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O.B. Tashyrev, V.M. Govorukha

Zabolotny Institute of Microbiology and Virology of the NASU,
Acad. Zabolotnogo Str., 154, Kyiv MSP, D 03680, Ukraine

THE WIDESPREAD OF Fe(III)-REDUCING BACTERIA IN NATURAL ECOSYSTEMS OF ECUADOR

The widespread of Fe(III)-reducing microorganisms in natural ecosystems of Ecuador of La Favorita, Tungurahua volcano and Papallacta areas was experimentally proved. High efficiency of microbial precipitation of soluble iron compounds was also demonstrated. Obtained results indicate the potential ability of Fe(III)-reducing microorganisms to influence the formation of carbon and iron vector fluxes in ecosystems, as well as development of effective biotechnologies of water purification from iron compounds.

K e y w o r d s: Fe(III)-reducing bacteria, natural ecosystems, Ecuador, water purification from iron.

Iron compounds are widely distributed throughout the earth's crust. But the role of microorganisms in biogeochemical cycles of iron compounds transformation is poorly understood [11, 12]. On the basis of conducted thermodynamic calculations of microbial iron compounds transformation microbial reduction of Fe(III) is assumed to be widespread in natural ecosystems.

The aim of the study was to investigate the spread of Fe(III)-reducing bacteria in natural ecosystems of Ecuador.

Materials and Methods. The samples of soil and lichens selected from natural ecosystems of Ecuador were investigated to detect Fe(III)-reducing bacteria. Description of the samples is given in Table 1.

Table 1.

Characteristics of the investigated samples

№	Description of the samples
	Group 1. La Favorita
1	Ecuador. 28.10.2013. Polygon-1. Sample №1
2	Ecuador. 28.10.2013. Polygon -1. Sample №2
3	Ecuador. 28.10.2013. Polygon -1. Sample №3
4	Ecuador. 28.10.2013. Polygon -1. Sample №4. Green-white lichen
5	Ecuador. 28.10.2013. Polygon -1. Sample №5. Green-white crustose lichen on the rock
6	Ecuador. 28.10.2013. Polygon -1. Sample №6. Cliff. Moss, roots, soil
7	Ecuador. 28.10.2013. Polygon -1. Sample №7. Le Favorita. Soil at the base of the cliff
8	Ecuador. 28.10.2013. Soil, tropical forest
	Group 2. Tungurahua Volcano
9	Tungurahua Volcano (acting). 06.11.2013. Sample №1. Volcanic soil
10	Tungurahua Volcano. 06.11.2013. Sample №2. Alluvium in the riverbed (pumice, volcanic sand)
11	Tungurahua Volcano. 06.11.2013. Sample №3. Soil in the riverbed
12	Tungurahua Volcano.06.11.2013. Sample №4. Soil, moss
	Group 3. Papallacta
13	4020 m Papallacta. 31.10.2013. Cliff-3. Sample № 10. Chamomile
14	4020 m Papallacta. 31.10.2013. Sample №11. Moss, soil
15	4020 m Papallacta. 31.10.2013. Sample №2. Soil
16	4020 m Papallacta. 31.10.2013. Sample №12. White crustose lichen
17	4020 m Papallacta. 31.10.2013. Soil, lichen
18	4020 m Papallacta. 31.10.2013. Lichen from the rock
19	3800 m Papallacta. 30.10.2013. Cliff-2. Sample № 8. Crumbling rock, lichen
20	3800 m Papallacta.30.10.2013. Polygon-2. Sample № 9. Black lichen, clay
21	Park. Botanical Orchid Garden (Puyo). 07.11.2013. Soil, wet forest

Ecuadorian ecosystems screening for the presence of Fe(III)-reducing microorganisms. Fe(III)-reducing bacteria were cultivated in liquid selective medium: nutrient broth (producer: HiMedia Laboratories Pvt. Limited, India) with Fe(III) citrate. Seeding of the samples was carried out into the tubes. The tubes volume was 20 ml. The samples (0,5 g) and 15 ml of nutrient medium with Fe(III) citrate (0,5 g/l of Fe(III)) were added. The tubes were capped with conical rubber stoppers. Nutrient medium without inoculum was used as a sterility control. Cultivation was carried out at 30°C during 7 days.

The following parameters served as the criteria of microbial metabolic activity: biomass growth, changes in the concentration Fe(II) and Fe(III), as well as changes in the gas phase composition.

