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REGULARITIES OF QUANTITATIVE DISTRIBUTION FOR FE(III)-REDUCING BACTERIA IN NATURAL ECOSYSTEMS

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The aim of the work was quantitative determination of Fe(III)-reducing bacteria in natural ecosystems of the Antarctic, the Arctic, the Dead and the Black Sea, middle latitude (Ukraine, Abkhazia) and the equatorial zone (Ecuador). It was used the method of quantitative determination of microorganisms by McCready and the colorimetric method for determination of Fe(II) compounds . Results. The systemic study of the number of Fe(III)-reducing bacteria of both hemispheres in the ecosystems of six geographic regions was carried out for the first time. High number of Fe(III)-reducing bacteria in natural ecosystems was experimentally shown. The number of Fe(III)-reducing bacteria ranged from $1.1 \cdot 10^2$ to $2.8 \cdot 10^7$ cells/g of absolutely dry sample. Conclusions. The presented data showed that Fe(III)-reducing bacteria are an integral part of natural ecosystems and can significantly affect the biogeochemical cycles of iron and carbon compounds transformation.

Key words: quantity of Fe(III)-reducing bacteria, natural ecosystems, biogeochemical cycles of iron.

The phenomenon of microbial Fe(III) reduction was discovered at the beginning of XX century [1, 2]. However, it was not considered to be important in biogeochemical cycle of carbon for a long time. Therefore proper attention was not paid to its study [3].

Iron compounds are widely distributed in soils, sediments, marine and fresh water. Their concentration ranges from tens of milligrams to 3–5 g per 1 kg of soil [4]. Microorganisms are able to transform iron compounds to soluble and insoluble form, as well as to reduce and oxidize them [2, 5].

In our opinion, Fe(III)-reducing bacteria (FRB) should be widely distributed in ecosystems, since Fe(III) compounds are present in high concentrations in ecosystem and therefore they can be used by microorganisms as terminal electron acceptors. Indeed, Fe(III) reduction occurs during organic compounds decomposition in soil, freshwater and marine sediments, formation of iron-containing minerals, soil gleying, etc. [6].

The rate and efficiency of microbial Fe(III) reduction depends not only on environmental conditions, but also on the quantity of microorganisms involved in the process. Therefore, quantitative determination of Fe(III)-reducing bacteria in microbial communities of natural ecosystems contribute to assessing the role of FRB in biogeochemical cycles of iron and carbon compounds transformation in ecosystems.

There are only a few data regarding FRB quantity in bottom sediments, groundwater and soils in literature [1, 7–9].

In this regard, the aim of our work was quantitative determination of FRB in natural ecosystems that differ by geographic location and complex of extreme factors.

Materials and Methods

Forty three samples (soil, clay, sediments, etc.) of six geographic zones (the Antarctic, the Arctic, the Dead Sea, the Black Sea, middle latitude (Ukraine, Abkhazia) and the equatorial zone of South America (Ecuador)) were studied (Table).

Nutrient broth (HiMedia Laboratories Pvt. Ltd., India) with addition of Fe(III) citrate (concentration 0,5 g/l of iron cations) was used for cultivation of heterotrophic microorganisms. Solution of Fe(III) citrate was used as the selective factor for FRB growth.

