

Geographical Distribution of Acidophilic Iron-Oxidizing Bacteria

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Abstract

Acidophilic iron-oxidizing bacteria are widely distributed in aquatic environments. To assess the correlation between their evolutionary relatedness and geographic distribution, we analyzed 267 16S rDNA sequences of obtained from the NCBI database and constructed phylogenetic trees based on these sequences. After eliminating redundant sequences by grouping sequences with identity more than 97% into a single one, we analyzed in detail total 83 16S rDNA sequences, including 58 from mine acidophilic iron-oxidizing bacteria and 25 from hot springs sequences, and belonging to bacteria. Phylogeny analysis based on these sequences suggested that the geographical distribution of acidophilic iron-oxidizing bacteria have certain regional distribution character. The acidophilic IOB derived from mine is different with that from spring. In contrast, the acidophilic IOB from similar habitats are closely related. It has been found that similar species have large geographic distribution and tend to adopt the similar habitats, morphotypes of acidophilic IOB were related to the growing environment conditions. Results revealed that acidophilic iron-oxidizing bacteria may have multiple evolutionary origins, and environment conditions play an important role in the long-term evolution of acidophilic iron-oxidizing bacteria.

Keywords: Acidophilic Iron-Oxidizing Bacteria; 16S rDNA Sequence Analysis; Phylogeny Analysis; Geographical Distribution

Introduction

Iron, the largest crust transition metal element, is widely found in fresh water, sea water, sediments and various mineral, and also the essential elements of all creatures [1]. Iron-oxidizing bacteria (IOB) were bacteria that they need to live and multiply by oxidizing dissolved ferrous iron. They catalyzed the oxidation of ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}) [2]. IOB were dominant ancient microorganisms in extreme environments, which from terrestrial mines to the ocean hydrotherm, from lava to the marsh, even in the habitat with high acidity and metal ion concentrations [3-5]. It has been reported that IOB can be classified into four types, i.e. acidophilic aerobic IOB, neutrophilic aerobic IOB, neutrophilic nitrate-dependent anaerobic IOB and anaerobic photosynthetic IOB [6].

The diversity of acidophilic IOB derived from different habitat, including acid rock drainage (ARD), acid mine drainage (AMD), metal mining, industrial leaching system, ground water lines and other acidic volcanic geothermal environment was studied by molecular biology techniques [7-13]. In the past few decades, more diverse, novel IOB have been discovered, such as *Acidithiobacillus ferrooxidans*, *Acidihalobacter prosperus*, *Leptospirillum ferrooxidans*, *Leptospirillum ferriphilum*, *Leptospirillum thermoferrooxidans*, *Ferrimicrobium acidiphilum*, *Ferroplasma acidiphilum*, *Ferroplasma acidarmanus*, *Sulfobacillus acidophilus*, *Sulfobacillus thermosulfidooxidans*, *Acidianus brierleyi*, *Acidianus infernus*, *Acidianus ambivalens*, *Metallosphaera sedula*, *Sulfurococcus yellowstonii*, *Sulfobacillus montserratensis*, *Thiomonas intermedia* and *Acidimicrobium ferrooxidans* [14-17].

Depends on the abundance and the biodiversity, the acidophilic IOB may play an important role in ecology. They may be participated in earth biochemical cycle such as Fe-cycle, C-, S- and N-cycle [18]. So far, the acidophilic IOB were found in China, the United States, India, Peru, Brazil, Russia, Japan and the Atlantic, etc. IOB has been widespread concerned due to the ecological significance and their diversity in morphology, physiology and phylogeny [19].

Aim of the Study

The aim of this study was to analyze the diversity difference of IOB from different environments. The 267 16S rDNA sequences from NCBI were analyzed by using Clustal1.83. The cladogram were built by Mega 6.0 and used to explore the geographical distribution of acidophilic IOB.

