Chapter

Quorum Sensing of Acidophiles: A Communication System in Microorganisms

Xueyan Gao, Jianqiang Lin, Linxu Chen, Jianqun Lin and Xin Pang

Abstract

Communication is important for organisms living in nature. Quorum sensing system (QS) are intercellular communication systems that promote the sociality of microbes. Microorganisms could promote cell-to-cell cooperation and population density to adapt to the changing environment through QS-mediated regulation that is dependent on the secretion and the detection of signal molecules (or called autoinducers). QS system is also discovered in acidophiles, a microorganism that is widely used in the bioleaching industry and can live in an acidic environment. An example is the LuxI/R-like QS system (AfeI/R) that has been reported in the chemoautotrophic species of the genus *Acidithiobacillus*. In this chapter, we will introduce the types and distribution of the QS system, and the biological function and regulatory mechanism of QS in acidophiles. We will also discuss the potential ecological function of QS system and the application value of the QS system in the control and regulation of the bioleaching process in the related industries and acid mine damage.

Keywords: quorum sensing, communication, signal molecules, environmental adaption

1. Introduction

Acidophiles is a microorganism that can live in an acidic environment and widely distributed in extremely harsh environments such as acid mines, sulfur-containing hot springs, and volcanic craters [1, 2]. The signal communication and cooperation of the bacterial flora could be conducive to the survival and propagation of acidophiles in an extremely harsh environment. Quorum sensing (QS), as an important part of sociomicrobiology, is a group behavior that enables bacteria to establish cell-to-cell communication by producing, secreting, and detecting signal molecules (also called autoinducers) [3–5]. With the increase of cell density, the concentration of signal molecules released by cells becomes higher. When the concentration of signal molecules accumulates to a threshold in the local environment, the signal molecules bind to the receptor protein to activate or inhibit the expression of specific genes and then allow bacteria to respond to population density and external environment [6, 7]. Diverse biological functions of bacteria are regulated by QS systems, such as the formation of biofilm, the production of antibiotics, the

expression of pathogenic virulence genes, the luminescence of marine organisms, the transfer of Ti plasmids, and so on [8–11].

The research of the QS system has a history of 50 to 60 years [12]. As early as 1965, Tomasz and Alexander reported the interesting phenomenon caused by QS [13]. Hormone-like cell products could control the competence of *Pneumococcus* and promote foreign DNA to enter the cell [13]. Subsequently, Nealson et al. found that the luminescence of marine bacteria was positively correlated with the quorum of bacteria [14]. The research of the QS system had a major breakthrough with the identification of the signal molecules of the QS system and the discovery of *luxI/R* operon in *Vibrio ficheri* and the regulatory mechanism of the QS system regulating the luminescence phenotype of *V. ficheri* was revealed [15–17]. 3-O-C_6-HSL was confirmed as the signal molecule of the QS system of *V. ficheri*, which was synthesized by the synthetase encoded by *luxI* gene. In contrast, the *luxR* gene encodes the transcription factor that binds to the signal molecules. LuxI/R-mediated QS system regulates the expression of the *lux* operon, thereby affecting the luminescence phenotype of *V. ficheri* [15–17]. In recent years, more and more signal molecules, regulatory mechanisms, and functions of QS system have been discovered, with the help of modern analytical experimental techniques such as bioinformatics, molecular biology, and chemical analysis.

Compared with the well-studied QS systems in model bacteria and some pathogenic bacteria, the studies on QS system in acidophiles are restricted due to the limitations in molecular manipulation techniques. In the present chapter, we will introduce the research on the QS system of acidophiles, outline the distribution and molecular mechanisms of the QS system in acidophiles, and discuss the function of the QS system involved in the control and regulation of the bioleaching process in the biomining industry and acid mine damage.

2. Quorum sensing of acidophiles

Acidithiobacillus spp. is an important member of the acidophilic and chemolithotrophic Gram-negative sulfur-oxidizing bacteria [18–20]. Members of *Acidithiobacillus* can grow on reduced inorganic sulfur compounds (RISCs), and some of them can use ferrous as the energy substrate [18–20]. In 2005, Farah et al. discovered the LuxI/R-type QS system (AfeI/R) in *Acidithiobacillus ferrooxidans* [21]. The AfeI/R system is composed of three genes located on one operon (*afeI-orf3-afeR*) [21, 22]. The AfeI is a homologous protein to LuxI, catalyzing the synthesis of signal molecules, while the AfeR is homologous to LuxR protein, functioning by recognizing and binding signal molecules and regulating gene expression. The *Orf3* locus, located between the *AfeI* and *AfeR*, has a mysterious existence and its function is presently unknown [21, 22].

