ISSN 1561-8889. Космічна наука і технологія. 2006. Т 12. № 5/6. С. 36—41.

UDC 579.65

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Optimization of plant mineral nutrition under growth-limiting conditions in a lunar greenhouse

Recieved 13.03.06

It may be assumed that the first plants in a lunar base will play a main role in forming a protosoil of acceptable fertility needed for purposively growing second generation plants like wheat, rice, tulips, etc. The residues of the first-generation plants could be composted and transformed by microorganisms into a soil-like substrate within a loop of regenerative life support system. The lunar regolith may be used as a substrate for plant growth at the very beginning of a mission to reduce its cost. The use of microbial communities for priming plants will allow one to facilitate adaptation to stressful conditions and to support the plant development under growth limiting conditions. Well-defined plant-associated bacteria were used for growing three cultivars to colonize French marigold (*Tagetes patula* L.) in anorthosite, a substrate of low bioavailability, analogous to a lunar rock. The consortium was composed of plant growth promoting rhizobacteria and the bacterium *Paenibacillus* sp. IMBG156 which stimulated seed germination, better plant development, and finally, the flowering of inoculated tagetes. In contrast, control plants grew poorly in the anorthosite and practically did not survive until flowering. Analysis of bacterial community composition showed that all species colonized plant roots, however, the rate of colonization depended on the allelopatic characteristics of marigold varieties. Bacteria of consortium were able to liberate some elements (Ca, Fe, Mn, Si, Ni, Cu, Zn) from substrate anorthosite. Plant colonization by mixed culture of bacterial strains resulted in the increase of accumulation of K, Mg, Mn by the plant and in the lowering of the level of toxic metal accumulation. It was assumed that a rationally assembled consortium of bacterial strains promoted germination of marigold seeds and supported the plant development under growth limiting conditions by means of bioleaching plant essential nutritional elements and by protecting the plant against hyperaccumulation of some toxic metals.

THE PROTOTYPE PLANT-BACTERIA MICROCOSM FOR A LUNAR BASE

The ability to grow plants in space self-perpetuating gardens is topical for providing an advanced life support system for humans while inhabiting a permanently manned lunar base (PMBL). Plants could provide fresh food, oxygen, and clean water for explorers living in PMLB. A lunar garden has to supplement less appetizing packaged food brought from the Earth. The ornamental plants will play a role in reducing stress and in recovering emotional potency in PMLB personnel. Lunar agriculture has the potential to earn the needed export of fresh food to other space locations at a decided fiscal advantage over fresh products brought up

from the Earth. To reduce a cost of early missions to the Moon, it would be practical to use local materials such as a lunar regolith for growing plants in lunar greenhouses. The use of bacteria to govern a decomposition of silicate rocks, a liberation of essential growth elements for plants, and to deliver them to the plant is a key idea in precursory scenario of growing pioneer plants for a lunar base [5—7]. The objectives of this study were to study bioleaching capacity of bacteria in batch experiments with anorthosite as a component of nutrient media, as well as in the model plant microcosms placed in plant growth chambers under controlled conditions.

The prototype plant. The ornamental plant French marigold (*Tagetes patula* L.), undemanding to growth conditions, has been chosen as a model plant system to demonstrate growth of plant with minimal expenses. The plant produces acceptable biomass which could be converted by microorganisms into a fertile protosoil assigned for growing first industrial plants. In future stages of lunar agroindustry, the marigold may be applied to recover a tired plant-growing environment by producing secondary metabolites (allelochemicals) [10]. Beside the pragmatic side, the marigold could perform a role in esthetic decoration of the hostile environment of PMBL: the beautiful image and delicate fragrance of marigold cultivars, familiar to everybody and lovely, would remind of an earthly spirit to habitants of PMLB and, accordingly, correct the emotional comfort of lunar explorers. Marigold flowers are consumed widely as aromatic tea, and this experience could be proposed to be used at PMLB in prophylactics of various diseases and protection from irradiation, to release pain [4, 18, 19]. Both marigold flowers and leaves are excellent spices that could appetize tasteless packaged food and in that way supply it with vitamins and microelements [11]. The set of these traits makes marigold a promising candidate for pioneer plant of multipurpose application at lunar base. In these experiments a middle-sized cultivar Carmen and two dwarf cultivars, Petite Harmony and Petite Gold, served as the plant-hosts for a consortium of plant growth promoting rhizobacteria.

