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ScienceDirect
Advances in Space Research 47 (2011) 1071–1078
ADVANCES IN SPACE RESEARCH (a COSPAR publication)
www.elsevier.com/locate/asr

Bioaugmentation in growing plants for lunar bases

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Received 17 March 2010; received in revised form 2 November 2010; accepted 16 November 2010
Available online 24 November 2010

Abstract

Microorganisms may be a key element in a precursory scenario of growing pioneer plants for extraterrestrial exploration. They can be used for plant inoculation to leach nutritional elements from regolith, to alleviate lunar stressors, as well as to decompose both lunar rocks and the plant straw in order to form a protosoil. Bioleaching capacities of both French marigold (*Tagetes patula* L.) and the associated bacteria in contact with a lunar rock simulant (terrestrial anorthosite) were examined using the model plant-bacteria microcosms under controlled conditions. Marigold accumulated K, Na, Fe, Zn, Ni, and Cr at higher concentrations in anorthosite compared to the podzol soil. Plants inoculated with the consortium of well-defined species of bacteria accumulated higher levels of K, Mg, and Mn, but lower levels of Ni, Cr, Zn, Na, Ca, Fe, which exist at higher levels in anorthosite. Bacteria also affected the Ca/Mg and Fe/Mn ratios in the biomass of marigold grown on anorthosite. Despite their growth retardation, the inoculated plants had 15% higher weight on anorthosite than noninoculated plants. The data suggest that the bacteria supplied basic macro-and microelements to the model plant.

Keywords: Anorthosite; Bioavailability; Bacterial consortium; Bioleaching; Marigold; Bioaugmentation

1. Introduction

Closed ecological life support systems (CELSS) will play a key role for future extraterrestrial explorations. From the very beginning of a Moon exploration, it seems to be practical and cost-effective to use local material (regolith) for plant growth as well as to transform plant biomass and regolith into protosoil with the assistance of microorganisms. This effort should be integrated closely with a bioregenerative life support system. The biotechnological process to extract some elements from the regolith for propellant and food manufacture (Brown et al., 2008) can be combined with processing regolith for fertile protosoil preparation.

The bioavailability of regolith for plant nutrition and its putative safety was tested following the delivery of lunar regolith. Botanical studies indicated that the lunar material from Apollo 11 and 12 outposts could provide mineral nutrients for germinating seeds, for liverworts growth, and plant tissue culture development (Walkinshaw et al., 1970; Weete and Walkinshaw, 1972; Johnston et al., 1975). The general conclusion was that the lunar rock used as a substrate to grow plants had low bioavailability and needed mineral additives. Since the amount of native lunar material is limited, terrestrial analogs can be evaluated in simulation experiments to define pioneer plant cultivation.

Ukrainian rocks from Korosten Pluton (Penizevitchi, Turchynka deposits, Zhytomyr oblast) provide a suitable test-bed for modeling biomobilization of plant-essential elements from the lunar-like rocks. The Penizevitchi anorthosite in addition to intermediate plagioclase, a

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doi:10.1016/j.asr.2010.11.014

low-calcium pyroxene and olivine, all contain minor quantities of ilmenite $FeTiO_3$, orthoclase $K[AlSi_3O_8]$, biotite $K(Mg, Fe)_3[AlSi_3O_{10}](OH,F)_2$, and apatite $Ca_5[PO_4]_3(F, OH, Cl)$ (Mytrokhyn et al., 2003). The Turchynka type anorthosite is composed of plagioclase, pyroxene of low-calcium content, and olivine (Mytrokhyn et al., 2003, 2008). As compared to an average composition of the lunar anorthosite, Ukrainian anorthosites contain a bit more SiO_2, Na_2O, FeO, MgO, TiO_2 and less CaO and Al_2O_3. It reflects more "alkaline" composition of plagioclase and a higher content of mafic minerals (Ashwal, 1993). There are other rock deposits on the Earth, like the Stillwater (MA, USA) and the Skaergaard Intrusion (East Greenland) complexes, which are counterparts to lunar anorthosites (Bérczi et al., 2008).

French marigold (*Tagetes patula* L.) could grow in a crushed terrestrial anorthosite in model experiments. However, a deficit of nutrients and probably toxicity of liberated ions did not allow marigold to grow normally (Kozyrovska et al., 2004). When a consortium of the well-defined plant-associated bacteria and mycorrhizal fungi was used for seed and substrate inoculation, the plant development improved (Kozyrovska et al., 2004, 2006; Lytvynenko et al., 2006). The purpose of our study was (i) to examine bioleaching capacity of bacteria and a plant, French marigold, in contact with anorthosite of the Turchynka deposit, and (ii) to evaluate the accumulation of released elements by plants in model plant microcosms under controlled conditions.