Bioinformatics analysis revealed that the AfeI/R-type QS system is widely distributed in the nine species of *Acidithiobacillus* reported so far (**Figure 1**) [23]. AfeI/R-like QS system could be identified from *Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferridurans*, and *Acidithiobacillus ferrivorans*, which means that in addition to *Acidithiobacillus ferrianus* and *Acidithiobacillus ferriphilus*, the AfeI/R system was found in almost all the sulfur- and ferrous iron-oxidizing species of *Acidithiobacillus*. However, among the sulfur-oxidizing species of *Acidithiobacillus*, only *Acidithiobacillus thiooxidans* was reported to have a QS system. It is worth noting that the conserved *afeR-orf3-afeI*-type operon can be found in *A. ferrooxidans*, *A. ferridurans,* and *A. thiooxidans*, while in *A. ferrivorans* were separated *afeI* and *afeR* genes. Besides, lower homology proteins were found in *A. thiooxidans* ATCC19377.

AfeI/R-like QS system also found in the genus of sulfur-oxidizing-only bacterium *Thiomonas* and the *Acidiferrobacter* reportedly produce acyl-HSL [24]. Further

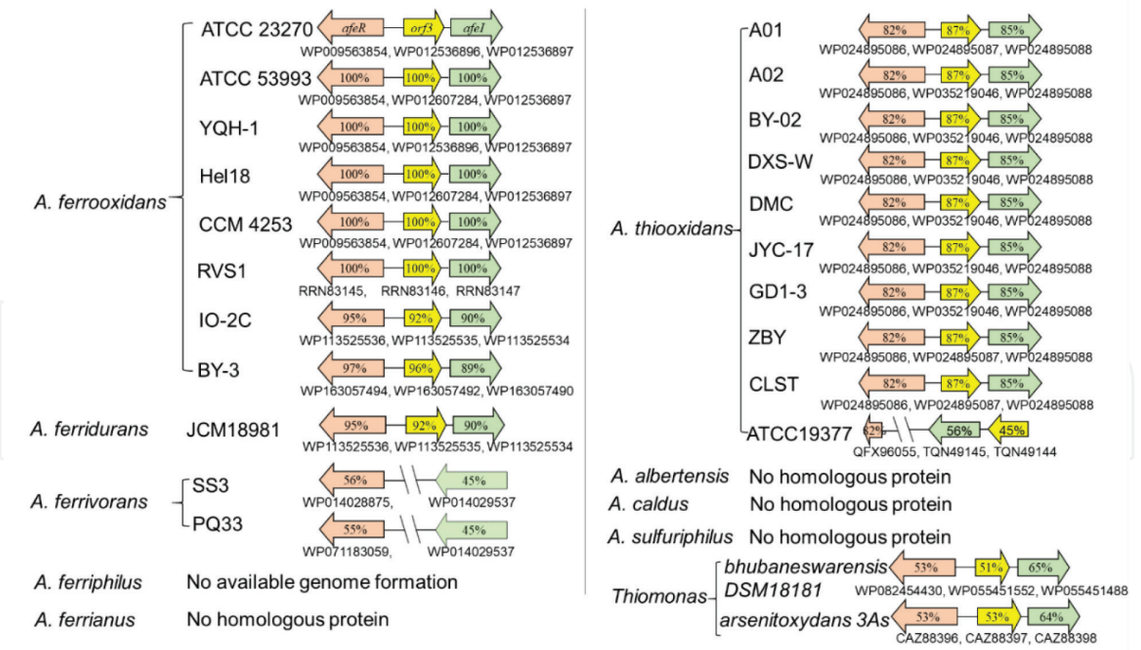

Figure 1.
Distribution of AfeI/R-like QS system in Acidithiobacillus and other acidophiles [23].

analysis of the acidophilus strains revealed that AfeI homologous proteins were found in 7 genera of acidophiles, and AfeR homologous proteins were present in 17 genera, and Orf3 homologous proteins were found in 12 genera of acidophiles (**Table 1**). AfeI/R-like QS system showed some variations at the gene arrangement and protein sequence in acidophilus strains.