The model consortium of bacteria. The rationally assembled bacterial community — *Pseudomonas* sp. IMBG163, *Pseudomonas aureofaciens* IMBG164, *Paenibacillus* sp. IMBG156, *Klebsiella oxytoca* IMBG26, and *Pantoea agglomerans* IMV56 — aims to support plant growth in a substrate of low bioavailability using several mechanisms: priming resistance in plant to stresses, stimulating seed germination by providing phytohormones, improving nutrition by leached or biologically fixed elements, "cleaning" plant environment, etc. Cultures of bacterial strains were applied for seed inoculation, except *Paenibacillus* sp., which was introduced into a substrate. Model consortium of bacteria needs both organogenic elements N, P, C, O and additional elements essential for physiological activity like K, Na, Mg, Ca, Fe, etc., as well as microelements. In accordance with the idea of cultivating healthy crops in a lunar garden using low-cost technology, in these experiments bacteria were not provided with nutrients, except a water or 1 mM solution of potassium phosphate (PP).

The analogs of a lunar rock. The lunar highland regolith is predominantly composed of alumino-silicate basic rocks, mainly anorthosites, noritic anorthosites, and gabbroic anorthosites [2]. The primary rock-forming minerals of lunar anorthosites are calcic plagioclase $Ca[Al_2Si_2O_8]$, pyroxene $(Mg, Fe, Ca)(Mg, Fe)[Si_2O_6]$, and olivine $(Mg, Fe)_2[SiO_4]$. Therefore, the plant could get the majority of elements essential for nutrition from the regolith, and the rest of it by lunar-sourced additions. The terrestrial anorthosites are usual rocks within the Precambrian Shields, for example, within the Ukrainian Shield. There are some differences between terrestrial and lunar anorthosites [2]. While the first are "dry", some earth rocks contain hydrated minerals. Another difference could be the absence of hydrocarbons. Despite these differences, terrestrial anorthosites may serve as simulants of lunar rocks in model experiments on plant cultivation under growth limited conditions. The Turchynka type anorthosite is composed of plagioclase $(Ca, Na)[Al_2Si_2O_8]$, pyroxene of low calcium content, and olivine. The Penizevitchi anorthosite in addition to intermediate plagioclase, low-calcic pyroxene and olivine, contains minor quantities of ilmenite $FeTiO_3$, orthoclase $K[AlSi_3O_8]$, biotite $K(Mg, Fe)_3[AlSi_3O_{10}](OH, F)_2$, and apatite $Ca_5[PO_4]_3(F, OH, Cl)$ [12]. These types of anorthosite appeared to be a poor support of marigold growth, and the idea was to use natural bacterial

residents of alumino-silicate rocks to leach the plant essential ions from a substrate and therefore to improve plant development [5]. The anorthosite of Turchynka deposit (Korosten Pluton, Ukraine) [9, 12], chemically and mineralogically similar to lunar anorthosites was used in model microcosms as the substrate for plant growth.

