REHABILITATION OF MINING SITES USING DIAZOTROPHIC BACTERIA AND GRAMINEOUS ASSOCIATIONS

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ABSTRACT

Degradation of mining sites resulting from deep and irreversible human action is a serious environmental problem. So, to rehabilitate mining sites, we studied the ability of diazotrophic bacteria to improve plant growth. Firstly, we isolated diazotrophic bacteria on *Cyperaceae* and *Poaceae* rhizosphere. After characterisation and partial identification of found bacteria, we decided to study the effect of presence and the activity of bacteria on plant growth. To compare with reference bacteria (*Azospirillum brasilense*), we had to test the effect of endemic bacteria on soil without limiting factors (Maré's soil). We think that if bacteria are able to help plants to grow on well-balanced soil, the association plant-bacteria could give best results on mining soils (without organic matter, nitrogen and with nickel). But, field tests should be realized in the future to confirm this hypothesis. In this experiment, it has been possible to show the significant effect of two bacteria on stage, length and dry matter production of *Chloris gayana* (Rhodes callide). So, with endemic diazotrophic bacteria-plant association we could:

- create a mulch to help *Cyperaceae* growth;
- restore microflora;
- or introduce *Poaceae* species if these two precedent solutions are not adapted to mining sites.

KEYWORDS

free diazotrophic bacteria**,** PGPR (plant-growth promoting rhizobacteria)**,** *Cyperaceae, Poaceae,* mining sites, nickel, rehabilitation, New-Caledonia.

INTRODUCTION

In New-Caledonia, nickel exploitation degrades the environment by removing surface material of mining sites. To fight against this degradation, more studies were realized to rehabilitate sites. The best regrowth results have been achieved with endemic *Cyperaceae* (Jaffré et al., 1994). However, these species are slow to take root and spread. Association of plants (*Cyperaceae* and *Poaceae)* and free diazotrophic bacteria can be used to increase yield (Fallik and Okon, 1996) 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 Caledonian ultramaphic 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 (V. van Tran, 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). The aim of this study was to find an impressive association between bacterial strain and herbaceous plants to rehabilitate mining sites quickly.

MATERIALS AND METHODS

Soils choice

Different soils of mining sites have been chosen for this study:

- Ouenarou site: ferritic ferralitic soil;
- Plum site: eutrophic hypermagnesium soil;
- Thio site: soil resulting from mining exploitation.

All these soils are without organic matter, without nitrogen and have high nickel concentration. Soils were dried and sifted at 2 mm and put in pots.

Plants choice

We studied rhizosphere of endemic plants (Family of *Cyperaceae)* and introduced herbaceous plants (Family of *Poaceae)*. We chose as well as *Cyperaceae, Baumea deplancheanum* and *Costularia comosa*, as well as *Poaceae*, *Chloris gayana* (Rhodes callide). Seeds were disinfected with antifungal product and put in different pots.

Methods

Isolation, partial identification and selection of diazotrophic bacteria. After 8 weeks of growth, roots were washed with water, disinfected by Chloramine-T and rinsed by sterile water. Pieces of root were cut (1 cm) and put on medium without nitrogen. After 48 hours of growth, bacterial trouble appears and it is put on complete medium (with nitrogen and yeast extract). Each colony was isolated to have pure strain and each strain with positive ARA test (Acetylene Reduction Activity means dinitrogen fixation ability) have been phenotypically described and identified using the API System 20 E, 20 NE and 50 CH (Biomérieux, France) according to methods defined by Berge et al. (1991).

In the end of this first stage, we have a collection of endemic free diazotrophic bacteria. We compared bacterial strains by χ^2 test and selected a few of them for second trial (cf. Table 1).

PGPR (plant growth-promoting rhizobacteria) test. For this experiment, we chose soil without limiting factor: soil of Maré (Loyalty Island dependent on New- Caledonia). The choice of plant was *Chloris gayana* (Rhodes callide) because the family of *Cyperaceae* is endemic on mining sites. Seeds of *C. gayana* were selected and shelled before being disinfected with saturated solution of Ca- hypochlorite for 60 min, were rinsed with sterile water for 30 and were put in H2O2 solution for 30 min, and were rinsed again with sterile water for 30 min. Seeds germination appeared after 24 hours on sterile water, at 30 °C. In the same time, inoculation was prepared: each strain was grown on nutrient broth during a certain time (growth curve). Each inoculum (cf. Table 1) was washed by centrifugation on phosphate buffer for 10 min at 15 000 rd/min. Germinated seeds were mixed with inoculum for 12 hours. Inoculated seeds were put in pot with Maré's soil in growth chamber (25 °C and constant hygrometry) for seven days. After that, plants were bedded out in PVC tube, under glasshouse and laboratory conditions for 4 weeks. Each treatment was replicated 30 times. Plant length and growth stage (number of leaves) were measured each week. Roots and shoots dry matter yield were weighed at the end of the experiment. Statistical treatment was realized over three factors (bacteria presence and state and growth conditions) and four variables.

