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Introductory Chapter: The Important Physiological Characteristics and Industrial Applications of Acidophiles

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1. The definition of acidophiles

Acidophiles are an important category of extremophiles that are defined by the environmental conditions in which they grow optimally. Acidophile is a broad definition that organisms can grow preferentially in environments with a pH at below 6. In 2007, Johnson proposed a generally accepted classification standard according to the optimal pH. The organisms with optimal pH at 3 or below are classified as extreme acidophiles, and those with an optimal pH of 3–5 are moderate acidophiles [1]. Although some organisms can grow at a pH lower than 5, they are recognized as acid-tolerant species because of their pH optima above 5. The research history of acidophiles started in the discovery of a sulfur-oxidizing bacteria isolated from a compost sample mixed with sulfur, rock phosphate, and soil by Waksman and Joffe in 1922 [2]. This bacterium has an optimal growth pH at 2.0–2.8 and is a strict autotroph that obtains energy by oxidizing inorganic sulfur substances (elemental sulfur, thiosulfate, and hydrogen sulfide). This bacterium was named as *Thiobacillus thiooxidans* by Waksman and Joffe, and later was reclassified as *Acidithiobacillus thiooxidans* by Kelly and Wood in 2000 [3]. With the development of microbiology and gene sequencing technology, more and more acidophiles have been discovered, identified, and sequenced. Until now, the most acidophilic organisms are from an archaeal genus of *Picrophilus*, firstly isolated from acidic hot springs and dry hot soil in Hokkaido in Japan [4]. Members in *Picrophilus* have optimal pH at 0.7 and the ability to grow at a pH of 0. Moreover, acidophiles are involved not in the domains of *Bacteria* and *Archaea*, but also in the *Eukarya* domains, such as some acidophilic fungi, algae, and yeast distributed in the acid mine environments.

2. The typical acidophilic bacteria and the applications of acidophiles

Acidithiobacillus is a kind of extensive research and wide application of gram-negative acidophiles. Members in this genus are broadly existed in the sulfur-containing acidic environments on land or in the sea, such as acid mine drainage (AMD), iron-sulfur mineral mines, hot springs, and sediments [5–10]. *Acidithiobacillus* spp., as the important sulfur and iron-oxidizers, participate in the element cycles of sulfur and iron, and promote the acid environment generation and acid microecosystem formation. All *Acidithiobacillus* strains have the capability of oxidizing various reduced inorganic sulfur compounds (RISCs)

Trait	<i>A. ferrooxidans</i>	<i>Acidithiobacillus ferrivorans</i>	<i>A. ferrophilus</i>	<i>A. ferridurans</i>	<i>Acidithiobacillus thiooxidans</i>	<i>Acidithiobacillus calvus</i>	<i>A. albertensis</i>
Gram stain	—	—	—	—	—	—	—
Cell size (µm)	1.0 × 0.5	2.4 × 0.5	1–2	1–2	1.0–2.0 × 0.5	1.2–1.9 × 0.7	1–2 × 0.4–0.6
Motility	+/–	+	+	+	+	+	+
Growth pH (optimum)	1.3–4.5 (2.0–2.5)	1.9–3.4 (2.5)	1.5– (2.0)	1.4–3.0 (2.1)	0.5–5.5 (2.0–3.0)	1.0–3.5 (2.0–2.5)	0.5–6.0 (3.5–4.0)
Growth T/°C (optimum)	10–37 (30–35)	4–37 (28–33)	5–33 (30)	10–37 (29)	10–37 (28–30)	32–52 (40–45)	10–40 (25–30)
Oxidation of S ⁰ , S ₄ O ₆ ²⁻ , S ₂ O ₃ ²⁻	+	+	+	+	+	+	+
Oxidation of Fe ²⁺	+	+	+	+	—	—	—
Growth on sulfide minerals	+	+	+	+	—	—	—
Growth on hydrogen	+	(+)	—	+	—	+	NR
Anaerobic growth with Fe ³⁺	+	+	+	+	—	—	—
N ₂ fixation	+	+	NR	NR	—	—	—
Mol% G + C	58–59	55–56	57.4	58.4	52	63–64	61.5
Thiosulfate- metabolic pathways	TSD enzyme; S ₄ I pathway.	Sox system; TSD enzyme; S ₄ I pathway.	NR	TSD enzyme; S ₄ I pathway.	Sox system; S ₄ I pathway.	Sox system; S ₄ I pathway.	Sox system; S ₄ I pathway.