AfeI homologous	**AfeR homologous**	**Orf3 homologous**
Acidithiobacillus	*Acidithiobacillus*	*Acidithiobacillus*
Acidocella	*Acidianus*	*Acidianus*
Frateuria	*Acidilobus*	*Acidomonas*
Metallosphaera	*Acidimicrobium*	*Aciduliprofundum*
Nitrosotalea	*Acidiphilium*	*Caldivigra*
Sulfurisphaera	*Acidisphaera*	*Ferrimicrobium*
Thiomonas	*Acidocella*	*Ferrovum*
	Acidomonas	*Nitrosotalea*
	Caldisphaera	*Picrophilus*
	Caldivigra	*Sulfolobus*
	Desulfosporosinus	*Thiobacillus*
	Ferrovum	*Thiomonas*
	Frateuria	
	Leptospirillum	
	Picrophilus	
	Thiobacillus	
	Thiomonas	

Table 1.
Distribution of AfeI/R- like QS system in acidophiles based on the AfeI/R sequence of Acidithiobacillus ferrooxidans alignment in acidophiles.

In 2007, another QS system (QS-II) of *A. ferrooxidans* was discovered [25]. This QS system includes four co-transcribed genes, *glyQ, glyS*, *gph*, and *act*, which encode α and β subunits of glycine t-RNA synthetase, phosphatase, and acyltransferase [25]. The reporter bacteria and GC–MS technology confirmed that acyl-HSL (C_{14}-HSL) could be synthesized by the *act* gene heterologously expressed in *Escherichia coli* [25]. Semi-quantitative RT-PCR experiments showed that the expression of *act* gene was higher when cultured in Fe^{2+}-enriched media than that in S^0-enriched media [25]. However, the regulatory protein that corresponds to Act has not been discovered, and whether the Act can synthesize acyl-HSLs or other types of signal molecules in *A. ferrooxidans* has not been reported up to now. Therefore, whether the Act system is a functional QS system is still controversial [26]. There are still many unsolved mysteries of *act* operon, which are worthy of in-depth study in the future.

It is worth noting that a thermophilic-like ene-reductase (*foye*-1) was discovered in the acidophilic iron-oxidizing bacterium *Ferrovum* sp. [27]. The *foye-1* gene is located directly upstream of *luxR* gene, and the encoded protein played a major role in intercellular communication [7]. Since the *luxI* gene is absent on the genome of *Ferrovum sp*. JA12, it is speculated that FOYE-1 may replace LuxI and participate in the QS process [27].

Interestingly, a diffusible signal factor (DSF) quorum sensing system was deciphered in the acidophilic, ferrous-oxidizing species, *Leptospirillum ferriphilum* [28]. This kind of QS system consisted of the *rpf* operon, which contains four genes, *rpfF-rpfC-rpfC-rpfG*, of which the *rpfF* gene encodes DSF synthase, the *rpfC* genes encode Hpt domain-containing protein and signal transduction kinase, and the *rpfG* gene encodes a two-component system response regulator [28]. Besides, three LuxR family transcriptional regulator proteins and another autoinducer-binding domain-containing protein were reported in *L. ferriphilum* [28]. Sequence alignment found the possible DSF perception protein *rpfR* and the homologous protein of *rpfC* in *A. caldus* and *Sulfobacillus thermosulfidooxidans* [29]. The homologous protein of *rpfG* was also found in *S. thermosulfidooxidans* [29].

Therefore, previous research results and bioinformatics analysis indicated that the QS system is universal and unique in acidophiles. Some of the acidophiles such as *A. ferrooxidans*, *A. ferridurans*, *and A. thiooxidans* have the complete AfeI/R-type QS system, which can synthesize and respond to signal molecules. This type of QS system is considered to be a fully functional and undisputed QS system. Some of the acidophiles only contain AfeI homologous protein that has the function of synthesizing signal molecules. Some acidophiles exist with the orphan LuxR family protein and the DSF-type QS system. Besides, more than one QS system were reported in some acidophiles.

