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A rock-weathering bacterium isolated from rock surface and its role in ecological restoration on exposed carbonate rocks

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ABSTRACT

Carbonate rock is widely distributed in the world. Most of the carbonate rock mining areas have been seriously degraded by destructive human activities, resulting in soil erosion, deterioration of vegetation cover, and bare rock. Microbial weathering of minerals is one of the important processes that can promote ecological restoration of such areas. However, bacterial strains vary in their mineral weathering abilities as well as their function in releasing nutrients. Thus, isolation of effective bacterial strains and studies of their mechanism of weathering processes are critical to promote ecological restoration on abandoned carbonate mine land. We isolated the *Bacillus megatherium* NL-7 from weathered rock surface and screened out as the most efficient strain for the ecological rehabilitation of rocky mine soil. In this study, we found that the rock sample released more Ca^{2+}, Mg^{2+} with a significant reduction of pH value in fermentation broth cultivated with NL-7 than with inactivated NL-7 (control group, CK). In combination with the results of high performance liquid chromatography (HPLC), acetic acid produced by bacterial metabolism was thereby considered as the effective component of promoting rock dissolution. This research also demonstrated that NL-7 releases essential elements (phosphorus and potassium) for plant establishment and growth from rocks. Our results indicated that *Bacillus megatherium* NL-7 can promote rock weathering and increase nutrient elements in the environment, therefore to improve soil formation and plant growth. We suggest its application may solve the present problems of environmental remediation technologies in environments damaged by carbonate rock mines.

1. Introduction

Mining has brought great economic benefits while causing serious ecological degradation around the world, especially in China with the high economic growth (Hubbard and Balfour, 1993), which reduce the original terrain stability, destroy vegetation cover, and erode the original natural soil (Hubbard and Balfour, 1993; Peng and Wang, 2012; Sweeting, 2012). As a result, the soil and water loss area increased by an average of 1.5×10^4 km^2 yr^{-1}, 20%–30% of which was area classified as rocky desertification (Balland-Bolou-Bi and Poszwa, 2012). Rocky desertification is a major issue in dissoluble rocky mountain regions mainly consisting of carbonate rocks (Liu, 2000). Because natural recovery in mining areas of rocky desertification would be nearly impossible, anthropogenic management to promote the recovery becomes the most favorable restoration strategy to these degraded ecosystems (Hobbs and Norton, 1996; Qi et al., 2013).

An efficient method of ecological restoration of the abandoned mine land is the spraying of a mixture of grass seeds, soil and nutrients on the exposed rock surface of the mines (Russell 2002). Plants need a continuous supply of nutrients and water from soil; however, such materials do not have the potential to break down rocks into soil, which is needed to provide nutrients and retain water (Fig. 1A). With the exhaustion of nutrients and water, plants stop growing and the sprayed seeding materials would eventually detach from the rock mass (Pratas et al., 2005). Therefore, soil formation is a question of fundamental importance to promote both plant growth and the fusion of the sprayed material to the rock surfaces in the rocky areas (Barker and Banfield, 1996).

Microbes have strong adaptability to the environment and capacity to grow quickly in population size (Prosser et al., 2007). Previous studies have reported that the biological effects on the loose rock was a significant factor in soil formation (Dietrich and Perron, 2006; Souza et al., 2013). Furthermore, a recent study showed that microbes promote rock weathering with three mechanisms: mechanical damage, water retention, and acidification by microbial metabolites (Gadd, 2010). Microorganisms have also been used improve the ecological degradation of stone mines in

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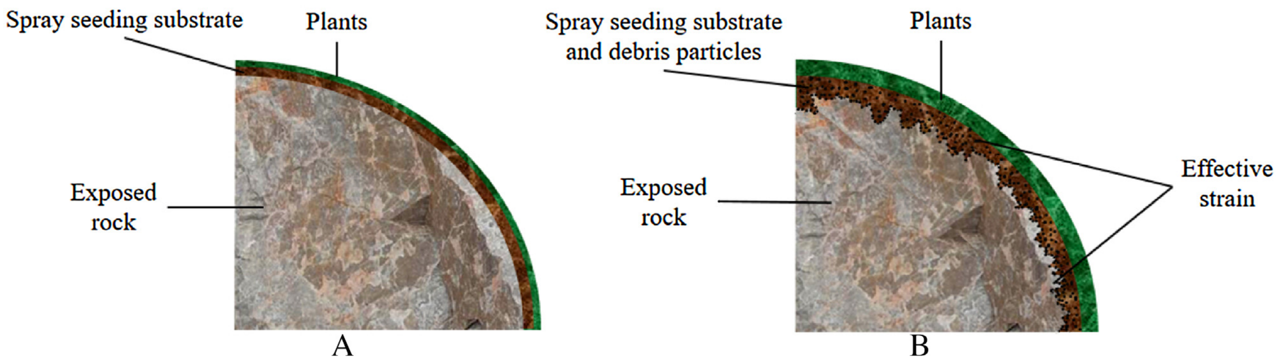

Fig. 1. Diagrams illustrating proposed spray-seeding methods with the current technology (A) and modified technology combined with effective strain (B). The traditional method of spraying with seeding substrate is difficult to integrate permanently into the rock and growth of plants is hard to establish (A). On the contrary, the modified technology shows evident remediation effect on exposed rock for the microbial weathering to release debris particles from rock thereby increasing the surface roughness of rock and thickening substrate layer (B).