For growth parameters measuring 5 ml of culture liquid was centrifuged at 2655 g during 15 minutes. The supernatant was decanted and used to determine the concentration of iron compounds. The precipitate consisting of bacterial biomass was diluted in the same volume of physiological solution and its optical density was measured. Biomass growth was determined by optical density of the cell suspension by the photoelectrocolorimeter (PEC) at λ = 540 nm and 0,5 cm of an optical path length.

The concentration of Fe(II) was determined by the colorimetric method based on the formation of compounds of Fe(II) with *o*-phenanthroline colored in red [6]. The solution of 0,25% *o*-phenanthroline (0,75 ml) was added to 1,5 ml of the supernatant. The presence of Fe(II) was indicated by the appearance of red-orange color. Quantitative determination of Fe(II) was performed using the PEC at λ = 490 nm and 0,5 cm of an optical path length.

Fe(III) compounds were determined by a colorimetric method based on the formation of colored in red compounds of Fe(III) with potassium rhodanide in acidic conditions [6]. 0,25 ml of 1,5 M KSCN solution and 0,75 ml of HCl (concentrated) were added to the culture medium (1,5 ml). The presence of Fe(III) was indicated by the appearance of the red color. Quantitative determination of Fe(III) was performed using the PEC at λ = 490 nm and 1 cm of an optical path length.

The gas composition in the tubes was determined by a standard method using the gas chromatograph LHM-8-MD [1]. The chromatograph is equipped with two steel columns – first (I) for H_2, O_2, N_2 and CH_4 analysis, second (II) – for CO_2 analysis.

Detector-katharometer column parameters: I – l = 3 m, d = 3 mm, with a molecular sieve 13X (NaX); II – l = 2 m, d = 3 mm, with Porapak-Q carrier. The column temperature is +60°C. The evaporator temperature is +75°C. The detector temperature is +60°C. The detector current is 50 mA. Argon is the carrier gas. The gas flow rate is 30 cm^3/min. Volume of the gas samples is 2,5 cm^3 for the first column and 1 cm^3 for the second.

The percentage of primary gases – H_2, CO_2, N_2 and O_2 – in the gas phase was determined by the standard procedure calculating the peak square of the gas phase components.

Results and their discussion. Metabolic activity of Fe(III)-reducing microorganisms was observed for all investigated samples. This was manifested by the increase of microbial biomass, changes of the gas phase composition, decrease of Fe(III) compounds concentration with the increase of Fe(II) concentration in the culture liquid.

As it is shown at Fig. 1, the most intense growth of microorganisms was observed in the samples № 8 (La Favorita), № 9 (Tungurahua volcano) and № 1 (La Favorita). The optical density of microbial cell suspension on the seventh day of cultivation reached 0,32 units. Microorganisms of the samples № 15 (Papallacta, 4020 m), № 21 (Papallacta, 3800 m) developed the least intensively. The optical density of cell suspension was 0,06 units. And for the sample № 17 (Papallacta, 4020 m) the optical density was 0,02 units.

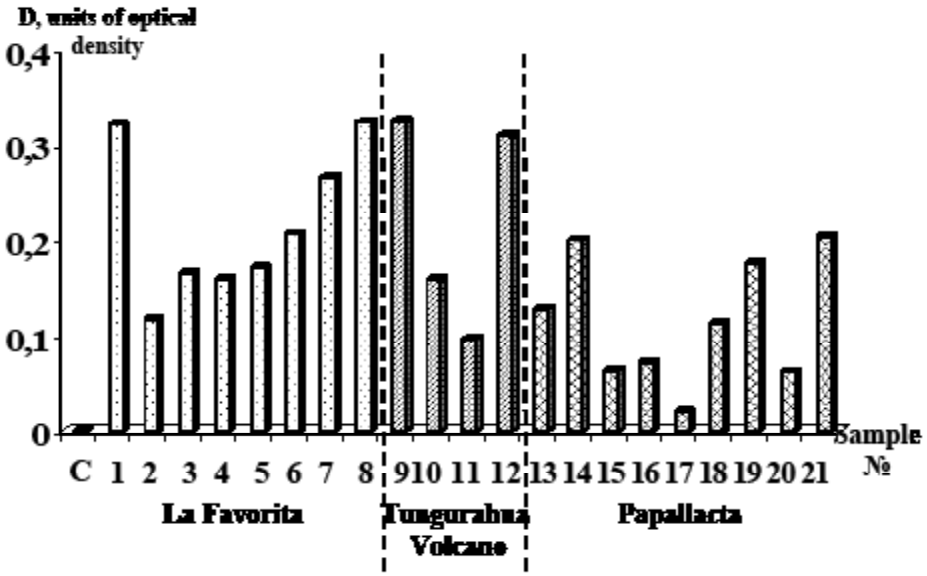

Figure 1. Microbial biomass increase of the investigated samples on the seventh day of cultivation. (C – sterility control).