The marigold growing in anorthosite. In our earlier experiments [5, 7] we observed a poor survival of marigolds in intact, the first-time-used anorthosite. In this research we re-used the substrate after sterilization, and marigold seed germination in such a substrate has been at the same level. However, control plants did survive in anorthosite, and they had a comparable level of survival with the inoculated marigolds. Nevertheless, inoculated marigold sprouts differed from control variants by a higher biomass, better branching of stems, and in more green color of leaves (see colour Fig. VII). Application of mixed populations of bacteria for seed inoculation resulted in 100 % seed germination and survival of sprouts in anorthosite. In contrast, watered seeds survived in 20—30 %, even when potassium and phosphorus were added to microcosm. After a period of 54±3 days of co-cultivating the French marigold with the consortium of bacteria, the plant began to flower. The noninoculated plants flowered 5—7 days later. There was practically no difference between varieties tested in survival rate and in duration of the period before flowering. As compared to plants grown in the soil, inoculated tagetes began to flower 5—6 days later in anorthosite and produced 4.3 time less dry biomass compared to plants grown in a fertile soil. Inoculated plants gained 1.8-time higher dry weight as compared to control plants that accidentally survived in anorthosite at an age of 4 weeks (Table 1).

The survival of bacteria in the marigold rhizosphere was tested in three cultivars with different allelopatic traits. There were no shifts in community composition in the roots of the Carmen cultivar within 6 weeks when plants were supplied by a PP solution or distilled water, in spite of putative deficit of nutrients in microcosms. At the beginning of the examination period, the *Paenibacillus* sp. IMBG156 came on roots from substrate anorthosite and generated a small-size population on the plant, and later, after 2 weeks, it was getting to rise the population rapidly to log 8/g of fresh roots [6, 7]. The partners of *Paenibacillus* were rather competitive on the marigold roots and gained the log 6–7 populations. In contrast, the roots of the dwarf marigold cultivars were less colonized by the bacterial assemblage. Total number of bacteria per gram of Petite Harmony root did not exceed log 7, and the most active colonizers were *Paenibacillus* sp. IMBG156 and *P. aureofaciens* IMBG164. Poor survival of some bacterial species on the dwarf marigold roots correlated with a higher rate of phenolcarbonic acids produced by plants which appeared to inhibit bacterial growth [10]. It was concluded that Carmen cultivar was more suitable than dwarfs for further experiments.

Bioleaching anorthosite by Paenibacillus sp. IMBG156 and by consortium of bacteria. Siliceous bacteria as well as other microorganisms

Table 1. Accumulation of elements from anorthosite by inoculated tagetes, μg/g

Elements	Anorthosite, control	Anorthosite, *Paenibacillus* sp.	Anorthosite, consortium	Soil, control	Soil, *Paenibacillus* sp.	Soil, consortium
Dry weight, g/plant	0.031±0.008	0.035±0.002	0.056±0.008	0.238±0.010	0.248±0.030	0.221±0.050
Zn^{+2}	80.4±14.3	30.1±10.4	29.4±6.5	69.4±4.0	58.2±11.4	71.0±2.7
Mn^{+2}	306±86	384±81	423±62	601±4	520±140	835±104
Fe^{+3}	340±17	143±42	81±13	95±0	70±22	75±3
Ni^{+2}	27.2±3.1	13.6±0.1	14.2±3.9	3.1±0.5	5.1±0.9	6.6±2.7
Cr^{+3}	35.4±11.8	3.1±1.4	18.0±5.5	2.7±3.0	1.9±1.1	2.0±2.2
Co^{+2}	< 0.5	2.6±0.9	1.5±0.4	0.6±0.1	0.9±0.5	1.3±1.1
Ca^{+2}	179050±51548	81505±3279	63373±13435	61710±5882	57392±7855	55966±7419
Mg^{+2}	1752±469	3005±652	1786±421	8940±1560	8493±2574	9604±382
Na^{+}	1162±71	678±148	383±23	451±76	435±63	373±58
K^{+}	19551±1948	20629±2285	23705±2780	12747±1443	15010±2870	14850±2335