3. Types of signal molecules synthesized by the QS system

There are many types of signal molecules synthesized and secreted by the QS system. The N-acyl homoserine lactone (acyl-HSL) is the prominent and widely studied signal molecule of the QS system and is composed of a homoserine lactone ring and an amide side chain (**Figure 2**) [4, 7]. The functional group of the third carbon atom has three forms: hydrogen, hydroxyl, and carbonyl [4, 7]. The R chain group can be 4–18 carbons, with or without an unsaturated C-C bond [12]. The terminal carbon has a branch in some bacteria, and the R chain group in some bacteria is an aromatic acid [12]. Furanosyl borate ester was reported to be the signal molecules used by the AI-2-type QS system [4]. Quinolone, diffusible signaling factor (DSF), hydroxyl-palmitic acid methyl ester (PAME), and small peptide have been reported as signal molecules for the QS system [4, 12].

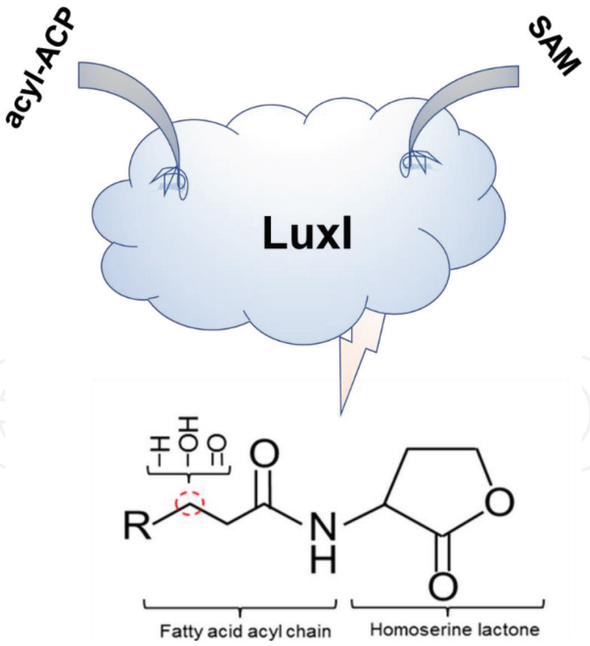

Figure 2.
Structure and synthesis process of acyl-HSL.

In 2005, Farah et al. reported that nine acyl-HSLs, namely 3-OH-C_8-HSL, 3-OH-C_{10}-HSL, C_{12}-HSL, 3-OH-C_{12}-HSL, 3-O-C_{12}-HSL, C_{14}-HSL, 3-OH-C_{14}-HSL, 3-O-C_{14}-HSL, and 3-OH-C_{16}-HSL, were detected in *A. ferrooxidans* ATCC 23270 cultured in ferrous, elemental sulfur, and thiosulfate energy [21]. The type and function of signal molecules produced by the AfeI/R system are confused due to the potential Act-type QS system in *A. ferrooxidans* [25]. In 2020, Gao et al. determined the types of signal molecules produced by AfeI by the construction of the gene mutant strain of *afeI* and *act* [23]. Different types and concentrations of acyl-HSLs were synthesized in S^0- and Fe^{2+}-enriched media of *A. ferrooxidans*, while the acyl-HSLs displayed different functions under different energy conditions. The homoserine lactone of acyl-HSLs is derived from S-adenosylmethionine (SAM), whereas its acyl side chain is derived from fatty acid metabolism and is provided by acyl carrier protein (acyl-ACP) (**Figure 2**) [30–32]. The difference in the molecular structure of acyl-HSLs depends on the length of the acyl side chain provided by the acyl carrier protein and the substituent on the third carbon atom [30–32]. The differences in the metabolism of sulfur and ferrous iron under different culture conditions may cause different kinds of acyl side chains produced in *A. ferrooxidans*, which in turn affects the type of signal molecules synthesized by AfeI [21, 31, 33]. Therefore, the synthesis process of acyl-HSLs by *A. ferrooxidans* and other acidophilic sulfide and iron-oxidizing bacteria under different energy sources will also be the key work of future research, which will help to clarify the close relationship between the AfeI/R system and energy substrates.

