BIOLOGICAL OXIDATION OF IRON IN FLOODED SOILS

A.A. VANKOVA

(Department of microbiology and immunology of Russian State Agrarian University - MTAA named after K A. Timiryazev)

Abstract. New strains of iron-oxidizing bacteria have been both isolated from paddy soils and identified. Bacterial iron oxidation intensity and influence on redox state of the environment were studied. It has been established the ability of bacteria to form iron-organic complexes, increasing mobility and availability of nutrients in plants.

Key words: soil, microorganisms, iron, trausformation.

Introduction

The problem of transforming elements with changing valence, in metals particular, is of great importance for understanding of geochemical regularities of its migration in the crust, soil and water, elaboration of environmental protection and use biotechnologies. The system Fe^{2+} — Fe^{3+} is considered to be the main redox one in paddy soils. Due to constant alternation of oxidizing and reducing conditions the transformation of iron is carried out quickly and related directly or indirectly to changing of elementary soil processes orientation that determine paddy fields evolution. Numerous studies of domestic and foreign scientists found the mobilization of iron in rice soils, the change of its mobility and availability to plants are the result of microbial activities [2,4, 6, 12, 17, 19, 20]. The investigation into new iron-oxidizing bacteria strains and their role in transformation of iron in the soil are provided in this article.

Materials and methods

Iron-oxidizing bacteria are isolated from meadow black rice soil in Krasnodar region by plating using agar medium, prepared on plant extract base with iron (II) oxalate [18]. Colonies of iron oxidizing bacteria are defined on their bright blue colour as a result of colour reaction Fe^{3+} with K_4 [Fe $(CN)_6$] under acid conditions. Isolation of pure cultures has been carried out with the aid of microscope by transferring entire colonies by means of capillar. Major cultural and morphological characteristics of bacteria strains isolated are described, using the methods of both light and electron microscopy. Iron-oxidizing activity of bacteria is defined by residual quantity of Fe^{2+} in the liquid medium in 20 days after inoculation colorimetricly with a-a-dipyridyl, pH and Eh (redox potential) — potentio-

* Author for correspondence: Anna A. Vankova, Ph.D., Associate Professor. E-mail: mbiol@timacad.ru

metrically according to standard techniques, complex formation ability — by changing of effective solubility of sparingly soluble iron hydroxide precipitate at various concentrations of bacteria — 10^3 and 10^5 cells / ml. Control — without inoculation. The iron content in solution has been determined in 7 days by atomic absorption spectrophotometer Perkin Elmer — 503 (Japan). To prepare samples for infrared (IR) spectroscopy the strains were grown on the solid medium with different concentrations of iron (II) citrate — 10 and 100 mg /1. In 10 days biomass was washed away from the medium surface and after agar removal by centrifugation dried at t 90 C° to constant weight. Samples for IR spectroscopy were obtained by pressing tablets from dry bacteria biomass with KBr.

Results and discussion

As a result of successive passages with subsequent microscopy and colour reaction, 8 strains of iron-oxidizing bacteria have been isolated and their morphological, cultural and physiological features have been studied. Pure cultures isolated form a characteristic dotted diphasic colonies reminding of "fried egg" (darker convex granular central area and a flat translucent peripheric zone), which differ in color and size in different strains. Transmission electron microscopy revealed cells deprived of cell walls and, obviously, extremely polymorphic because of that, varying in shape from oval and more or less correct form rods to very curved, fabiform ones and filaments of different length (fig. 1). The analysis showed that their diameters ranged from 0.3 to 1.2jxm and their lengths varied from 0.9 to 3.Оцт. Bacterial cells are encapsulated, a thickness of a capsule is up to 0.5цт. The cells are surrounded by three-layer membrane, which is clearly visible. Inclusions of 2 types were found: electron-light ones placed in the peripheric parts of cytoplasm with diameter of 0.1 цт and very electron-dense, randomly located with diameter up to 0.25 цт. In intercellular space traces of organic matter fixation, probably capsular material or slime, are observed.