Characterization of the studied samples

Geographic zone	№ of the sample	Description of the sample
The Antarctic	1	Galindez island, soil
	2	Barrientos island, soil
	3	Deception island, stream sediments
	4	Deception island, freshwater lake sediments
	5	Deception island, moss, soil, lichen
	6	Galindez island, cliff
	7	Rasmussen peninsula, soil, sample № 1
	8	Rasmussen peninsula, soil, sample № 2
The Arctic	9	Svalbard archipelago, moss and soil
	10	Svalbard archipelago, wet soil, surface layer, under the moss
	11	Svalbard archipelago, soil (sample № 1)
	12	Svalbard archipelago, soil (sample № 2)
	13	Svalbard archipelago, soil (sample № 3)
	14	Svalbard archipelago, soil (sample № 4)
The Dead sea	15	Negev desert (Israel), soil
	16	Israel, salt crust
	17	Cliff of the Dead sea (Israel), soil, grass
	18	Israel, stream, upper layer, salted soil
	19	Israel, sludge from the bank
	20	Israel, stream, sulphidogenic black layer
	21	Israel, stream sediments
The Black sea	22	Marine sediments (depth 1705 m)
	23	Marine sediments (depth 2000 m)
	24	Marine sediments (depth 1800 m)
	25	Marine sediments (depth 2000 m)
	26	Marine sediments (depth 2000 m)
	27	Marine sediments (depth 1800 m)
	28	Marine sediments
	29	Pomorie (Bulgaria), sludge
	30	Pomorie (Bulgaria), sludge
Middle latitude	31	Grun river (Sumy region, Ukraine), swamp, bay sludge
	32	Kyiv (Ukraine), humus
	33	Kyiv, garden and park complex of NASU “Feofania” (Ukraine), meadow soil
	34	Kyiv, garden and park complex of NASU “Feofania” (Ukraine), forest soil
	35	The Biosphere Reserve “Askania-Nova” (Kherson region, Ukraine), soil
	36	Kuybyshevskaya cave (Abkhazia), clay
	37	Muskarova Yama cave (Ternopil region, Ukraine), clay
	38	Mlynky cave (Ternopil region, Ukraine), clay
	39	Zolushka cave (Chernivtsi region, Ukraine), clay
The equatorial zone (Ecuador)	40	La Favorita region, mountain jungle (Ecuador), soil
	41	Tungurahua volcano (Ecuador), volcanic soil
	42	Tungurahua volcano (Ecuador), alluvium in the riverbed
	43	Papallacta region, the Andes, 4020 m (Ecuador), soil

The solution of Fe(III) citrate was prepared in two stages. At the first stage 3 g of iron powder was brought into the 100 ml volumetric flask and dissolved in minimal volume of concentrated HCl in the stream of argon. To speed up the process of iron dissolution the flask was heated in the water bath (t = 100 °C). After that solution was evaporated on the open flame of the burner to the condition of the wet crystalline precipitate. The purging of the solution was carried out in continuous argon flow. After the cooling the solution of chelator ($C_6H_6O_7Na_2 \cdot 1,5H_2O$) was added to the flask. The chelator solution was prepared by dissolution of 15 g of Na_2-citrate in 70 ml of distilled water. Obtained acidic solution of Fe(II) citrate was neutralized by adding of 6 g of $NaHCO_3$ in argon stream. As the result neutral solution of Fe(II) citrate was obtained and the volume of the solution was adjusted to 100 ml. The second stage was to oxidize Fe(II) to Fe(III). The solution of Fe(II) citrate was boiled during 30 min and hydrogen peroxide (35%) was added ensuring complete oxidation of Fe(II) to Fe(III). The initial solution was colored in brown due to the formation of Fe(III) citrate. A control reaction with *o*-phenanthroline [10] showed the absence of Fe(II) compounds in the solution. To sterilize the obtained solution of Fe(III) citrate it was tightly closed and boiled in the water bath during 10 min.

Microbiological counts of Fe(III)-reducing bacteria in natural ecosystems was carried out on the liquid nutrient medium by McCready method. [11] For this purpose, we prepared serial tenfold dilutions of the samples (10^{-2}–10^{-9}). Sterile nutrient broth (9 ml) with Fe(III) citrate (0,5 g/l of iron cations) and 1 ml of microbial suspension of the appropriate dilution of the samples were added to the tube (20 ml volume). Each dilution was seeded in triplicate. The tubes were sealed with rubber stoppers. Cultivation of microorganisms was carried out during 7 days at t = 30 °C. Nutrient medium without inoculum was used as sterility control, as well as control of absence of Fe(III) chemical reduction by the components of nutrient medium.

Growth of Fe(III)-reducing microorganisms was determined by the presence of cells by microscopy, the increase of the optical density of culture medium, visual changes of cultivation medium — the films and the gas bubbles formation at the surface, changing its color from brown typical for Fe(III) compounds to light yellow, due to Fe(III) reduction. Moreover, the growth of FRB was evaluated by their ability to reduce Fe(III) to Fe(II). Accumulation of Fe(II) was determined using a colorimetric reaction of Fe(II) with *o*-phenanthroline [10]. For this purpose, 0,15 ml of 0,25% aqueous solution of *o*-phenanthroline was added to the tubes containing culture medium. Its red staining indicated the presence of Fe(II) compounds, i.e. Fe(III) reduction by microorganisms.