It has been reported in many papers that the phenotype of acidophilus bacteria such as *A. ferrooxidans*, *A. ferrivorans*, *L. ferrooxidans*, and *Acidiferrobacter sp*. strain SPIII/3 was affected by acyl-HSLs addition and these acyl-HSLs that influenced the growth and metabolism of acidophiles were not synthesized by the strain itself [24, 26, 29, 34, 35]. Besides, the synthetic tetrazole analog of acyl-HSLs could also stimulate the differential gene expression in *A. ferrooxidans* [36]. Therefore, it can be inferred that cross-communication exists in acidophilus bacteria and related research work needs to be carried out in-depth. In addition to the classic acyl-HSLs-type signal molecules, the DSF was also described in *L. ferriphilum* [28]. Whether there are other types of signal molecules in acidophilus bacteria remains to be studied to enrich the types of signal molecules of the QS system in acidophiles.

4. The regulatory function of the QS system in acidophiles

4.1 QS system and biofilm formation

The quorum sensing system is an important way to regulate extracellular polymeric substance (EPS) synthesis and biofilm formation [37–39]. Transcriptome data of *A. ferrooxidans* show that the tetrazole analog of acyl-HSLs stimulated the differential expression of more than 100 genes, and 42.5% of the differentially expressed genes are related to biofilm synthesis [36]. Laser confocal microscopy and atomic force microscopy observed that the addition of acyl-HSLs could affect the formation of biofilm formed by acidophiles on the surface of the pyrites [23, 24, 26]. It is worth noting that the influence of signal molecules on the formation of biofilms was specific to the types of acyl-HSLs and bacterial species [14, 24]. Studies have shown that the addition of C_{14}-HSL promoted the aggregation of *A. ferrooxidans* and *A. ferrivorans* cells, and enhanced the formation of biofilms on the surface of pyrite [24]. However, although the addition of C12-HSL increased the biofilm formation of *A. ferrooxidans*, it inhibited the biofilm formation of *A. ferrivorans* under the same conditions [24]. Gao et al. found that overexpression of *afeI* could not only promote the EPS synthesis and biofilm formation, but also increased the sulfur oxidation ability of cells and enhanced the erosion effect of *A. ferrooxidans* cells on sulfur [23]. In *A. thiooxidans*, studies confirmed that the QS system positively regulated the *pel* operon, participated in regulating the exopolysaccharide biosynthesis, and then affected the formation of biofilms [35]. The *pelD* gene is located in the *pel* operon and encodes the c-di-GMP-binding protein [35]. The interaction between QS system and c-di-GMP pathway to regulated EPS synthesis and biofilm formation had been displayed in other bacterial species [40–42]. It is reported that the QS system regulates the expression of *pelD* gene and may also regulate some gene-encoding proteins with c-di-GMP synthase activity and/or c-di-GMP degradation activity, resulting in the change in intracellular c-di-GMP levels and in turn affecting the formation of biofilm in *A. thiooxidans* [35].

The regulation of the QS system on the dispersion of biofilms has been confirmed in many bacteria such as *Xanthomonas campestris*, *Pseudomonas aeruginosa*, *and Staphylococcus aureus* [43]. The phenomenon of biofilm dispersal is also found in acidophilus bacteria. The addition of DSF family signal molecules caused biofilm dispersal of *L. ferriphilum* and *S. thermosulfidooxidans*, which strongly inhibited the growth and metabolism of bioleaching bacteria [29].

4.2 The regulatory function of AfeI/R in different energy substrate environments of *A. ferrooxidans*

Compared with the QS system in other acidophiles, the research of QS system in *A. ferrooxidans* is more in-depth. As early as 2005, semi-quantitative RT-PCR measurements found that the expression levels of *afeI* and *afeR* genes in S^0-enriched media were higher than those in Fe^{2+}-enriched media [21], which suggested that the AfeI/R system may function optimally in an environment or medium containing sulfur. Confocal laser microscopy and atomic force microscopy techniques have observed that the AfeI/R-mediated QS system can enhance the formation of biofilms of *A. ferrooxidans* on elemental sulfur or metal sulfide surfaces [26]. The *lux-box* (LuxR family protein-binding sequence) in *A. ferrooxidans* was predicted *via* a bioinformatic approach [44], and the *lux-box* sequence upstream of the *afeI* gene was confirmed *via in vitro* experiments [36]. Due to the difficulty of gene manipulation of *A. ferrooxidans* and other acidophiles, it was not until 2020 that

Gao et al. successfully knocked out and overexpressed the *afeI* gene to determine the regulatory role of AfeI/R in the sulfur and iron culture system [23].