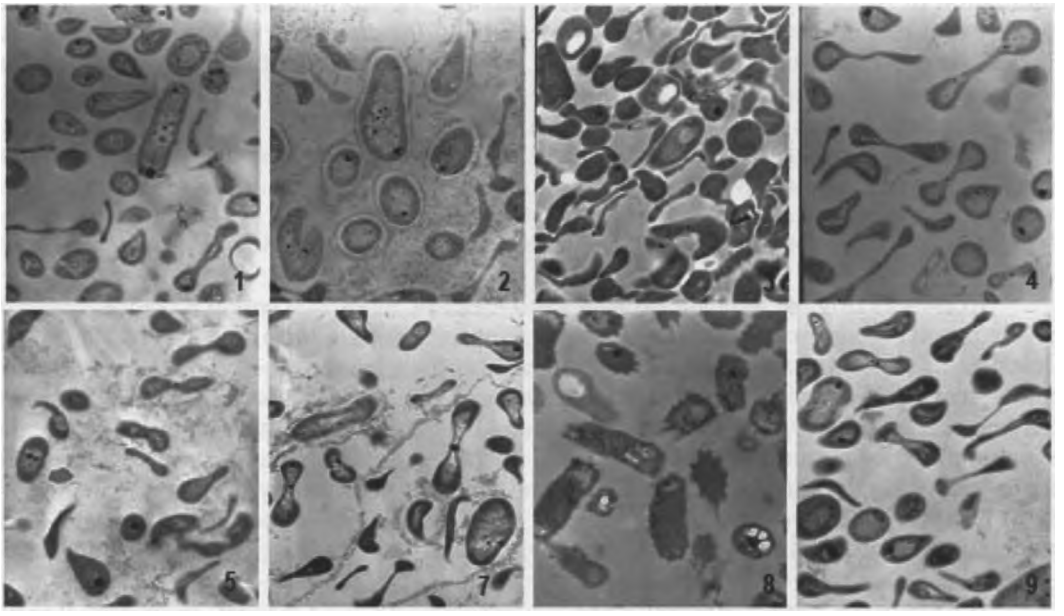

Fig. 1. Morphology of iron-oxidizing bacteria (1-5, 7-9 strains), isolated from rice soil, x18000

The strains isolated use the circumscribed number of organic compounds. Most of them are not capable of growth on rich organic mediums, but do it best on poor ones, such as potato agar with formation of pink pigment, Tyler medium and "hungry" agar. All cultures reveal the high requirement for moisture, peroxidative activity, aerobes (microaerophils), mesophils.

Found morphological and physiological characteristics of pure cultures testified significant similarities of these organisms with mycoplasmas. On the base of identification carried out [3], they are referred to as mycoplasmalike bacteria g.*Siderococcus.*

All strains studied are capable of oxidation of iron, which sediments on cells surface in capsules. Bacterial iron oxidation intensity, the influence on pH and Eh and the ability to form complexes have been studied.

The analysis of Fe^{2+} in the medium in 20 days after inoculation shows that the oxidation of iron occurs with varying intensity (table). The culture 9 is the most active — for a specified space of time, it oxidizes about 8 mg / 1 Fe^{2+}. The oxidation process takes place without virtually changing pH and Eh in the medium. Integral characteristic of redox processes intensity in environment with different meanings of pH and Eh is considered the oxidation degree of system — rH_2 [11]. Calculation of this index shows that the environment becomes more oxidized during cultivation of strains 1, 2, 7-9 in it.

Effect of iron bacteria on the Fe^{2}* content, pH and Eh in the medium

Variants	The content of Fe^{2+} in the medium		pH	Eh, mV	rH_2
	mg/l	% to control			
Control	16.50		7.97	279	25.2
1	12.83	78	7.96	305	26.1
2	19.33	117	8.38	287	26.3
3	11.33	69	7.78	278	24.8
4	14.00	85	8.16	265	25.2
5	10.58	64	7.90	267	24.7
7	15.83	96	8.46	266	25.8
8	10.41	63	8.40	267	25.7
9	8.66	53	8.53	265	25.9
LSD*	3.02		0.41	17.66	

* The least statistical difference.