Japan, but few studies have focused on the specific type of effective materials which had remarkable acid soluble effect on different mineral rocks (Uroz et al., 2009; Wood, 2013). In China, microorganisms are mainly combined with ecological restoration technologies to decrease the heavy metal pollution in metal mines (Wang et al., 2007; Wei et al., 2009; Wong, 2003), whereas the function of microorganisms to accelerate rock weathering is still ignored.

The objective of our study is to improve the existing technologies for restoring soil and vegetation in destroyed mining areas with exposed carbonate rocks. Specifically, we screened the microbial strains that are effective to rock dissolution, investigated the weathering mechanism, and proposed to mix the strain into spray seeding materials so as to increase surface roughness of rocks and promote soil formation. By this method, the fusion between spray seeding substrates and rocks would be firmer and the plants would keep growing (Fig. 1B). Therefore, we believe these findings could contribute to the revegetation of damaged carbonate rock mines.

2. Materials and methods

2.1. Rock sample

The fresh rock samples used in experiments were obtained from an abandoned carbonate rock mine of the Mufu Mountain, located in the northern suburb of Nanjing, China (32°07′15.23″N, 118°46′33.99″E). Rocks were fully rinsed in distilled water and dried overnight at 80 °C, then autoclaved at 121 °C for 20 min after grinding in a mill for 8 min (Olsson-Francis and Cockell, 2010). The rocks were sieved with a 200 mesh sieve, to a fraction size of <90 μm. Microprobe analysis showed that the rocks were mainly composed of dolostone, the chemical formula of which was $CaMg(CO_3)_2$, with a small amount of calcite and clay mineral. The major element compositions of rock samples (Table 1) were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optimal 2100 DV, Perkin Elmer) (Leake et al., 1969).

Table 1
Elemental compositions of the carbonatite used in the experiments.

Elements	Content (% wt oxides)
CaO	32.30
MgO	18.92
Fe_2O_3	0.67
K_2O	0.62
SiO_2	0.50
Al_2O_3	0.32
P_2O_5	0.31
Na_2O	0.03
LOI[#]	46.19
Other	0.14

[#] Loss on ignition (i.e., LOI), refers to the weight percentage lost by igniting raw material under 1000–1100 °C, which is typically employed to characterize gaseous heat decomposition products (such as H_2O, CO_2, etc) of the raw material.

2.2. Isolation, purification and screening of bacteria strains

The bacterial strains were isolated from rock-surface soils, which were collected in triplicate from Mufu Mountain, Nanjing, using 10-fold serial dilutions (Xi and others 2014). Serially diluted soil samples (up to 10^{-6}) were spread over isolated medium plates and cultivated at 28 °C for 72 h. The isolation medium contained: sucrose (5.0 g L^{-1}), Na_2HPO_4 (2.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), $FeCl_3$ (0.05 g L^{-1}), $CaCO_3$ (0.1 g L^{-1}), agar (18.0 g L^{-1}), and rock sample (1.0 g L^{-1}), and was sterilized at 121 °C for 20 min following the adjustment of the pH value to 7.0. Single bacterial colonies were streaked on culture medium plates (beef extract-peptone agar medium) and incubated at 28 °C for 48 h. The morphologies of colonies were used for the confirmation of different strains.

Subsequently, isolated colonies were extracted, purified using Scribing, inoculated on beef extract peptone agar slant and stored at 4 °C. Moreover, every strain was cultivated with a rock sample for 7 days (180 rpm, 28 °C), following which the concentration of Ca^{2+} and Mg^{2+} in the fermented liquid was investigated to select the bacterial strain that possessed the capacity to significantly expedite the rate of rock dissolution. The strain NL-7, which demonstrated the highest enhancing effect on mineral dissolution (see Fig. S1 in Supplementary material), was selected for more detailed dissolution studies of dolostone, as described below.

2.3. Colony morphology and gram-staining test

The NL-7 colony was streaked on the beef extract peptone agar plate and incubated at 28 °C for 48 h to study the morphological aspects of the colony. Gram-staining of the strain NL-7 was performed with the Vincent method (Oxford, 1970).

2.4. Identification of the bacteria

2.4.1. Biolog microstation system

Strain NL-7 was removed from storage, subcultured thrice on beef extract peptone agar. The single colony was inoculated BUG + B agar medium and cultured at 33 °C for 24 h. The inoculum to be used for testing was prepared by rolling a cotton swab over the agar plate and preparing a suspension. The turbidity of inoculums was compared with standard bacteria suspension to control the