+, positive; –, negative; +/–, the positive or negative result from different reports; (+), some strains have the ability to oxidize hydrogen; NR, not reported; Tm, temperature.

Table 1.
Taxonomic traits of species in the genus of *Acidithiobacillus*.

and elemental sulfur, and some of them also have ferrous iron oxidation ability [11]. By the oxidation of sulfur and ferrous, *Acidithiobacillus* spp. obtains electrons to generate the bioenergy (ATP) and reducing power (NADH/NADPH) to fix carbon dioxide for autotrophic growth. More and more species have been identified based on their physiological characters and 16S rRNA gene sequences (**Table 1**) [2, 12–16]. Species in *Acidithiobacillus* can be divided into two groups according to their energy-substrates: the sulfur-oxidizing only species, including *A. thiooxidans*, *Acidithiobacillus caldus* and *A. albertensis*, and the sulfur and ferrous-oxidizing species, including *A. ferrooxidans*, *Acidithiobacillus ferrivorans*, *A. ferriphilus*, and *A. ferridurans* (**Table 1**).

Acidithiobacillus spp. and other chemoautotrophic acidophilic bacteria have an important application in bioleaching. The bioleaching technology is originated from the biohydrometallurgy industry, and has become a great potential and broad-prospects in non-ferrous metal extraction (golden, silver, copper et al.) from various sulfide ores. *Acidithiobacillus* spp. have the remarkable capabilities of metabolizing the sulfur and iron in ores and adapting to extremely acidic environments, thus they have become the most active and preponderant bacteria used in biomining [17, 18]. *A. ferrooxidans*, *A. thiooxidans*, and *A. caldus* are the wide used ore leaching species in biomining for mineral extraction from ores [19, 20]. In recent years, based on their abilities to produce acid and heavy leaching metals, *Acidithiobacillus* spp. have been used from biohydrometallurgy to the treatment of wastes containing heavy metals, such as sewage sludge, spent household batteries, mine tailings, and printed circuit boards [21–25]. Moreover, these bacteria have been widely studied in microbial desulfurization of coal and gas [26–28]. In a word, the great application values of *Acidithiobacillus* spp. have been exploited from the biohydrometallurgy industry to the environmental pollution treatments.

3. The physiological feature of chemoautotrophic acidophiles

Sulfur oxidation is a characteristic physiological feature for many acidophilic microorganisms and is an important biochemical process that promotes the generation of the acid environment and the formation of acidophilic microbial communities. *Acidithiobacillus* spp., as the first-discovered and the most widespread used acidophile, has been attracted extensive attention and has been used as model sulfur-oxidizing bacteria to research microbial sulfur metabolism [11, 29–40]. The oxidation states of element sulfur are range from -2 to $+6$, resulting in different kinds of RISCs (tetrathionate ($S_4O_6^{2-}$), thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), sulfide (S^{2-}) et al.), and elemental sulfur (S^0). Thus, many microbes, particularly autotrophic sulfur-oxidizing microbes, have evolved a variety of enzymes and proteins participating in the oxidation of RISCs and S^0 . Research shows *Acidithiobacillus* spp. have a high-efficient and sophisticated sulfur-metabolizing network that could oxidize RISCs and S^0 to sulfate. Based on metabolic substrates, the sulfur-metabolic enzymes in *Acidithiobacillus* spp. could be categorized as elemental sulfur oxidation enzymes, enzymes in thiosulfate oxidation pathways, sulfide oxidation enzymes, and sulfite oxidation enzymes. These enzymes work cooperatively in different cellular compartments to oxidize the RISCs and S^0 to the final product sulfate (**Figures 1** and **2**) [11]. As shown in **Figure 1**, the extracellular elemental sulfur (S_8) oxidation in *A. caldus* starts from the activation and transportation of S_8 by special outer-membrane proteins (OMP), generating the persulfide sulfane sulfur in the periplasm; then the persulfide sulfane sulfur is oxidized to sulfite that can directly enter the sulfur oxidizing enzyme (Sox) system or form $S_2O_3^{2-}$ via a nonenzymatic reaction; the generated thiosulfate is then metabolized by the truncated Sox pathway or catalyzed by thiosulfate:quinol oxidoreductase (TQO or DoxDA) to