To assess the activity of iron bacteria in the soil it is necessary to know mechanisms of their interaction with the substrate. Influence of iron bacteria on the state of soil iron compounds is caused by secretion of their specific metabolites into environment. On the basis of studying of both morphology and physiology of iron bacteria isolates, it is possible to offer some variants of their interaction with substrate, which results in oxidation and sedimentation of iron on the cell surface of bacteria.

Most isolates have peroxidative activity, so the sedimentation of iron oxides on the surface of cells can be result of reaction of peroxide, the final or intermediate product of organic substances oxidation, with iron ions in coupled oxidation reaction with peroxidase participation [5]. Heterotrophic nutrition type of microorganisms isolated allows to suppose their ability to decompose iron-organic compounds with subsequent use of organic part in metabolic processes and sedimentation of iron oxides in capsules or on cell surface [2].

In presence of cell surface compounds with a negative charge: mucopolysaccharides, polysaccharides and phospholipids, physical and chemical adsorption of iron is not ruled out [5]. So far as most of the strains studied have a mucous capsule, the presence on their cell surfaces of organic compounds mentioned above is rather possible. Besides, sedimentation of iron in the form of hydroxide on the cells is possible in connection with a local increase of a redox potential or pH, due to bacteria secretion of alkaline metabolites, temporary surplus of ions (Mn^{4+}, $S0_4^{2-}$, $N0_3^-$, etc.) or molecular oxygen. Highly dynamic redox state of the bacterial cell surfaces is quite possible, in view of the absence at the majority of strains of true cell walls and specific conditions of their habitats. As for interrelation of ion density and Eh, they were considered by us [15] for the root cover of plants growing under anaerobic conditions and soil system [16].

One of the mechanisms of iron accumulation by bacteria studied can be complex formation, which reduces allocation of specific chelating compounds to the environment — specific "carriers" of iron to the cell [14]. Complex formation ability of isolated strains is especially probable that iron oxidation occurs without change of redox potential and acidity.

Finally, the processes considered above, lead to migration of iron from environment to bacterial cell as "sedimentation center" and its local accumulation. It is quite possible simultaneous existence of several mechanisms of iron sedimentation or accumulation at one organism. Prevalence of one or combined functioning of several mechanisms will depend on both specific environmental conditions and physiological characteristics of bacterial species.

Application of IR spectroscopy method for the analysis of biomass of pure cultures bacteria grown with different concentrations of iron (II) in nutrient media, allows to judge, to some extent, a possible mechanism of iron binding by bacterial cell. The IR spectrum, in most cases, serves as a "fingerprint" of a molecule. Absorption in the IR area is characteristic for individual atomic groups, and the absorption intensity is directly proportional to their concentration. Thus, measuring absorption intensity it is possible to quantify any component in the sample investigated. Comparing characteristic IR spectrum peaks of one strain biomass grown at concentrations 10 and 100 mg / 1 iron (II) citrate in media, it is possible to assume, what atomic groups include iron at absorption by cell. It should be noted that interpretation of IR spectrums of bacterial biomass is difficult because it is multicomponent. However, following the order of viewing range of wavelengths [8], one can identify the main functional groups roughly. Quantifying of functional groups on IR spectrum of dry biomass of five strains iron-oxidizing bacteria was conducted by the ratio of characteristic peak intensities, which serves as reliable criterion of changes occurring in a microbial cell at absorption of iron.

The analysis of IR spectrums of iron-oxidizing bacteria pure cultures biomass showed that at absorption of iron by microbial cells the part of free carboxylic, alcoholic and phenolic functional groups is reduced, that testifies their possible blockading by metal cation and suggests the ability of the studied strains to a complex formation (fig. 2).