Data sources

All 267 16S rDNA sequences acquired from National Center for Biotechnology Information searching database (NCBI). *Acidophilic IOB*, *neutrophilic IOB*, *neutrophilic nitrate-dependent anaerobic IOB*, *Acidithiobacillus ferrooxidans*, *Leptospirillum Ferrooxidans*, *Acidimicrobium ferrooxidans*, *Ferrimicrobium acidiphilum*, *Sulfobacillus acidophilus*, *Acidianus brierleyi* were used as keywords. All possible acidophilic IOB 16S rDNA sequences were included and the non-iron-oxidizing bacteria 16S rDNA sequences were removed.

Methods

The similarity analysis was performed through Clustal1.83 (<http://www.clustal.org/download/1.X/ftp-igbmc.u-strasbg.fr/pub/ClustalX/>). It has been assumed that 16S rDNA similarity over than 97% may belong to the same species, the same sequence of submission repeated only keeping one [20]. All sequences were classified and respectively counted according to habitat (mining and spring), taxonomic status, place and country and the length of 16S rDNA sequences, *et al.* The abundance of IOB was calculated in mining and spring.

The full length (about 1500 bp), front-end (about 1000 bp), middle (about 500 bp), and end-piece (about 800 bp) of 16S rDNA sequence were phylogenetically analyzed using MEGA 6.0 due to the various lengths of submitted 16S rDNA sequences of IOB [21]. Meanwhile, the distributive character of acidophilic IOB derived from different continents and environments were also analyzed.

Results

Original data processing

The 267 16S rDNA sequences of IOB were acquired from NCBI and fragments of IOB derived from mining and spring were found to be 182 and 85, respectively. Sequences similarity alignment were carried out by Clustal1.83 (<http://www.clustal.org/download/1.X/ftp-igbmc.u-strasbg.fr/pub/ClustalX/>), when the similarity over than 97% means the same sequence and the sequence submitted repeatedly just use one. After removal of repetitive sequences, mining and spring acidophilic IOB sequences were 58 and 25, respectively (Table 1). Among these sequences, the γ -Proteobacteria and *Clostridia* were major sequences (24 and 22, respectively), followed by *Thermoprotei* (11), β -Proteobacteria, *Nitrospira*, *Thermoplasmata* and *Actinobacteria* (Figure 1). It can be seen from the table 1 that IOB were found mainly in China, United States, India, Peru, Chile and Iran. *Clostridium* is the dominant IOB in China, while *Thermoprotei* is the major IOB in other countries.

Taxonomic status	Habitat	The total sequences ¹⁾	97%ID ²⁾	Find locations
<i>β-Proteobacteria</i>	Mine	15	4	China, Chile
	Spring	3	2	China, America
<i>γ-Proteobacteria</i>	Mine	61	21	Peru, Iran
	Spring	12	3	Peru
<i>Nitrospira</i>	Mine	18	7	China
	Spring	0	0	
<i>Thermoprotei</i>	Mine	7	2	China, America
	Spring	26	9	China, India
<i>Thermoplasmata</i>	Mine	23	8	China, India
	Spring	0	0	
<i>Actinobacteria</i>	Mine	17	5	China
	Spring	1	1	America
<i>Clostridia</i>	Mine	63	11	America, Russia, Africa
	Spring	21	11	China, America, South America
Total		267	83	

Table 1: Acidophilic iron-oxidizing bacteria statistics (according to the 16SrDNA submitted to the NCBI).
 1) Full-length sequence: 97% ID of 16SrDNA full-length or near full-length sequence, about 1500 bp.
 2) 97% ID: In the same place, the same stretch of time to find and submit the NCBI 16SrDNA sequence similarity comparison, 97% sequence similarity may be the same kind of iron-oxidizing bacteria, according to a Statistics conducted.