could be used for bioleaching the lunar regolith in a program of growing pioneer plants. Some species of *Bacillus*/*Paenibacillus* and *Pseudomonas* genera are known as destructors of alumino-silicates [1, 13]. In this respect *Paenibacillus* sp. IMBG156, isolated from a silica rock, has been chosen as the model bacterium in the simulation of bioleching anorthosite. In batch experiments with monoculture, *Paenibacillus* sp. IMBG156 cells attached to anorthosite fragments and changed electrokinetic potential of the rock surface apparently due to exopolysaccaride (EPS) capsule [5]. Paenibacilli caused the corrosion of anorthosite resulted from formation of iron(III) oxide of the rock within 28 days of incubation in the presence of the Pinizevitchi anorthosite fragments. No signs of changes on the anorthosite surface were observed in the variant without bacteria. Cultural media were examined by flame atomic adsorption spectrophotometry using C115-M1 (Selmi, Ukraine). SiO_3^{-2} was detected with the colorimetric method. The results represented in Table 2 show that strain IMBG156 as well as the consortium of bacteria were able to deliberate Fe^{+3}, Ca^{+2}, and SiO_3^{-2} from anorthosite under normal pH within the 6-week period of incubation with a rock in a minimal medium. The level of released elements increased 2—8-fold compared to control in both *Paenibacillus* sp. IMBG156 and model bacterial consortium. In a pellet of mixed culture concentration of liberated ions increased 1.5—4.5 times. In contrast to IMBG156, the consortium of bacteria were able to release and accumulate cations of Zn, Mn, Cu and Ni. Results of this series experiments clearly demonstrated that both monoculture *Paenibacillus* sp. IMBG156 and mixed bacterial culture were able to leach anorthosite.

Bioleaching anorthosite by plant microcosm. The marigold is known to accumulate metal cations [3]. In this study results revealed that *T. patula* cv. Carmen accumulated macroelements K, Ca, Na and microelements Fe, Zn, Ni, Cr in anorthosite substrate in a higher concentration than when grew in a 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) (see Table 1). In association with bacteria, tagetes accumulated more K^+, Mg^{+2}, Mn^{+2} in anorthosite. Bacteria corrected hyperaccumulation by the plants of Ca, Zn, Fe, Cr, Ni, and in such a way prevented the intoxication by these elements. The consortium of plant root microinhabitants enhanced accumulation of Cr^{+3} in a higher rate than the plant colonizer *Paenibacillus* sp. alone. Bacteria promoted accumulation of Co^{+2} by marigold, and this phenomenon may be connected with a resistance to cations of toxic metals known for some species of bacteria [17], for example, some representatives of *Pseudomonas* and *Klebsiella* genera are tolerant to toxic concentrations of heavy metals [15]. Bacteria have developed a variety of resistance mechanisms to counteract heavy metal stress. These mechanisms include the formation and sequestration of heavy metals in complexes, reduction of a metal to a less toxic species, and the direct efflux of a metal out of the cell [14]. Mobile genetic elements (MGE) are responsible for such activity in some cases [16]. Either deleting MGE or substituting some species

Table 2. Concentration of elements released by bacteria from anorthosite, mg/l

Microcosm	Zn^{+2}	Mn^{+2}	Fe^{+3}	Cu^{+2}	Ni^{+2}	Ca^{+2}	SiO_3^{-2}
Consortium of bacteria*:							
A cultural medium	0.133	0	2.340	0.036	0	1010.000	Not determined
A pellet (bacterial cells)	0.310	0.223	10.129	0.049	0.072	1309.000	Not determined
Paenibacillus sp. IMBG156 (a cultural medium)	0	0	2.250	0	0	1730.000	12.000
Control (a nutrient medium without bacteria)	0	0	0	0	0	1010.000	0.800
Control (a nutrient medium without bacteria and anorthosite**)	0	0	0	0	0	0	0

*Titre of bacteria log 6-7 CFU/ml.

**Anorthosite rocks of the Turchynka type contain (ppm) Fe (46722—75426), Ca (52226—65746), Si (228326—240499), Mn (924—693), Zn (44.0—24.0), Cu (18.2—16.7), Ni (68.8—42.7)

of bacteria, possessing resistance to cromium and cobalt, can resolve the problem with accumulation of undesirable elements.

