTO bioreactor[1] using the native effluent microflora as the biological agent, during 135 days and testing four different hydraulic retention times (HRT): 4d, 2d, 1d and 0.5d. Denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rRNA gene fragments was used as a monitoring tool to evaluate the structural microbial community shifts that occurred along the biotreatment and in a close association with step-decreases on HRT. Selected bands that were correlated with apparently dominant phylotypes were excised, sequenced and identified, revealing affiliations with *Proteobacteria*, *Actinobacteria* and *Firmicutes*. Length heterogeneity analysis by PCR (LH-PCR) allowed distinguishing 30 16S rDNA fragments. Seven of these fragments were coincident with high COD and total phenols removal rates (80 %). When HRT was changed to 0.5d, these removal rates decreased to 48 % and 32 % respectively, and the bacteria corresponding to the seven referred fragments were not detected, strongly suggesting their association with the most active COD and phenol removals.

Acknowledgments: FCT post-doc grants for S. Chaves (BPD/20819/2004) and M. Gadanho (BPD/17391/2004). Funding from FCT Projects JACTO (POCTI/BIO/41253/2001) and MOTIVE (PPCDT/AMB/56616/2004). The authors also thank Prof. António Correia and his co-workers, from CESAM/University of Aveiro, Portugal, for their collaboration with DGGE analysis.

1. Eusébio, A. *et al.* (2004) *J Ind Microbiol Biotechnol* **31**:29-34.