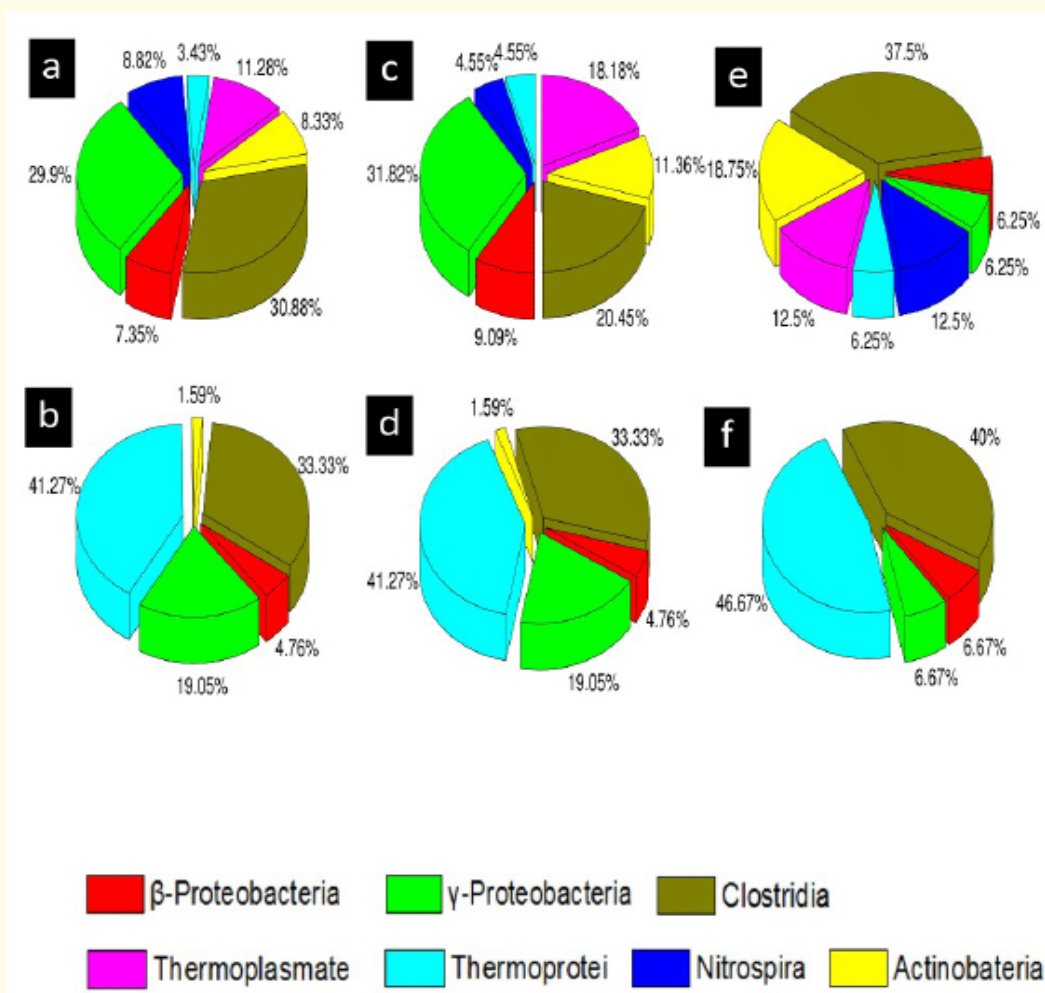


Figure 1: Classification proportion of acidophilic iron-oxidizing bacteria (a, b: each acidophilic iron-oxidizing bacteria respectively class proportion of the total sequence in mining and springs. c, d: each acidophilic iron-oxidizing bacteria respectively class proportion in mining and spring 97% ID. e, f: acidophilic iron-oxidizing bacteria respective class proportion of full-length sequence in mining and spring).

Phylogenetic analysis of acidophilic iron-oxidizing bacteria

In this study, 65 16S rDNA sequences were screened and phylogenetic trees were constructed with the front-end (about 1000 bp), middle (about 500 bp), and end-piece (about 800 bp) of sequences, respectively (Figure 2 and Figure 3). The results shown that three phylogenetic trees were almost unanimous, and the sequences were selected in the tree had same location. It can be seen that the cluster subregion of phylogenetic tree were obvious in the mining and spring. And the cluster subregion was aggregated distribution.