To test the hypothesis about the possibility of iron bacteria cells or their metabolites react to iron in helatization reactions, complex formation ability of bacteria on the effective solubility of $Fe(OH)_3$ at initial concentration of 10^3 and 10^5 bacterial cells/ ml was evaluated in model experiment. The results show that the iron-oxidizing bacteria naturally alkalize solution, and the increasing of pH and titer bacteria introduced correlated for all strains. Redox potential growth in medium is noted at inoculation of the majority of studied strains. For strains 3 and 8 it is observed already at concentration of 10^3 cells / ml, for

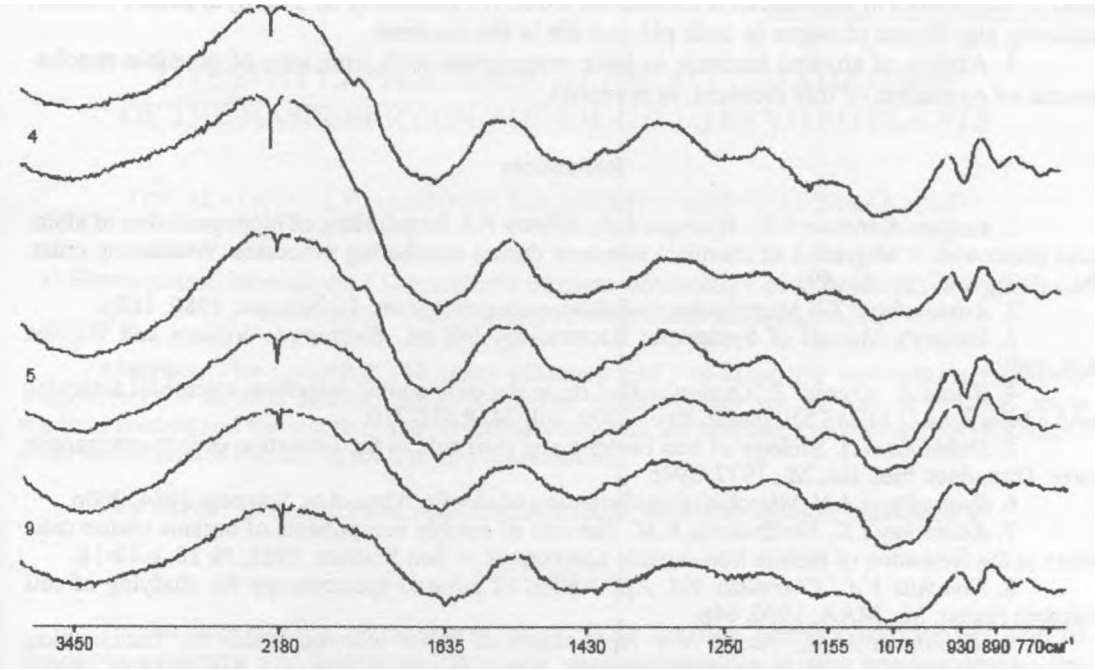

Fig. 2. IR-spectrums of 4, 5, 9 strains biomass, grown at concentrations 10mg/l (upper curve) and 100mg/i (lower curve) iron (II) citrate in medium

strains 2, 4—7, 9 — at higher titer of microbial cells. For the strain of 1 Eh changes in the medium are not registered.

The increase in effective solubility of iron hydroxide in solution is observed in the presence of strains 1, 2, 5 at initial concentration of 10^3 cells / ml and for strains 1 and 4 at concentration of 10^5 cells / ml. Strains 3 and 7 display only a tendency for increasing soluble iron content in the medium. Strains 8 and 9 do not affect solubility of iron hydroxide. Calculation of the complex formation ability [15] of iron-oxidizing bacteria towards iron has shown that strains 1, 2, 4, 5 possess this ability.

Ability of bacteria to form complexes with iron facilitates the mobilization of this element from almost insoluble natural compounds. It is known that as a part of complex and chelate (chelated) compounds iron loses properties characteristic for cation: formation of almost insoluble phosphates, sedimentation in the form of hydroxide, etc. [1, 7, 9, 10, 13]. Due to good solubility, stability and high mobility in a wide range of pH iron organic complexes are of great importance in weathering, migration of elements in soil profile and supply of plants microelements and multivalent cations.