RESULTS AND DISCUSSION

In this first experiment, it has been possible to show the existence of diazotrophic bacteria in ultramaphic 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 found bacteria were Gram positive 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 and Berge (1994). However, biochemical characteristics of diazotrophic *Bacillus* isolated in Caledonians soils can not be compared with those of four known species (*B. azotofixans, B. circulans, B. macerans et B. polymyxa*). The *Bacillus* genus is common as not only does its sporulation capacity enable it to survive in extreme conditions but it also synthesizes 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 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 *Poceae* roots.

In the second experiment (cf. Table 2), we quantified the P.G.P.R. effect of endemic and reference strains (*Azospirillum* genus) by inoculation of free diazotrophic bacteria on the rhizosphere of *Chloris gayana* in order to rehabilitate mining sites. In this study, we show significant effect of bacteria ($p < 0.01$), effect of condition ($p < 0.05$) and effect of state ($p < 0.01$) on *Chloris gayana* growth. Two of the six bacteria have a significant effect ($p < 0.05$) on plant growth. These activities and presence stimulate development of shoots (dry matter and length) and roots (dry matter). One comes from the site of Thio and the second is the reference bacteria (*Azospirillum brasilense*). Endemic bacteria give better results on greenhouse conditions than under laboratory conditions. These results are reverse for reference bacteria. The strain named RT (cf. Table 1) have the same or more effect ($p<0.05$) on plant growth as *Azospirillum brasilense* (reference strain). There are two reasons: provenance and type of association. The strain RT:

- comes from soil of Thio and can resist under difficult conditions. When this strain was reintroduced on rich soil (Maré's soil), it grew better. The presence of organic matter may explain this result;
- has been found on the rhizosphere of *Chloris gayana*. Exudates of plant seem to be well adapted to strain metabolism: an homologous association.

Results about the reference strain (*Azospirillum brasilense*) confirm observations made in other studies (Bashan and Levanony, 1990).

To conclude, it would be necessary to centre our efforts on future research concerning native strains. It would be better not to introduce in caledonian soil allochton strains! With endemic diazotrophic bacteria-plant association we could:

- create a mulch to help *Cyperaceae* growth;
- restore microflore;
- or introduce *Poaceae* species if these two precedent solutions are not adapted to mining sites.

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Table 1
Characteristics of native diazotrophic bacteria

Name	Site	Plant	Strain (family or genus)
CCT	Thio	*Costularia comosa*	*Enterobacteriaceae*
OC	Ouénarou	*Costularia comosa*	--------------------
OB	Ouénarou	*Baumea deplanchei*	*Bacillus*
BP	Plum	*Baumea deplanchei*	*Enterobacteriaceae*
RT	Thio	*Chloris gayana*	*Enterobacteriaceae*

Table 2
Quantification of bacteria effect on *Chloris gayana* growth

Strain	**CCT (N=110)**	**OC (N=108)**	**Sp7 (N=124)**	**OB (N=112)**	**BP (N=106)**	**RT (N=122)**	**SEM**	**Effect**
Variables	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**		
Length	$9,2^{x,y}$	$6,8^{y}$	$10,8^{x}$	$7,8^{y}$	$7,9^{y}$	$11,2^{x}$	3,1	S**, E**, C**, SE**, SC**, EC*
Growth stage	$4,3^{x,y}$	$4,0^{y}$	$4,4^{x}$	$4,1^{y}$	$4,2^{x,y}$	$4,6^{x}$	0,5	S**, E**, C**, SE*, SC**, ECns
Root dry matter	$0,6^{x,y}$	$0,3^{y}$	$0,9^{x}$	$0,5^{y}$	$0,4^{y}$	$0,9^{x}$	0,4	S**, E**, Cns, SE**, SC**, EC**
Shoot dry matter	$2,4^{x,y}$	$1,6^{y}$	$3,6^{x}$	$1,9^{y}$	$1,9^{y}$	$3,3^{x}$	1,3	S**, E**, C**, SE**, SC**, EC**

* P<0.05 — S: strain
** P<0.01 — C: condition (greenhouse or laboratory)
ns: no significant — E: state (dead or alive)