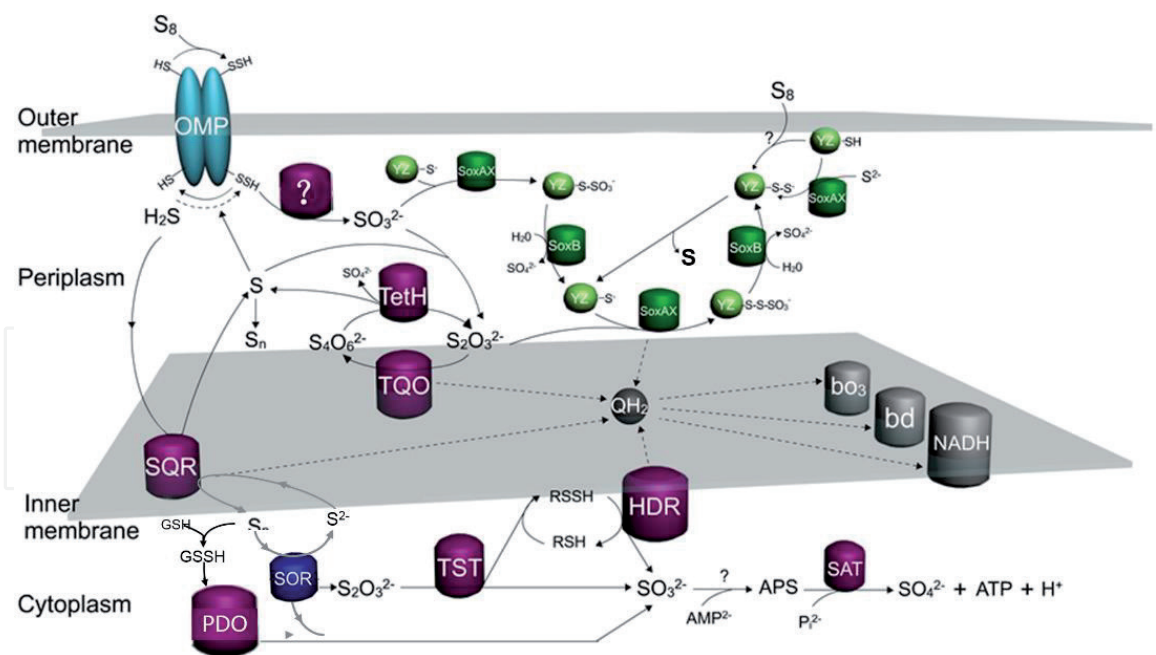


Figure 1. The model of sulfur oxidation in *Acidithiobacillus caldus*. OMP, outer-membrane proteins; TQO, thiosulfate quinone oxidoreductase; TetH, tetrathionate hydrolase; SQR, sulfide:Quinone oxidoreductase; PDO, persulfide dioxigenase; SOR, sulfur oxygenase reductase; TST, rhodanese; HDR, Hdr-like complex; SAT, ATP sulfurylase; bd, bo₃, terminal oxidases; QH₂, quinol pool; NADH, NADH dehydrogenase complex I.