Besides microbial biomass accumulation changes of the gas phase composition in tubes were observed. Along with the decrease of O_2 concentration CO_2 and H_2 were synthesized (Fig. 2). Accumulation of CO_2 evidenced an active growth of microorganisms. Hydrogen was intensively accumulated in the samples № 3 (La Favorita) – 15,94%, № 10 (Tungurahua Volcano) – 13,27% and № 13 (Papallacta, 4020 m) – 13,21 %. Synthesis of hydrogen may indicate functioning of low potential redox-enzymes. Along with the decrease of oxygen concentration it promotes rapid and efficient reduction of Fe(III).

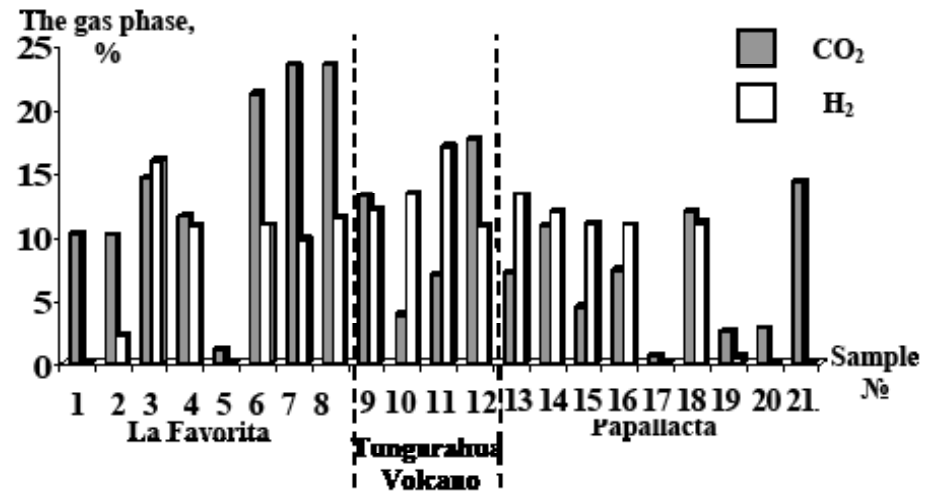

Figure 2. Concentration of CO_2 and H_2 in the tubes on the seventh day of cultivation.

Special attention was paid to the ability of microbial associations to reduce Fe(III) compounds to Fe(II). The data obtained indicate that microorganisms of all studied samples are capable to reduce Fe(III) compounds. The concentration of Fe(II) on the seventh day of cultivation was from 8 to 55 mg/l.

Effectiveness of iron reduction, i.e. the ratio of Fe(II) compounds concentration accumulated in the culture medium on the 7th day of Fe(III)-reducing bacteria cultivation to the initial concentration of Fe(III), ranged from 2 to 12% (Fig. 3).