Conclusions

1. New strains of iron-oxidizing microorganisms are isolated from meadow black rice soil and identified as mycoplasmalike bacteria of the genus *Siderococcus.*

2. The strains studied are capable of oxidation of iron, which sediments on cells surface in capsules. The oxidation of iron occurs with varying intensity. It has been established

that pure cultures of iron bacteria exceed the effective solubility of iron hydroxide, without causing significant changes in both **pH** and Eh in the medium.

3. Ability of studied bacteria to form complexes with iron, one of possible mechanisms of oxidation of this element, is revealed.

References

1. *Antipov-Karataev I.N., Tsyurupa I.G., Alferov V.A.* Regularities of biodegradation of albite and muscovite. // Migration of chemical elements during weathering processes. Weathering crust. M.: 1966, vol.7, pp. 53-89.
2. *Aristovskaya T. V* Microbiology of soil-forming processes. L.: Science, 1980, 188p.
3. Bergey's Manual of Systematic Bacteriology 9th ed., Baltimore, William and Wilkins Co, 1989.
4. *Brune A., Frenzel P., Cypionka H.* Life at the oxic-anoxic interface: microbial activities and adaptations / / FEMS Microbiol. Rev., 2000, vol. 24, P.691-710.
5. *Dubinina G.A.* Biology of iron bacteria and their role in the formation of iron-manganese ores. Diss. doct. biol. Sci. M., 1977, 394p.
6. *Ilyaletdinov A.N.* Microbial transformation of metals. Alma-Ata: Science, 1984, 268p.
7. *Kaurichev I.S., Nozdrunova E.M.* The role of soluble components of organic matter residues in the formation of mobile iron-organic compounds. // Soil Science, 1961, № 10, p. 10-18.
8. *Konchits V.A., Chernikov V.A.* Application of infrared spectroscopy for studying of soil organic matter. M.: MAA, 1990, 64p.
9. *Kononova M.M., Titova N.A.* Application of paper electrophoresis for fractionation of humic substances and study their complex compounds with iron. / / Soil Science, 1961, № 11, p.81-88.
10. *Moore P.A., Patrick tV.H.* Iron availability and uptake by rice in acid and sulfate soils. / / Soil. Sci. Amer. J,, 1989, vol.53, № 2, p.471-476.
11. *Orlov D.S.* Soil chemistry. M.: MSU, 1985, 376p.
12. *Pinevich A. V* Microbiology of iron and manganese. SPb.: SPbSU, 2005, 373p.
13. *Plehanova I.O.* Mobilization of iron and manganese bacteria in rice soils. Diss. cand. biol. sci. M., 1986, 24p.
14. *Ratledge C., Patel P.* Lipide-soluble, iron binding compounds in *Nocardia* and related organisms. / / The biology of Nocardia, London, 1976.
15. *Savich VI., Trubitsina E. V, Muradi N.M. II* Complexformating ability of soil solution and soil organic matter. Appl. TAA, 1988, vol. 1, p.73-80.
16. *Savich V.I., Vankova A.A., Gushin VP II* Electrochemical and concentration fields in soil and their regulation. Appl. TAA, 1989, vol. 2, p.63-71.
17. *Sidorenko O.D., Sidibe G., Savich VI.* Effect of rice monoculture on biological activity of meadow black soil. //Appl. Acad. Sci. ser. biol., 1990, № 3, p.470-473.
18. *Vankova A.A.* State and biogenic transformation of iron compounds in the meadow black soil of rice fields. Diss. cand. biol. sci. M., 1991, 150p.
19. *Zaitseva T.F., Tabakov S.I.* The dynamics and mechanisms of mobilization of iron in the irrigated black soils. // Soil fertility and problems of irrigated agriculture. Barnaul: 1989, p.64-72.
20. *Zavarzin G.A.* Lectures on natural sciences of microbiology. M.: Science, 2003, 348p.

Аннотация. Из затопленных почв рисовников выделены и идентифицированы новые штаммы железоокисляющих бактерий. Изучена интенсивность окисления железа и влияние этого процесса на окислительно-восстановительное состояние среды. Установлена способность бактерий к образованию железоорганических комплексов, повышающих подвижность и доступность растениям элементов питания.