Analysis of the results of this study shows that inoculated marigold plants have got in full practically all elements, except magnesium and mangan which were in deficit. However, due to bacteria, it was possible to save up to 70 % of needed Mn^{+2}. Summarizing advantages of marigold inoculations by mono- or mixed culture, we can conclude that application of consortium of bacteria is more profitable than *Paenibacillus* sp. alone.

CONCLUSIONS

In model experiments, the rationally assembled consortium of bacterial strains promoted the growth of *T. patula* L. and supported the plant development under growth limiting conditions by stimulating seed germination (1); bioleaching and delivering essential nutritional elements to the plant (2); preventing intoxication of the plant by excess of metal cations released from anorthosite. Due to the bacterial consortium, the model plant was supplied with an additional amount of basic macro- and microelements. French marigold grown without bacteria appears to be intoxicated by theshold accumulation of some metals, and the presence of bacteria on the plant roots protected it against excessive accumulation of some elements. So, the bacteria were able to correct both hyperaccumulation and deficit of elements needed for plant nutrition. Growing first generation plants such as the French marigold in the presence of a community of microorganisms, including eubacteria, cyanobacteria, mycorrhiza fungi, etc. and converting the plant residues by microorganisms into a soil-like substrate may give the beginning of agro-industry at PMBL, however, bioaugmentation strategy of growing plants for lunar bases needs comprehensive study and a wider body of evidence.

ACKNOWLEDGMENTS

The authors are thankful to O. Nimenko for technical assistance. The project has been fulfilled on a voluntary basis.

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ОПТИМІЗАЦІЯ МІНЕРАЛЬНОГО ЖИВЛЕННЯ РОСЛИН В НЕСПРИЯТЛИВИХ УМОВАХ ВИРОЩУВАННЯ У МІСЯЧНІЙ ОРАНЖЕРЕЇ

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Передбачається, що перші рослини відіграватимуть важливу роль в утворенні протогрунту достатньої родючості, потрібної для цільового вирощування другого покоління рослин, таких як пшениця, рис, соя тощо. Залишки рослин першого покоління могли б бути компостовані і перетворені мікроорганізмами у подібний до грунту субстрат в регенеративній системі життєзабезпечення. Місячний реголіт може бути використано як субстрат для вирощування рослин на початку місії, щоб скоротити витрати. Використання мікробних спільнот для праймування рослин дозволить полегшити адаптацію до стресових умов і забезпечити розвиток рослини в несприятливих умовах. Добре охарактеризовані, асоційовані з рослинами бактерії використовувалися для вирощування трьох сортів чорнобривців (*Tagetes patula* L.) в анортозиті, субстраті низької біодоступності, аналогічному місячній гірській породі. Консорціум складався з бактерії *Paenibacillus* sp. IMBG156 та ризобактерій, що сприяють росту рослин, який стимулював проростання насіння, поліпшував розвиток рослин і, в кінцевому результаті, цвітіння інокульованих чорнобривців. На противагу цьому, контрольні рослини погано росли в анортозиті і практично не доживали до цвітіння. Аналіз складу бактерійної спільноти показав, що всі види бактерій колонізували коріння рослин, проте рівень колонізації залежав від алелопатичних характеристик видів чорнобривців. Бактерії консорціуму здатні вивільняти деякі елементи (Ca, Fe, Mn, Si, Ni, Cu, Zn) з анортозиту. Колонізація рослин змішаною культурою бактерійних штамів призводила до збільшення накопичення рослиною K, Mg, Mn і зниження рівня накопичення токсичних металів. Припускається, що раціонально підібраний консорціум бактерійних штамів сприяв проростанню насіння чорнобривців і підтримував розвиток рослин в несприятливих для росту умовах за допомогою вилужування необхідних для рослин поживних елементів і захисту рослин проти наднакопичення деяких токсичних металів.