The quantity of FRB in samples was calculated using McCready table [11]. Total FRB number was counted per 1 g of absolutely dry sample according to the formula:

$$N = X \cdot K, \qquad (1)$$

where N — number of cells per 1 g of absolutely dry sample;
X — number of cells in 1 g of wet sample, calculated using McCready table;
K — coefficient of moisture of samples.

The coefficient of moisture of samples was determined by the formula:

$$K = \frac{100}{100 - A}, \qquad (2)$$

where A — moisture of samples.

Moisture of samples was determined by gravimetric method by the difference of their weights before and after drying to constant weight at 100 °C. The moisture content was calculated by the formula:

$$A = \frac{B}{(P - B)} \cdot 100\%, \qquad (3)$$

where B — mass of evaporated water;
P — mass of the sample [12].

Statistical analysis of experimental data was performed using Exel, $P \leq 0,05$.

Results and Discussion

To study the quantity of FRB in natural ecosystems serial tenfold dilutions of 43 samples were seeded in selective medium with Fe(III) citrate by McCready method. Microorganisms were cultured under conditions of limited aeration. Such selective conditions are favorable for the development of several physiological groups of FRB. Firstly, aerobic FRB develop in the surface layer of the medium that contacts air. Secondly, conditions for the development of facultative anaerobic bacteria forming inside the medium layer, and for obligatory anaerobic FRB at the bottom. Under this conditions oxygen concentration sharply decreased, and oxygen could not inhibit Fe(III) reduction. Thus, conditions for the quantitative estimation of FRB groups present in samples were created.

Increasing optical density of medium, its color change and positive *o*-phenanthroline reaction indicated by red coloration were the criteria for FRB growth (Fig. 1).

The number of FRB in all studied ecosystems ranged from hundreds to millions of cells per 1 g of absolutely dry sample (Fig. 2).

Each ecosystem has a particular set of conditions that influence microbial communities: climate, temperature, humidity, UV radiation, mineral composition, etc. The Antarctic and the Arctic environments are characterized by low temperatures and intensive ultraviolet radiation. Such extreme factors as high temperatures, lack of moisture, UV radiation and high salinity influence microbial communities of the Dead Sea ecosystem. Microorganisms of the Black Sea depths exist under high pressure, lack of oxygen and lack of mass transfer in the bottom sediments. Ecosystems of middle latitudes are characterized by moderate influence of physical and chemical factors (temperature, UV radiation, salinity, etc.). Studied ecosystem of Ecuador have warm, humid climate, and mountainous areas are characterized by high levels of ultraviolet radiation. In contrast to the above-mentioned regions, caves are unique "closed chambers" isolated from the effects of extreme factors. Caves have a stable temperature, pressure, salt composition, lack of ultraviolet radiation.

It should be noted that the quantity of Fe(III)-reducing bacteria in the samples of the Black Sea, middle latitude and Ecuador geographic zones is 1–2 orders higher than in the samples from the Antarctic, the Arctic and the Dead Sea regions.

Microorganisms of ecosystems of the Antarctic, the Arctic and the Dead Sea are intensively influenced by such extreme factors as ultraviolet radiation, low temperature (the Antarctic, the Arctic), high temperature, lack of moisture, high salinity (the Dead Sea). That is why the quantity of FRB in the samples from these regions, with a few exceptions, do not exceed $n \cdot 10^2$–$n \cdot 10^4$ cells/g of sample.

The number of FRB in samples from middle latitudes (Ukraine) and the equatorial zone (Ecuador) made $n \cdot 10^5$–$n \cdot 10^6$ cells/g of sample. This fact can be explained by the wide variety of microbial communities in the soil of these geographic zones, as well as the moderate climate and influence of physical and chemical factors.