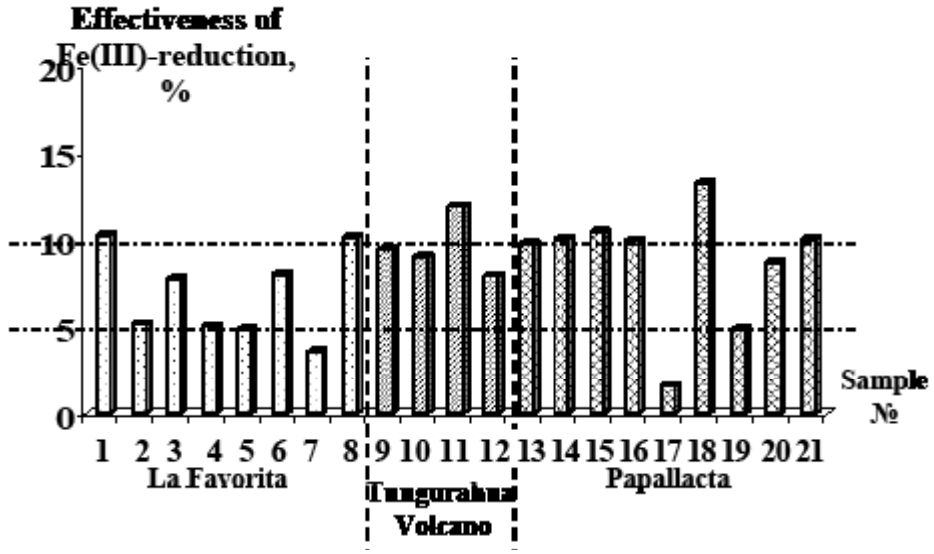

Figure 3. Effectiveness of microbial Fe(III)-reduction as the ratio accumulated Fe(II) and initial Fe(III).

Low efficiency of Fe(III) compounds reduction was demonstrated for 19 % of the investigated samples. It did not exceed 5 %. The average level of microbial iron reduction (from 5 to 10 %) was determined for 38 % of the samples. Effectiveness of Fe(III)-reduction over 10 % was found for 43 % of investigated samples.

Microorganisms of Ecuadorian natural ecosystems showed very high efficiency of soluble iron compounds precipitation (Fig. 4). Low activity of microorganisms was showed by two samples: № 5 (La Favorita) and № 17 (Papallacta, 4020 m). Effectiveness of microbial iron precipitation was 23 %. The average level of microbial activity (from 40 to 80 %) was typical

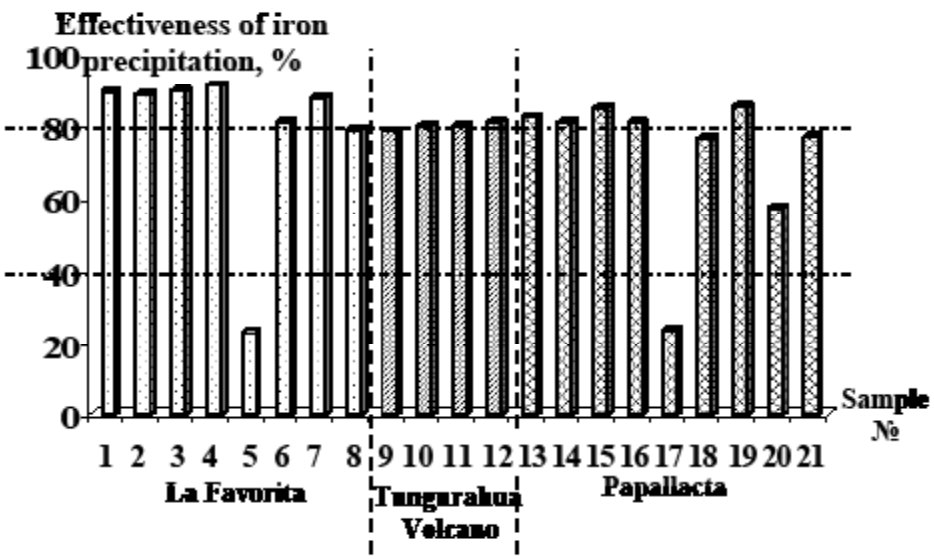

Figure 4. Effectiveness of iron compounds precipitation by microorganisms.

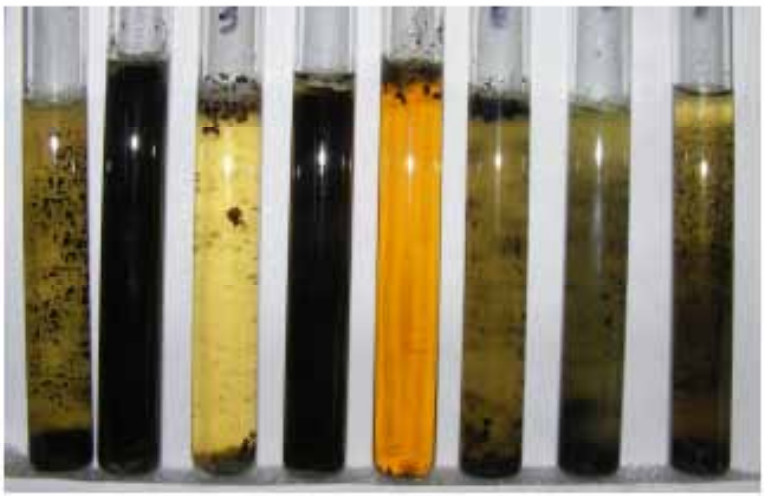

Figure 5. Microbial reduction of Fe(III) compounds in the selective medium with Fe(III) citrate.

for six samples. High efficiency of iron precipitation by microorganisms (over 80 %) was detected for 57 % of the samples.