Gao et al. revealed that AfeI/R not only played an important role in S^0-enriched media, but also had a more significant regulatory role in Fe^{2+}-enriched media [23]. In S^0-media, overexpression of *afeI* could increase the cell concentration and acid production capacity of the strain in the lag phase and exponential phase, but did not affect the final population density of the culture system, Therefore, AfeI/R can be used as an "accelerator" for *A. ferrooxidans* cultured in S^0-enriched media. Besides, the effect of *afeI* overexpression on EPS synthesis was consistent with that on the sulfate yield and cell density of *A. ferrooxidans* in S^0-enriched media. Therefore, the regulation of AfeI/R on EPS synthesis was the key strategy for AfeI/R to regulate cell growth, metabolism, and population density of *A. ferrooxidans*. Moreover, the AfeI/R-regulating EPS synthesis could also be an important way for *A. ferrooxidans* to adapt to the sulfur substrate in the environments. In Fe^{2+}-enriched media, overexpression of *afeI* significantly inhibited the cell concentration and the ferrous oxidation capacity of the strain. Therefore, AfeI/R can be used as an "inhibitor," regulating cell metabolic growth and the final population density in Fe^{2+}-enriched media. The overexpression of *afeI* significantly inhibited the expression of the hydrogenase synthesis gene cluster (AFE_0700–0719) in *A. ferrooxidans* in Fe^{2+}-enriched media, suggesting that AfeI/R may regulate hydrogen metabolism to influence the growth, metabolism, and quorum of the *A. ferrooxidans* cells during ferrous culture. The results indicated that the QS system may have a new regulation function when *A. ferrooxidans* is cultivated with ferrous iron, and research on the related molecular mechanism is needed. Energy substrates can affect the acyl-HSLs synthesized by AfeI and the regulatory effects of AfeI/R in *A. ferrooxidans*, and the substrate-dependent regulation strategy of the AfeI/ R was proposed based on these research findings.

5. The application of QS in bioleaching

The bioleaching bacteria, as an important class of acidophiles, are widely distributed in the acid mine environments [2]. The progress of mineral dissolution and metal leaching requires the consortium of the bioleaching bacteria and the attachment of cells to the surface of the ores [45, 46]. The QS system regulates cell aggregation and adsorption, EPS synthesis, and biofilm formation; thus, the QS-mediated regulation could be involved in the regulation of the bioleaching process. Therefore, the QS system has important application value in the bioleaching industry and the treatment of acid pollution.

In 2013, González et al. found that the addition of C12/C14-HSLs can promote the biofilm formation of *A. ferrooxidans* on the surface of pyrites [26]. Bellenberg et al. reported that the acyl-HSLs addition caused different effects on the pyrite dissolution of *A. ferrivorans*, *Acidiferrobacter sp*., and *L. ferrooxidans* [24]. Gao et al. found that the *afeI/R* gene cluster overexpression significantly enhanced the adhesion and erosion ability of the cells [47]. A model of the QS system participating in the regulation of the bioleaching progress was proposed [47]. As shown in **Figure 3**, the QS system regulated the EPS synthesis and the biofilm formation, stimulating the planktonic cells to transform to the sessile state. Simultaneously, the sulfur metabolism, bioerosion capacity, and bioleaching efficiency were enhanced by the QS system regulation. The discovery of AfeI/R in regulating the bioleaching process indicated that the QS system plays an important role in regulating the biooxidation process of minerals, and the QS-regulating bioleaching model provides the theoretical basis for studying the control strategy and technologies of acidophilus bacteria in the bioleaching process.

