Review Paper: Iron-Reducing Bacteria and Iron Nanostructures

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ABSTRACT

Iron Reducing Bacteria (IRB) are one of the most applicable microorganisms in various industrial and environmental activities. These bacteria play a main role in the natural iron transformation. They act in a reverse metabolic pathway in contrast to iron oxidizing bacteria. In the anaerobic conditions IRB are capable to use ferric ion as the final electron acceptor and reduce Fe^{3+} to Fe^{2+}. What makes these bacteria interesting in bionanotechnology is that IRB are able to synthesize iron nanostructures. In this mini review we have a quick look on the diversity, metabolism, and cultivation of IRB. Finally, we discuss iron nano structures which biosynthesized by IRB.

1. Introduction

Iron reducing bacteria (IRB) are among the most important groups of bacteria that are presented in every environment. These are a group of bacteria which act in reverse direction in contrast to iron oxidizing bacteria. Iron oxidizing bacteria transform ferrous ions to ferric while IRB convert this reaction and reduce ferric ions to ferrous (Figure 1). Starkey and Halvorson were among the very first people who introduced the concept of IRB in 1927. They highlighted the importance of microorganisms in the natural transformation of iron from solutes to precipitates and vice versa [1]. Iron reducing metabolic pathway is perform under anaerobic and or micro aerobic conditions. Meanwhile, microbial oxidization of iron can be done in both aerobic and anaerobic environments [2, 3].

To date, more than 71 facultative IRB have been identified and they are present in different morphologies from cocci to comma and rod shapes [4]. From gram staining point of view IRB are not restricted in one category and both gram-positive and gram-negative bacteria can be found among them [5-10]. This group of bacteria is now

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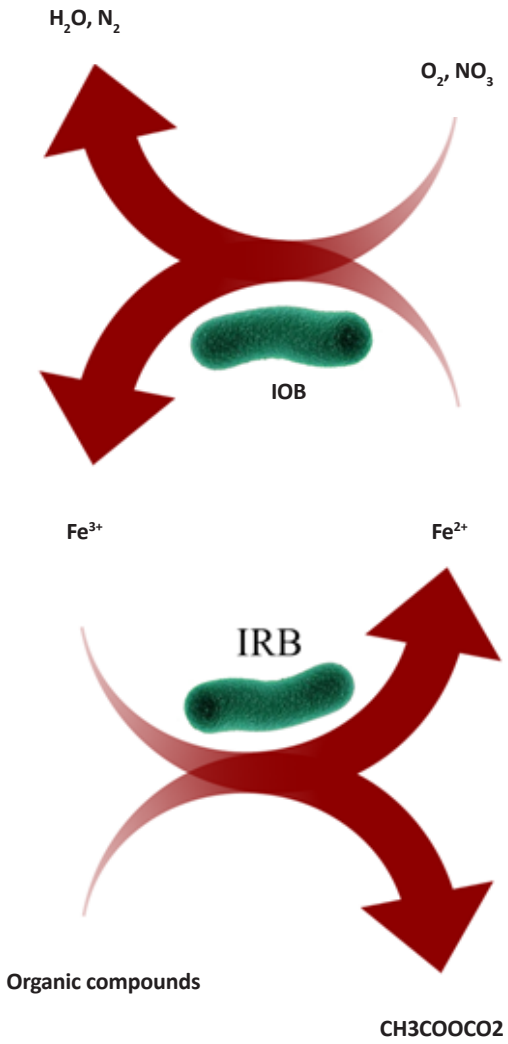

Figure 1. The role of Iron Oxidizing Bacteria (IOB) and Iron Reducing Bacteria (IRB) in natural iron transformation [3]

so divergent and belong to gamma and delta Proteobacteria including verity of genus and species such as Stenotrophomonas maltophilia, Brachymonas denitrificans, Paracolobacterum aerogenoides, Serratia marcescens, Aerobacter aerogenes, Citrobacter freundii, Bacillus circuhzns, Bacillus polymyxa, Bacillus alvei, Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bacillus cereus, Bacillus sphaericus, Pseudomonas aeruginosa, Pseudomonas denitrificans, Pseudomonas stutzeri, Pseudomonas fluorescens, and Pseudomonas putida [11-13].