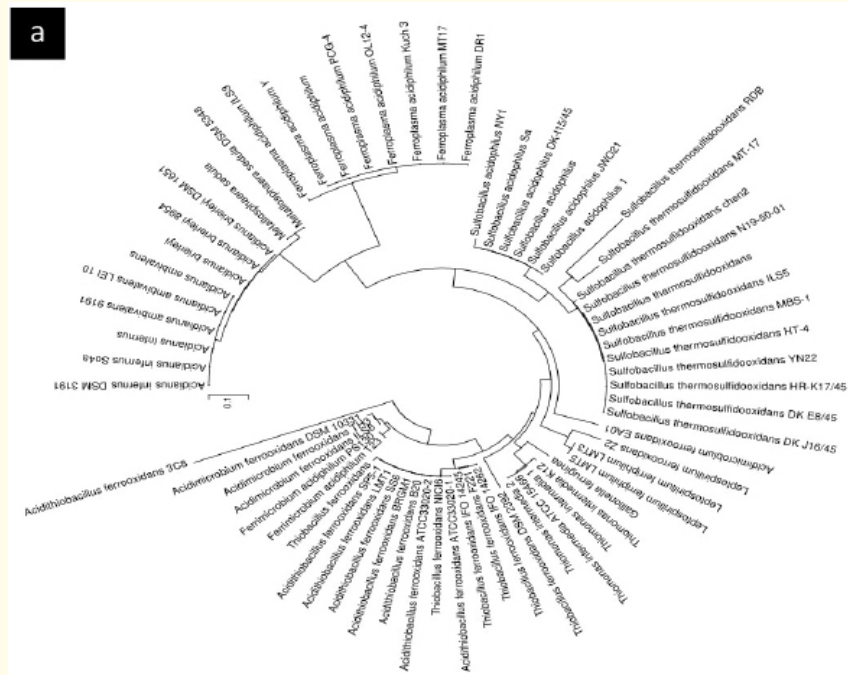


Figure 2: Phylogenetic trees based on partial sequence of 16SrDNA of acidophilic iron-oxidizing bacteria (a: 16S rDNA front section (1000 bp) of the phylogenetic tree, b: 16SrDNA middle (500 bp) of the phylogenetic).

Distribution of acidophilic IOB in different regions

Distribution of acidophilic IOB in mining and spring

Figure 4 shows that the γ -Proteobacteria (36%) and *Thermoprotei* (3%) in the mines were the dominant and least IOB, respectively (Figure 4a). For acidophilic IOB derived from spring, *Clostridium* (44%) and *Thermoprotei* (36%) were dominant, while *Nitrospira* and *Thermoplasmata* were rarely found (Figure 4b). It can be seen from figure 4 that *Nitrospira* and *Thermoplasmata* acidophilic IOB could survived in the highly acidic environments associated with metal ore mining. Results indicated that the similarity of the acidophilic IOB derived from mines and spring was low and their phylogenetic position was found to be significant difference.