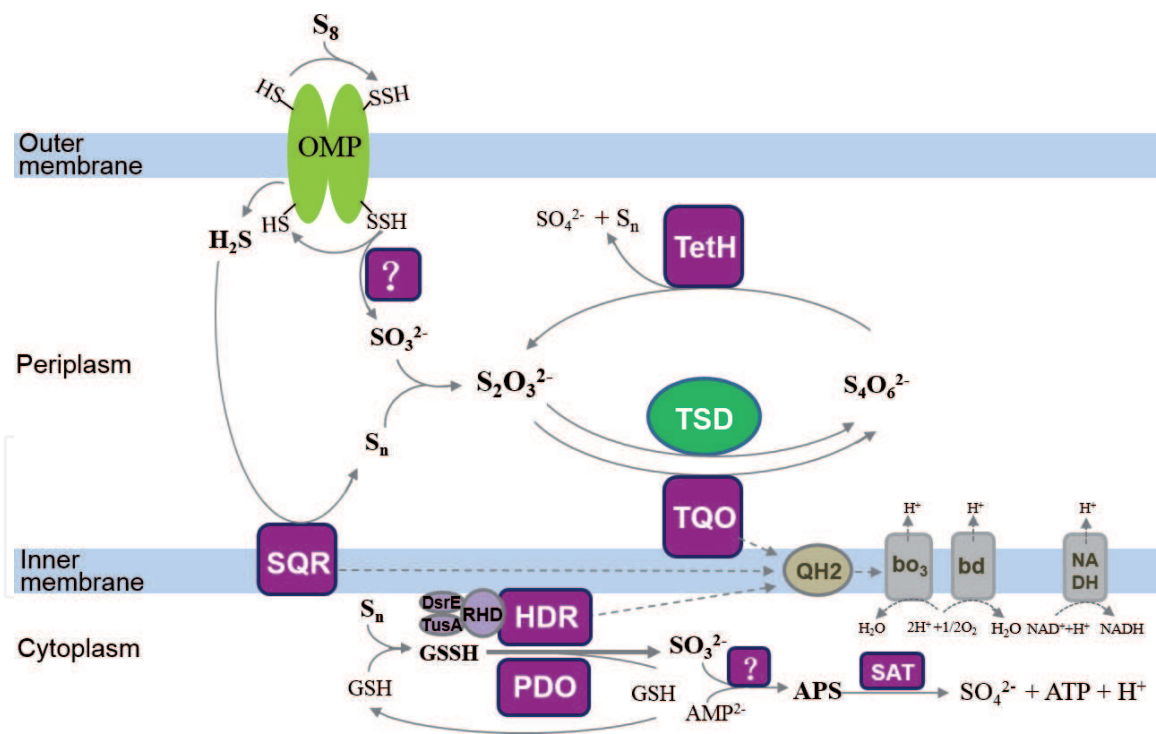


Figure 2. The model of sulfur oxidation in *A. ferrooxidans*. OMP, outer-membrane proteins; TQO, thiosulfate quinone oxidoreductase; TSD, thiosulfate dehydrogenase; TetH, tetrathionate hydrolase; SQR, sulfide:Quinone oxidoreductase; PDO, persulfide dioxygenase; HDR, Hdr-like complex; SAT, ATP sulfurylase; bd, *bo*₃, terminal oxidases; QH₂, quinol pool; NADH, NADH dehydrogenase complex I.

generate $\text{S}_4\text{O}_6^{2-}$; $\text{S}_4\text{O}_6^{2-}$ is further hydrolyzed by tetrathionate hydrolase (TetH); the H_2S produced during the activation of S_8 can be oxidized by sulfide:quinone oxidoreductase (SQR) located in the inner membrane; the periplasmic elemental sulfur (S_n) produced from Sox pathway, tetrathionate hydrolysis and sulfide oxidation, could be re-activated at the outer membrane region, or be mobilized into the cytoplasm

where Sn could be used by cytoplasmic elemental sulfur oxidation enzyme persulfide dioxygenase (PDO) and Sulfur oxygenase reductase (SOR); the metabolites from the reaction of PDO and SOR could be utilized by cytoplasmic sulfur-metabolic enzymes, including the $S_2O_3^{2-}$ metabolism via by rhodanese (TST) and the Hdr-like complex (HDR), the degradation of SO_3^{2-} via the APS pathway and the oxidation of S^{2-} by SQR. During the sulfur metabolic process, the periplasmic sulfur-oxidizing pathways (Sox and TetH) are responsible for electron acquisition, thus they are important for the sulfur metabolism in *A. caldus*. Different from '*A. caldus*' like sulfur metabolism network, some sulfur-oxidizers, such as *A. ferrooxidans*, did not have the Sox pathway, but rather a thiosulfate dehydrogenase (TSD) (**Figure 2**). Interestingly, *A. ferrivorans* possesses both Sox system and TSD enzyme (**Table 1**). The proposal of sulfur metabolism models provides new knowledge and insights in understanding the metabolism and adaptation mechanisms of acidophilic sulfur-oxidizing microorganisms in extreme environments.

4. The significance of studying and understanding acidophiles

Acidophiles, as important extremophiles, have presented important scientific significance and industrial application values. Researches on acidophiles do not only help us understand the diversity and adaptation of life on earth, but also be conducive in developing various new biotechnologies to resolve the problems of resource exploitation, pollution treatment, and human health. This book provides some new breakthroughs and insights on the researches of acidophiles: the two-component system (TCS) in the regulation of sulfur metabolic process; the adaptation mechanisms of acidophiles to low pH; the regulation mechanism and the application strategy of quorum sensing in bioleaching bacteria; *Lactobacillus acidophilus* and its application in the human health.

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