These bacteria have applications in many environmental processes for pollutant removal such as mercury methylation, uranium and phosphate removal and mineralization of organic carbon in anoxia conditions [14, 15]. On the other hand iron oxidizing bacteria are classified as aerobic and anaerobic bacteria. The aerobic group classified as acidophilic and neutrophilic. Anaerobic group of iron oxidizing bacteria are neutrophilic (nitrate-dependent) and photosynthetic bacteria [2].

2. Metabolism of IRB

In anaerobic environments IRB reduce ferric ions as final electron acceptor for anaerobic decomposition of organic compounds such as fumarate, formate, succinate, acetate, pyruvate, propionate, succinate, malate, propanol and ethanol [6, 11]. Consumption of organic compounds and production of CO_2 are the key parameters for metabolic assays [16]. Some IRB such as Shewanella putrefaciens, Shewanella algae, and Pseudomonas spp. have the capacity to use a wide variety of electron acceptors such as oxygen. However, by using ferric ions as the final electron acceptor, their ability to use organic electron donors extremely reduces. In this condition organic compounds such as lactate and pyruvate oxidize to acetate. For instance, Geospirillum barnesii, is capable to used ferric ions as the electron acceptor and grow by oxidation of hydrogen or incomplete oxidation of lactate to acetate [17].

IRB can divert carbon and electron flow away from methanogenic food chain in the presence of ferric ions. In fact, addition of synthetic amorphous ferric oxyhydroxide to methanogenic sediments results in about 50 to 90% reduction in methane production. The decrease in electron flow to methane production was completely compensated by increase in electron flow toward ferric ion reduction. Ferric is not toxic to methanogenic bacteria, as this ion does not affect the methane production from hydrogen and acetate when these substrates are in excess [18].

3. Cultivation of IRB

Different genus and species of IRB also possesses different growth rates. Some of them grew faster and produce colonies with about 5 mm diameter after 14 day of cultivation. Whereas, some others are produce colonies with less than 1 mm diameter after the same period of incubation [11]. Iron minerals which are used as electron acceptor by IRB can give color to bacterial cells, for instance using fumarate as the electron acceptor will give a red color to cells [6]. Klebsiella oxytoca cells which grown on the ferric citrate agar media produce colonies with a metallic shine (Figure 2) [19]. However, it is not universal rule and the red color of some strains of IRB such as Geobacter bremensis and Geobacter pelophilus is due to the presence of c-type cytochromes [6].

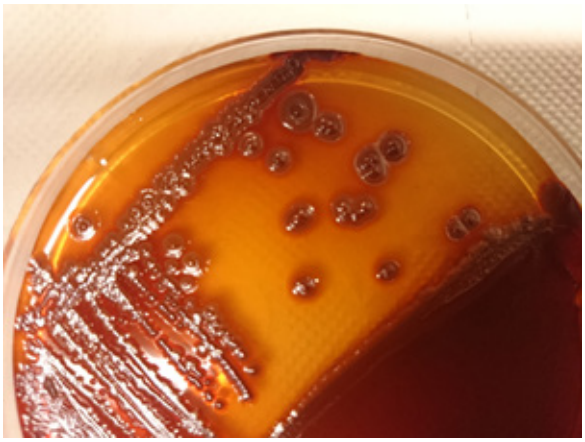

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Figure 2. Colonies of Klebsiella oxytoca with metallic shine on the ferric citrate agar media [19]

Different culture media were used for cultivation of IRB and ingredients of some of these media are presented in Table 1. All of these media are used for anaerobic cultivation of IRB while having bicarbonate as the buffering agent (i.e. 30 mM, pH 7.0). The media are usually enriched with vitamins and trace elements [19-21]. For instance, some researchers have used a vitamin and mineral mixture enriched media for cultivation of Klebsiella oxytoca. The media was supplemented with 50 mM of ferric citrate which provides ferric ions as electron acceptor and also citrate as organic substrate for fermentation [10,19]. The bacteria consume citrate as energy and carbon sources in anaerobic culture condition [8-10]. The cultivation is usually performed in the dark or under dim light at room temperature and under protected atmosphere against oxygen. Under aerobic conditions, bacterial growth depends on appropriate transport system and a functional tricarboxylic acid cycle [10, 22].