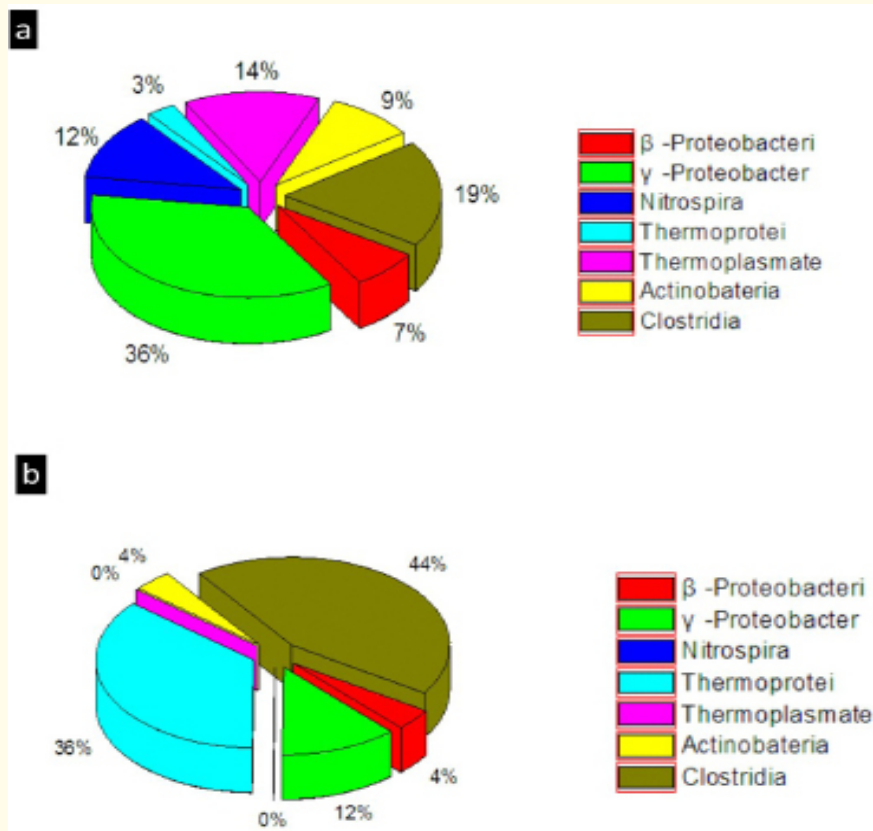


Figure 4: Different environments of acidophilic iron-oxidizing bacteria (a: Mining of iron-oxidizing bacteria in the environment, b: Spring of iron-oxidizing bacteria in the environment).

Distribution of acidophilic IOB in different mining environments

Figure 5 shows the acidophilic IOB distributed in different mines located in Asia, North America, Africa, South America, Europe and Oceania. It can be found that the dominant IOB were γ -Proteobacteria and *Nitrospira* in all mines. The acidophilic IOB of 16S rDNA from the mining were 17 sequences in Asia. They belonged to *Clostridia*, *Nitrospira*, *Thermoplasmata*, *Thermoprotei*, γ -Proteobacteria and β -Proteobacteria in Asia. They were *Thermoplasmata*, *Thermoprotei*, γ -Proteobacteria and β -Proteobacteria, *Actinobacteria* except *Nitrospira* respectively in Europe. Acidophilic IOB in Africa was only γ -Proteobacteria. The acidophilic IOB from Oceania was *Actinobacteria*. The acidophilic IOB of γ -Proteobacteria was distributed in South America. Species was concerned with distribution and metallic properties; the geographical location of the mining is not necessarily related.