Fig. 5 illustrates the activity of Fe(III)-reducing microorganisms. It manifested itself by changing the culture medium color from dark brown that is typical for Fe(III) compounds to yellow since Fe(II) compounds are colorless. Also Fe(III)-reducing microorganisms promoted iron sulfides formation and iron precipitation.

Figure 5

The participation of microorganisms in reduction of Fe(III) compounds has been known for a century (Runov, 1926) [7]. However, the role of microorganisms in biogeochemical cycles of iron compounds transformation is not determined.

Until recently, the process of microbial Fe(III)-reduction was not considered to be important in the circulation of organic matter [9]. It is now considered that Fe(III) is one of the most spread electron acceptors in soils and second after sulfate in marine sediments [10].

There is evidence of Fe(III)-reducing microorganisms presence in soil, sediments of lakes, seas, groundwater, oil fields [2, 4, 7], coal and ore dumps, in anaerobic bioreactors, in water and corrosive deposits of heat networks pipes, etc. [7, 8].

The results obtained confirm the literature data [2, 3, 4, 5, 7, 8] on the widespread of Fe(III)-reducing microorganisms in natural ecosystems. Furthermore, the results of studies provide evidence to suggest that Fe(III)-reducing microorganisms can significantly affect the biogeochemical cycles of carbon and iron transformation. Because, regardless of geographical location and complex of extreme factors in ecosystems, microbial associations are able to effectively reduce compounds of Fe(III) to Fe(II).

Thus, we have shown that Fe(III)-reducing bacteria are an integral part of natural ecosystems of Ecuador. The widespread of Fe(III)-reducing bacteria in Ecuador ecosystems was experimentally confirmed. Furthermore, high effectiveness of soluble iron compounds precipitation by microorganisms was demonstrated. The results obtained indicate the potential effect of Fe(III)-reducing microorganisms on biogeochemical cycles of carbon and energy in natural ecosystems of Ecuador. The results can be useful for developing of effective biotechnologies of water purification from iron compounds.

Таширев О.Б., Говоруха В.М.

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, ГСП Д03680, Україна

ПОШИРЕННЯ ЗАЛІЗОВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ У ПРИРОДНИХ ЕКОСИСТЕМАХ ЕКВАДОРУ

Резюме

Експериментально доведено широке поширення залізовідновлювальних мікроорганізмів у природних екосистемах Еквадору районів Ла-Фаворита, вулкана Тунгурауа і Папаллакта. Продемонстрована висока ефективність мікробного осадження розчинних сполук заліза. Отримані результати свідчать про потенційну можливість впливу залізовідновлювальних мікроорганізмів на формування векторних потоків вуглецю і заліза в екосистемах, а також про можливість створення ефективних біотехнологій очистки води від сполук заліза.

Ключові слова: залізовідновлювальні бактерії, природні екосистеми, Еквадор, очистка води від заліза.

Таширев А.Б., Говоруха В.М.

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины,
ул. Академика Заболотного, 154, Киев, ГСП Д03680, Украина

РАСПРОСТРАНЕНИЕ ЖЕЛЕЗОВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ В ПРИРОДНЫХ ЭКОСИСТЕМАХ ЭКВАДОРА

Резюме

Экспериментально доказано широкое распространение железовосстанавливающих микроорганизмов в природных экосистемах Эквадора районов Ла-Фаворита, вулкана Тунгурауа и Папаллакта. Продемонстрирована высокая эффективность микробного осаждения растворимых соединений железа. Полученные результаты свидетельствуют о потенциальной возможности влияния железовосстанавливающих микроорганизмов на формирование векторных потоков углерода и железа в экосистемах, а также о возможности создания эффективных биотехнологий очистки воды от соединений железа.

Ключевые слова: железовосстанавливающие бактерии, природные экосистемы, Эквадор, очистка воды от железа.

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