2. Materials and methods

2.1. Prototype plant

The ornamental plant French marigold (*Tagetes patula* L.), cultivar Carmen, was chosen as a model plant system. Plastic transparent containers (160 by 90 mm) were used to establish a substrate-plant microcosm. The growth containers were placed into a controlled environment room under a 16/8 day (light/dark) photoperiod with light supplied at an intensity of 55 μmol m^{-2} s^{-1} and 22 °C. Anorthosite of the Turchynka deposit (Zhytomyr region) was used as a substrate for plant growth. In these experiments plants were not provided with nutrients. Sterile, distilled water was used to wet the microcosms. The microcosms either received 10 seeds or were left unplanted. Seeds were disinfected with 1.0% sodium hypochlorite supplemented with 0.02% Tween-20 for 1 min, followed by incubation in 70% ethanol (1 min) and washing 3 times (1 min each time). Plants were grown for 70 days. Plants were weighed at several periods: after forming of pseudo leaves, first, second and third true leaves, budding and flowering (stages 1–6).

2.2. Model substrate

Rock samples were obtained from the inner fragments, which were not in contact with the environment. The rock contained (in ppm) Fe (46722–75426), Ca (52226–65746), Si (228326–240499), Mn (924-693), Zn (44.024.0), Cu (18.2–16.7), Ni (68.8–42.7) (Mytrokhyn et al., 2008). The rock was crushed and the size range from 3.0 to 10.0 mm was used. A regular soil (podzol soil; organic matter, 1.2%; pH 6.2; N-4.3; P-7.6; K-8.4 mg in 100 g of a soil) mixed with sand 1:1 was used as an alternative substrate. The sand has been collected on the Dniper river and washed off by running tap water. All substrates were heated twice for 2 h at 170 °C before being autoclaved at 112 °C for 40 min. Portions of 260 g of either substrate were used in the containers.

2.3. Model consortium of bacteria

The bacterial consortium consisted of *Pseudomonas* sp. IMBG163, *Pseudomonas aureofaciens* IMBG164, *Stenotrophomonas maltophilia* IMBG147, *Paenibacillus* sp. IMBG156, *Klebsiella oxytoca* IMBG26, and *Pantoea agglomerans* IMV56 (kindly provided by Prof. R. Gvozdyak, Institute of Microbiology and Virology, Kyiv) (Kozyrovska et al., 2004; Lytvynenko et al., 2006). Bacteria supported plant growth by increasing stress tolerance, stimulated seed germination by providing phytohormones, and improved nutrition by leached or biologically fixed elements. For inoculation bacteria were cultivated overnight in liquid medium as follows: *Paenibacillus* sp. IMBG156 grew in MZ (Kozyrovska et al., 2005), *Pseudomonas* sp. IMBG163 grew in KB (King et al., 1954), the other species grew in LB medium (Miller, 1972), up to density of 10^6 CFU (colony forming units)/ml before being spayed onto seeds. To estimate external root colonization, root sections were vortexed in 0.9% NaCl. Then the serial dilutions were plated on LB plates to discriminate between *Ps. aureofaciens* and *P. agglomerans* (determination was based on color differences between orange and pale yellow colonies, respectively), or on KB to identify *Pseudomonas* sp. Siliceous bacterium *Paenibacillus* sp. was grown on the selective medium MZ. To isolate *K. oxytoca* and *S. maltophilia*, chloramphenicol and rifampicin (both of 50 μg/ml, Sigma) were added to LB agar, respectively.

Batch experiments on bioleaching were performed in 50 ml stationary cultures with crushed rock material (10%) in MZ. The evolution of bacterial populations was monitored for a 48-day period. Samples of whole population (unattached and sessile bacteria; the latter were washed off with Tween 20 (0.02%) on shaker during 30 min) were in parallel spread on selective agar media as described in (Lytvynenko et al., 2006). Minimal inhibitory concentration (MIC) of heavy metal salts ($CdSO_4$, $CuSO_4$, $ZnSO_4$ – all from Sigma–Aldrich, USA) were determined in 10 ml of an appropriate broth at 28 °C. Overnight cultures were used for inoculations of liquid media containing different concentrations of 0.22 μm filter-sterilized salt solutions (0.05–3 mM $ZnSO_4$, $CuSO_4$, $CdSO_4$). Optical density of populations was controlled with a NanoDrop ND-1000 (NanoDrop Technologies, USA) at 620 nm.