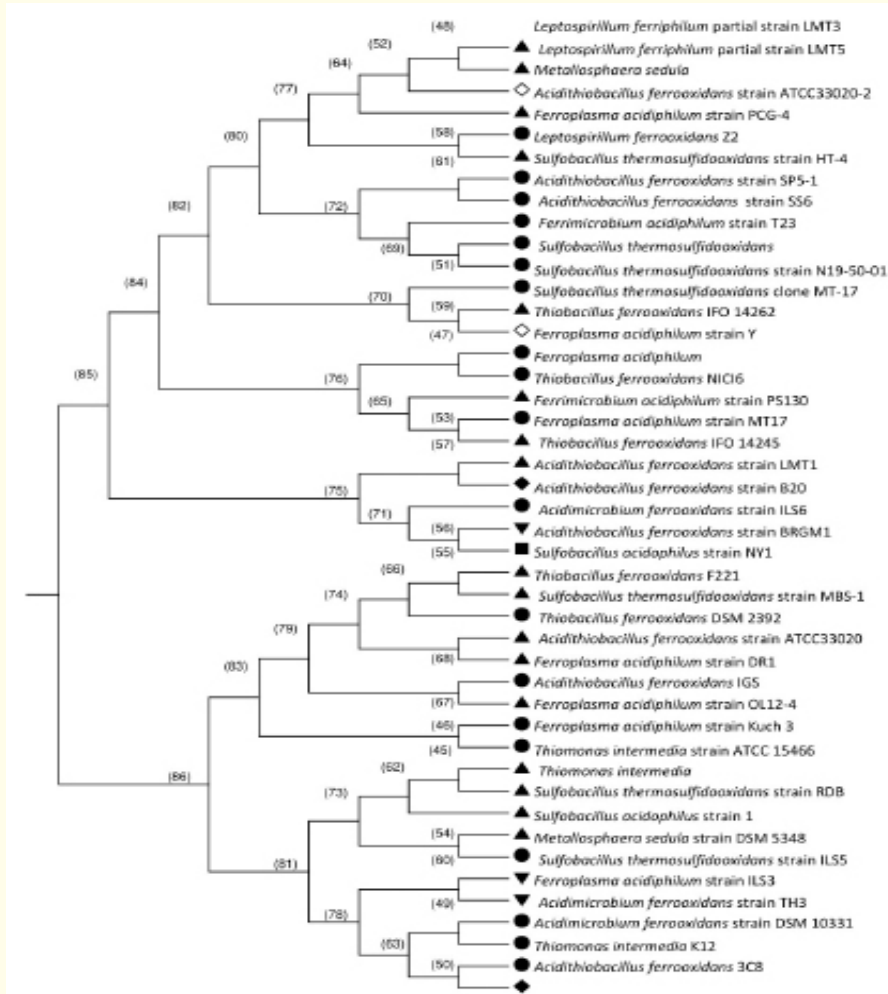


Figure 5: Phylogenetic tree based on of acidophilic iron-oxidizing bacteria in different mining environment (▲ Asia, ● Europe, ▼ Oceania, ◇ North America, ■ Africa, ◆ South America).

Distribution of acidophilic IOB in different spring environments

The distribution of acidophilic IOB in different springs located at Asia, North America and Europe are shown in figure 6. The acidophilic IOB derived from North America spring belonged to *Actinobacteria*, *Clostridia*, *Thermoprotei* and γ -Proteobacteria. It can be found that *Clostridia* and *Thermoprotei* derived from China were dominant, while *Clostridia*, *Thermoprotei* and β -Proteobacteria were dominant IOB in springs located in Europe including Russia, Iceland and Italy.