Lack of oxygen in the deep Black Sea sediments may contribute to the high number of FRB that use Fe(III) for anaerobic respiration. Oxygen can inhibit Fe(III) reduction. There is an example of reaction equations, where H_2 acts as an electron donor and O_2 and Fe^{3+} are electron acceptors:

$$4H_2 + O_2 = 2H_2O$$
$$\Delta E_0 = +0{,}814 + 0{,}414 = 1{,}228\ V; \quad (4)$$

$$H_2 + 2Fe^{3+} = 2H^+ + 2Fe^{2+}$$
$$\Delta E_0 = +0{,}771 + 0{,}095 = 0{,}866\ V. \quad (5)$$

Potential difference between the donor and the acceptor of electrons determines the efficiency of microbial reduction of the acceptor. Increase of difference of potentials between donor and acceptor leads to the increase of energy production by microorganisms. The difference of potentials between the acceptor (O_2) and electron donor (H_2) is 1,228 V (pH = 7) (reaction 4). The difference of potentials between Fe^{3+} and H_2 equals 0,866 V (pH = 1,6) (reaction 5). Reaction 5 shows the quantity of ΔE_0 at the pH = 1,6 since at the pH > 1,6 Fe^{3+} is precipitated: $Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3$ [13].

The difference of potentials between hydrogen and oxygen is higher than that between hydrogen and iron. Therefore, oxygen

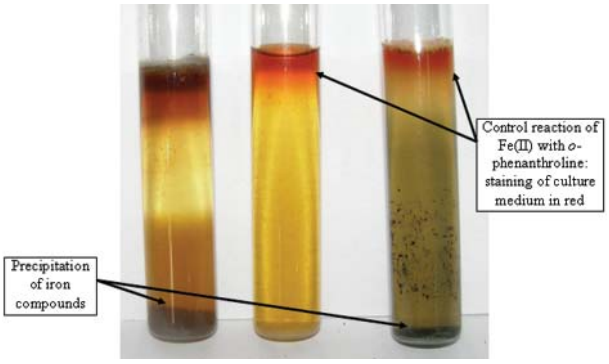

Fig. 1. **Growth of Fe(III)-reducing bacteria in selective medium with Fe(III) citrate**

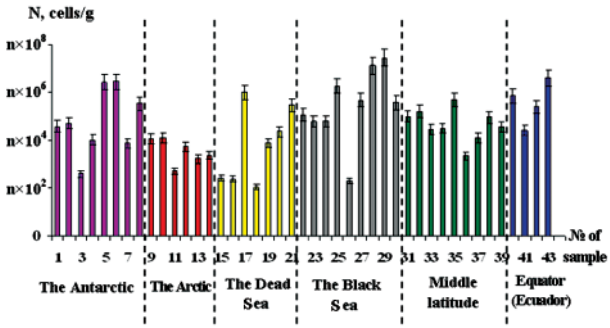

Fig. 2. **The quantity of Fe(III)-reducing bacteria in samples ($P \leq 0{,}05$)**

is an energetically favorable electron acceptor for microorganisms. Oxygen can compete with iron for the electron flow from the substrate and inhibit Fe(III) reduction.

Therefore, the lack of oxygen in the deep sediments of the Black Sea can contribute FRB development. It is confirmed by obtained results. The quantity of FRB in the samples from anaerobic zones of the Black Sea is $n\cdot10^4$–$n\cdot10^7$ cells/g. It is 1-2 orders higher than their number in other studied environments.

There are few references concerning the FRB quantity in some ecosystems in literature. For example, Slobodkin showed that the number of thermophilic FRB in the black smokers of the Pacific Ocean was $n\cdot10^7$ cells/ml [1]. The work of Weiss demonstrated that FRB quantity in soils of Virginia was about $n\cdot10^6$–$n\cdot10^7$ cells/g [9]. Shekhovtsova showed that the number of FRB in groundwater of Finland and Sweden did not exceed $n\cdot10^4$ cells/ml, and in the Ural ultradeep wells it was $n\cdot10^2$ cells/ml [7]. According Kostka the quantity of FRB in soils of rice paddies was $n\cdot10^4$–$n\cdot10^6$ cells/g [8].