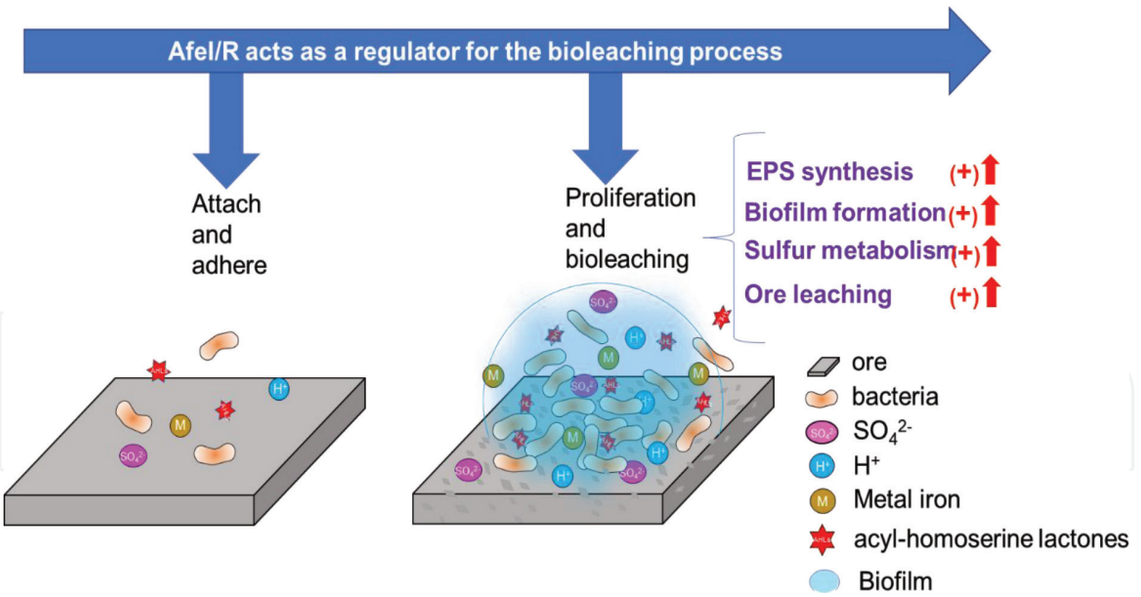

Figure 3.
The regulation of AfeI/R on the bioleaching process of Acidithiobacillus ferrooxidans [47].

6. Conclusion

The regulation function of QS system is an important research content in the study of the co-evolution of microbial community and environment. This chapter systematically describes the QS system in acidophiles including the distribution of QS system, the types of QS system signal molecules, the regulatory function, and application of QS system. Current research shows that the quorum sensing system is involved in the process of cell growth, energy metabolism, the interaction between bacteria and minerals, and the co-evolution process of acidophiles and the extreme environment.

The research of QS system in *A. ferrooxidans* is relatively more extensive than the other acidophiles. Taking *A. ferrooxidans* as an example, the discovery of the energy-dependent regulatory strategy of the AfeI/ R in *A. ferrooxidans* indicated that some chemoautotrophic sulfur-iron-oxidizing bacteria may use the QS system to build the co-evolution process from the response to energy substrates to the regulation on cell growth and population density in the sulfur-and-iron-contained environments. This QS-regulated adaptive strategy may be an important way for chemoautotrophs to adapt to their growth environments and to obtain an ecological competitive advantage.

Due to the complex metabolism and difficulty in the genetic manipulation of acidophiles, the research progress of the QS system in acidophiles has been slow. There is still a lot of room for the research of the QS system in acidophiles. Is there another QS system different from the LuxI/R? In addition to the reported acyl-HSLs type of signal molecules, are there other types of signal molecules in acidophiles? What kind of interspecies regulatory role of the QS system exists in various acidophiles? Moreover, the regulatory functions and molecular mechanisms of the QS system in acidophiles need to be further explored and analyzed. The answers to these questions will not only help to recognize the regulatory functions and mechanisms of the QS system in acidophiles but also help reveal the survival adaptation strategies of microorganisms in extreme environments.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Xueyan Gao[1,2*], Jianqiang Lin[1], Linxu Chen[1], Jianqun Lin[1] and Xin Pang[1]

1 State Key Laboratory of Microbial Technology, Shandong University, Qingdao, P.R. China

2 Medical Science and Technology Innovation Center, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, P.R. China

*Address all correspondence to: gaoxueyan@sdu.edu.cn

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