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Study of microbial diversity by SSCP and ssuDNA sequencing

P. Colin-Morel, F. Giraud, L. Sage, **R. A. Geremia**

Laboratoire d'Ecologie Alpine, Domaine Universitaire, Grenoble, France. E-mail**:** roberto.geremia@ujf-grenoble.fr

Although characterisation of microbial diversity in environmental samples such as soil is essential for understanding biogeochemical cycles, the measure of this biodiversity is not straightforward. Molecular DNA-based methods are of choice because they take into account microbes that are difficult to cultivate in vitro. Once that clean DNA extraction is achieved, two main strategies are available: Shot-Gun Sequencing (SGS) and electrophoresis-based techniques. While SGS is the more precise method, it requires sequencing of several Giga bases of DNA; the cost of such sequencing precludes the use of this technique for routine studies. Electrophoresis-based methods rely on the different behaviour of molecular markers; being the ssuDNA the most used one. The later methods are less expensive and more convenient for routine studies. However, they do not reflect the real diversity of the sample, mainly because the lack of resolution of very similar molecules. Single Strain Chain Polymorphism (SSCP) is one electrophoretic method that can be upscale for the analysis of hundreds of samples in a few weeks.

We report here the set-up of a routine method for the analysis of environmental samples. Our goal was to establish the biodiversity index for bacteria and fungi. To achieve this, we have optimised: 1) the DNA isolation method, 2) PCR conditions for SSCP, 3) SSCP conditions, 4) ssuDNA cloning and sequencing. The improvements that we have introduced to the SSCP study allow led to a higher resolution of the species. The sequencing of ssuDNA, besides allowing the identification of microbial species, is used to model the weight of each species in the sample.