4. Iron Reducing Bacteria and Iron Nanoparticles

A significant fraction of iron minerals in the geological subsurface is supposed to be presented as nano-sized colloids including iron oxides (hematite, magnetite) iron oxyhydroxides (goethite, akaganeite, lepidocrocite, feroxyhyte) and iron hydrous oxides (ferrihydrite, hydrohematite maghemite). Due to their wide occurrence, tendency to nucleate and grow on the surfaces of other phases, important redox capabilities, and relatively high reactivity iron nanostructures have important roles in biogeochemical processes [23]. On the other hand iron nanostructures have gained wide applications in various sciences and particularly in biomedical sciences. These nanostructures are now used in Magnetic Resonance Imaging (MRI), magnetic transfection, hyperthermia, DNA and cell labelling, tissue engineering, and targeted drug delivery [24-34]. Biosynthesized nanostructures are more interesting for these biomedical applications due to high biocompatibility and high physicochemical stability [35-42].

As the most abundant crystal transition metal (representing 6% of the chemical composition of the Earth's crust), Fe is commonly leached from minerals by both inorganic weathering processes and biological activity, resulting in the concentrated FeOX phases found in nearly all surficial soils and sediments [43]. IRB can couple the reduction of ferri-hydrite, goethite and other FeOX phases to the oxidation of organic carbon. All such nano-particulate phases are expected to have different properties from bulk crystallites. Nanocrystals have large surface to volume ratio which results in large reactive surface areas for biochemical reactions and hence increase the bioavailability of iron compounds. The surface structure of

Table 1. Ingredients of various media for cultivation of iron reducing bacteria

Ingredients Per Liter of Media	Reference
$NaHCO_3$, 2.5 g; $CaCl_2.2H_2O$, 0.1 g; KCl, 0.1 g; NH_4Cl, 1.5 g; $NaH_2PO_4.H_2O$, 0.6 g; NaCl, 0.1 g; $MgCl_2.6H_2O$, 0.1 g; $MgSO_4.7H_2O$, 0.1 g; $MnCl_2.4H_2O$, 0.005 g; $NaMoO_4.2H_2O$, 0.001 g; $NaCH_3COO$, 2.7 g; yeast extract, 0.05 g; Fe(III) in the form of amorphic Fe(III) oxide at ca. 250 mM of ferric ion	[16]
$NaHCO_3$, 30 mM, NH_4Cl, 28 mM, $NaH_2PO.H_2O$, 4.4 mM, NaCl, 1.7 mM; KCl, 1.3 mM; $CaCl_2.2H_2O$, 0.68 mM, $MgCl_2.6H_2O$, 0.49 mM; $MgSO_4.7H_2O$, 0.41 mM; $MnCl_2.4H_2O$, 0.025 mM; $Na_2MoO_4.2H_2O$, 0.004 mM; pH adjusted to 7.0	[20]
Glucose-asparagine broth; glucose, 20 g; asparagine, 5.0 g; K_2HPO_4, 3 g; KH_2PO_4, 0.8 g; KCl, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; yeast extract, 0.5 g; Fe_2O_3, (reagent grade, powdered), 1 g; pH adjusted to 7.0	[13]
KCl, 0.1 g; NaH_2PO4, 1 g; NH_4Cl, 1.5 g; $NaHCO_3$, 2.5 g; ferric citrate, 0.5 mM; mineral solution, 10 ml; vitamin solution, 10 ml	[19]
$NaHCO_3$, 2.5 g, NH_4Cl, 1.5 g, NaH_2PO_4, 0.6 g, KCl, 0.1 g, ferric citrate, 50 mM; vitamin mixture, 10 mL; mineral mixture, 10 mL	[10]

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iron nanoparticles differs from those of larger crystallites which leads to altered reactivity or varied crystal chemistry [44]. For instance, the size-dependent bioavailability of hematite (α-Fe_2O_3) nanoparticles to obligate aerobic Pseudomonas mendocina bacteria was examined by using the natural siderophore-producing wild type strain and a siderophore mutant strain. Results demonstrated that Fe from hematite with nano size scale appears to be considerably more bioavailable than Fe associated with larger particles. This increased bioavailability is related to the total available particle surface area, and depends in part on greater accessibility of the Fe to the chelating siderophores [45].

