

## Study of diazotrophic bacteria in the rhizosphere of Poaceae, Leguminosae and Cyperaceae for the purpose of rehabilitating mining sites (New Caledonia)

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### Introduction

Degradation of mining sites resulting from deep and irreversible action is a serious environmental problem. The best regrowth results have been achieved with endemic *Cyperaceae*. However, these species are slow to take root and propagate.

Association of plants (*Cyperaceae*, *Poaceae* and *Leguminosae*) and free diazotrophic bacteria can be used to increase yield and enhance root development. In this plant/free diazotrophic bacteria association, micro-organisms enable plants to use dinitrogen and optimise growth (P.G.P.R. effect : Plant Growth-Promoting Rhizobacteria).

The usual process is to inoculate bacteria which are not naturally adapted to the environmental conditions. Due to the specific nature of New Caledonian ultramafic sites, the opposite approach is adopted. Firstly we have isolated local populations of rhizospheric bacteria with phenotypical and biochemical characteristics adapted to the local environment (Tran Van Van, *et al.* 1994). Secondly, to select efficient plant growth promoting bacteria, screening of rhizobacteria based on their nitrogen fixing ability has been carried out (Omar *et al.* 1992).

### Experimental protocol

Isolation of bacteria has been achieved from pieces of different roots (*Cyperaceae*, *Leguminosae* and *Poaceae*) and different soils (Ouénaou site : ferrallitic ferritic soil ; Plum site : brown serpentinic soil). Strains with positive ARA test (Acetylene Reduction Activity means dinitrogen fixation capacity) have been phenotypically described then identified using the API System 20 E, 20 NE and 50 CH (Biomérieux, France) according to methods defined by Berge *et al.* (1991).

### Results and discussion

In this first experiment, it was possible to identify the existence of diazotrophic bacteria in ultramafic soils. Numbers of these bacteria differ according to soil : 36% of isolated bacteria at the Ouénarou site and 62% of isolated bacteria at the Plum site.

Most bacteria found were positive Gram type and belong to the *Bacillus* genus : 85% at the Ouénarou site and 66% at the Plum site. This observation is in accordance with that of Heulin & Berge (1994). However, biochemical characteristics of diazotrophic *Bacillus* isolated in New Caledonian soils can not be compared with those of four known species (*B. azotofixans*, *B. circulans*, *B. macerans* and *B. polymixa*). The *Bacillus* genus is common as not only does its sporulation capacity enable it to survive in extreme conditions but it also synthesises antibiotic compounds that inhibit the propagation of other bacteria (Berge *et al.* 1991). One other family of micro-organisms, *Enterobacteriaceae* (Bilal *et al.* 1993), was found in New Caledonian soils.

Although diazotrophic bacteria have little effect on the addition of nitrogen in soil, they help maintain fertility in a disturbed ecosystem and increase the level of nitrogen in *Poaceae* roots.

At present, we are carrying out research on a collection of diazotrophic bacteria found in New Caledonian soils : quantification of P.G.P.R. effect and of dinitrogen fixation («spermosphere model», Heulin *et al.* 1989) of endemic and reference strains (*Azospirillum* genus) and the combined effect of low fertilization and inoculation of free diazotrophic bacteria on plant growth (Zaady *et al.* 1994) for the purpose of rehabilitating mining sites.

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