We conducted systemic study of Fe(III)-reducing bacteria quantity of both hemispheres in ecosystems of six geographic zones (the Antarctic, the Arctic, the Dead and the Black Sea, middle latitudes — Ukraine, Abkhazia, the equatorial zone — Ecuador). These ecosystems fundamentally differ in the location and complex of extreme factors influencing microorganisms. The study complements and significantly expands the knowledge of distribution and amount of FRB in ecosystems. It is shown that Fe(III)-reducing bacteria are an integral part of natural ecosystems, where their quantity is $n\cdot10^2$–$n\cdot10^7$ cells/g of sample.

It is demonstrated that a particular set of conditions of natural ecosystems affects the number of FRB. The number of FRB is the lowest ($n\cdot10^2$–$n\cdot10^4$ cells/g) in the Antarctic, the Arctic and the Dead Sea ecosystems, since the influence of extreme factors (temperature, UV radiation, etc.) is the highest. The number of FRB makes $n\cdot10^5$–$n\cdot10^6$ cells/g in the ecosystems of middle latitudes and the equatorial zone where are the most diverse microbial communities, and the impact of extreme factors is moderate. The highest number of FRB (up to $n\cdot10^7$ cells/g) was found in samples of bottom sediments of the Black Sea, where the lack of oxygen may contribute to FRB growth.

The results obtained indicate the potential of Fe(III)-reducing bacteria to influence the biogeochemical cycles of iron and carbon transformation and energy flows in natural ecosystems. High concentrations of iron compounds in soil and water, along with the widespread and high quantity of FRB in natural ecosystems should contribute to the rapid and efficient reduction of Fe(III) compounds.

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ЗАКОНОМІРНОСТІ КІЛЬКІСНОГО РОЗПОДІЛЕННЯ ЗАЛІЗОВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ У ПРИРОДНИХ ЕКОСИСТЕМАХ

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Метою роботи був кількісний облік залізовідновлювальних бактерій у природних екосистемах Антарктики, Арктики, Мертвого і Чорного морів, середніх широт (Україна, Абхазія) та екваторіальної зони (Еквадор). Використано метод кількісного обліку мікроорганізмів за Мак-Креді, колориметричний метод визначення сполук Fe(II) у культуральній рідині. Вперше проведено системне дослідження чисельності залізовідновлювальних бактерій обох півкуль в екосистемах шести географічних областей. Експериментально показано високу чисельність залізовідновлювальних бактерій у природних екосистемах. Їх кількість становила від $1{,}1\cdot10^2$ до $2{,}8\cdot10^7$ кл/г абсолютно сухого зразка. Подані результати свідчать, що залізовідновлювальні бактерії є невід'ємною складовою природних екосистем і можуть суттєво впливати на біогеохімічні цикли трансформації сполук заліза та вуглецю.

Ключові слова: чисельність залізовідновлювальних бактерій, природні екосистеми, біогеохімічні цикли заліза.

ЗАКОНОМЕРНОСТИ КОЛИЧЕСТВЕННОГО РАСПРЕДЕЛЕНИЯ ЖЕЛЕЗОВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ В ПРИРОДНЫХ ЭКОСИСТЕМАХ

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Целью работы был количественный учет железовосстанавливающих бактерий в природных экосистемах Антарктики, Арктики, Мертвого и Черного морей, средних широт (Украина, Абхазия) и экваториальной зоны (Эквадор). Использованы метод количественного учета микроорганизмов по Мак-Креди, колориметрический метод определения соединений Fe(II) в культуральной жидкости. Впервые проведено системное исследование численности железовосстанавливающих бактерий обоих полушарий в экосистемах шести географических областей. Экспериментально показана высокая численность железовосстанавливающих бактерий в природных экосистемах. Их количество составило от $1{,}1\cdot10^2$ до $2{,}8\cdot10^7$ кл/г абсолютно сухого образца. Представленные результаты свидетельствуют о том, что железовосстанавливающие бактерии являются неотъемлемой составляющей природных экосистем и могут существенно влиять на биогеохимические циклы трансформации соединений железа и углерода.

Ключевые слова: численность железовосстанавливающих бактерий, природные экосистемы, биогеохимические циклы железа.