It has been shown that siderophore bacteria readily acquire Fe from particles with less than 10 nm in diameter. The bacteria neither produce a diffusible Fe-mobilizing agent nor accumulate a reservoir of dissolved Fe in supernatant solutions. Hence, bacterial cells must be in direct physical proximity to the nanominerals. One possible pathway for microbial Fe acquisition from iron nanostructures is that ultra-small particles with diameters less than 10 nm appear to be capable to penetrate the bacterial cell wall. In addition, other cell-surface-associated molecules and processes such as a cell-wall associated reducing capability could also be important in this regards [45]. Even the size of iron nanoparticles has a significant effect on the Fe bioavailability. In an experiment the rates of iron reduction by Geobacter sulfurreducens was measured in the presence of hematite nanoparticles with various sizes (i.e. 10, 30, and 50 nm). The mass-normalized reduction rates of particles with 10 and 30 nm diameters were comparable to each other and higher than the rate for the 50 nm particles [46].

IRB are also capable to convert iron ions to iron nanoparticles. Klebsiella oxytoca is one of these bacteria which produce a secretory exopolysaccharide. The polysaccharide is attached to the bacterial cell surface and composed of galactose, glucuronic acid, and rhamnose that display metal-binding properties [9, 47]. As mentioned above, in anaerobic environments, Klebsiella oxytoca ferments citrate to CO_2 and acetic acid joined with reduction of ferric ions to ferrous. The secretory polysaccharide has a capability to entrap ferric ions and forms polysaccharide-iron hydrogel. Transmission electron microscopy analysis shows that the complex of iron ions with the exopolysaccharide can form iron nanostructures known as Fe (III)-exopolysaccharide (Fe-EPS). In fact, the Fe-EPS is composed of ultra-small (about 1.8 nm) iron nanoparticles which are entrapped in the bacterial exopolysaccharide. These nanoparticles are noncrystalline (amorphous) and have some very slight

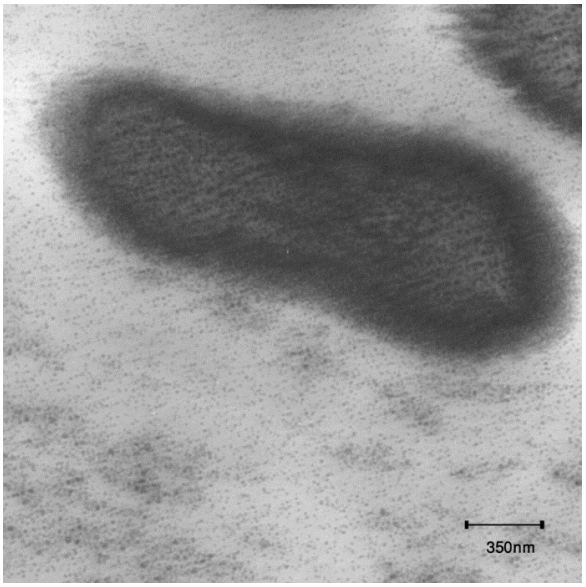

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Figure 3. TEM micrograph of a Klebsiella oxytoca cell that surrounded by INPs shell with high electron density [19]

magnetic properties. This nanocomposite of exopolysaccharide and iron nanoparticles usually forms a shell with high electron density around the bacterial cells. This shell can be seen as a dark hollow in the transmission electron micrographs (Figure 3) [19].

4. Extraction of Iron Nanostructures

From biotechnological and bio-industrial point of view, extra cellular production of a microbial product is a major advantage over an intracellular product. Extra cellular production significantly reduces the cost and steps of downstream processing. On the other hand, cell lysis is one of inevitable steps in the extraction of almost all intracellular products. In biotechnological industries the cells are your micro factory. It is not interesting that you have to destroy your factory to obtain the product, while cells with extracellular products can use for production in various batches. In contrast to magneto tactic bacteria which produce iron nanoparticles as an array of intracellular magnetosomes IRB have overcome all these disadvantages. A simple protocol have been developed for extraction of iron nanostructures which produced by IRB [19]. Short and mild sonication have any significant impact on the bacterial cells [39]. Extracellular iron nanostructures which produced by IRB are loosely attached to the bacterial cell surface and so can be detached with a short and mild sonication. In the next step bacterial cells can be separated from nanostructures simply by low speed centrifugation [19].

5. Conclusion

IRB are so divergent microorganisms with different microbial physiologies. These bacteria are capable to convert iron ions to INPs. The advantage of nanoparticles which produce by IRB is that the particles are protected with a biologic material such as bacterial exopolysaccharides. This protection significantly improves the physicochemical and biological properties of INPs. So, IRB can be introduced as a biologic reactor for sustainable production of INPs in future.

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Conflict of Interest

The authors declared no conflict of interests.

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