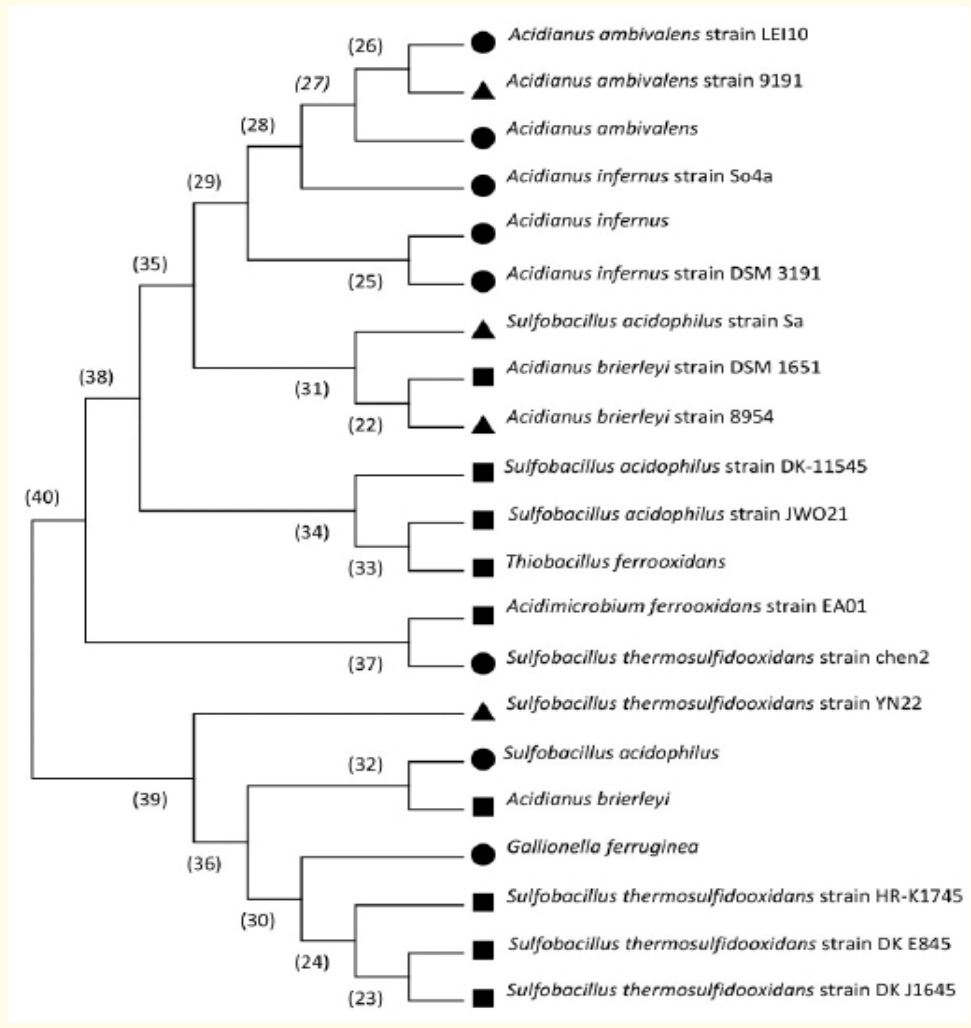


Figure 6: Phylogenetic tree based on of acidophilic iron-oxidizing bacteria in different spring environment (● Europe, ▲ Asia, ■ North America).

Discussion

Phylogenetic analysis of acidophilic IOB derived from different regions showed that high similarity IOB lived in the similar surroundings. In addition, the acidophilic IOB were most found in the spring outlet. Results suggested that the distribution of acidophilic IOB may have regional characteristic, and these IOB may be as indigenous microorganisms living in the world [22-24]. The acidophilic IOB can evolve to adapt the local environment in the extreme conditions.

Most of similarity acidophilic IOB were grown in similar habitats. *Thermophilic bacterium* and *Acidimicrobium ferrooxidans* strain similarities were 99%, they were found in the mining. However, *Leptospirillum ferriphilum* in metal mining and *Sulfobacillus acidophilus* in spring similarities were 92% [25,26]. The two 16S rDNA sequences of acidophilic IOB were closed in different ecological environments. This variation may be occasional and may be environmental factors in certain environments (such as pH, temperature, and salinity, etc.) [27]. Environmental conditions prompted the similar acidophilic IOB to evolve forward the same direction. Different environment catalysed the different types of acidophilic IOB to evolve [28]. For acidophilic IOB and other specific functions of bacteria, the classification of functional genetics or whole genomes may be to have a greater significance than the 16S rDNA phylogenetic tree.

The results of this study are limited to the number of acidophilic IOB 16S rDNA sequence iron-oxidizing bacteria from NCBI. The distribution of acidophilic IOB in different ecological regions could give people more comprehensive information of extreme microorganism and provide a better understanding of the roles they play in iron cycling and biotechnology [29-31].

Conclusion

The acidophilic IOB of γ -Proteobacteria in the mining was dominant and *Thermoprotei* was less. The acidophilic IOB from spring belonged to *Clostridium* and *Thermoprotei*. The acidophilic IOB distributed Asia and Europe in the mining, while acidophilic IOB from the spring located in North America. Phylogenetic analysis revealed that the distribution of acidophilic IOB in mining and spring environment were different. Results reflected that the environmental factors are correlative with the type of the acidophilic IOB. These observations suggest that environmental conditions were important evolutionary pressure in the evolution. They can be prompted that the similar acidophilic IOB were most in the same environment, while the different environments were formed different types of acidophilic IOB. The acidophilic IOB distribution revealed that the origin of acidophilic IOB may be multiple.

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