微生物介导的铁氧化机制及应用研究进展

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摘 要: 微生物介导的铁氧化过程是铁循环的重要组成部分。参与铁氧化过程的微生物主要是细菌和古菌,依据生长环境及电子受体情况可将其分为四类:嗜酸铁氧化菌、中性微氧铁氧化菌、中性厌氧光合铁氧化菌和中性厌氧硝酸盐还原铁氧化菌。铁氧化微生物驱动着环境中 C、N、O、S 等生源要素的元素地球化学循环。目前已经阐明的微生物铁氧化电子传递机制都有着一个共同范式,即亚铁都是在外膜细胞色素上被氧化,细胞从亚铁获得电子,由细胞外膜经周质电子传递蛋白,而后传递至细胞内膜各蛋白上,用于电子受体还原或固碳。现代环境中铁氧化微生物的研究已被广泛运用于地球早期生命演化研究、环境污染修复、新材料合成及生物浸矿等领域。铁氧化菌的分离鉴定、铁氧化代谢途径及其地球化学效应的详细解析及铁氧化在环境污染治理等方面还有大量的工作有待开展。

关键词: 铁氧化微生物;电子传递机制;早期生命演化;生物修复

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作为生命必需的营养元素,铁在生命活动过程中扮演着至关重要的角色。例如,含铁元素的铁血红素(heme)是细胞色素(Cytochrome)的关键结构成分之一[1]。铁还可作为电子供体参与微生物的铁氧化代谢,而这类利用亚铁作为电子供体的微生物称为铁氧化微生物。对铁氧化微生物的研究最早可追溯至 19 世纪中叶[2]。由铁氧化微生物介导的铁氧化过程是环境中铁循环的重要组成部分。铁氧化微生物在自然环境中具有强大的生态适应性,它们能够利用亚铁、光、氢气、硝酸盐或有机物等作为其能源物质[3]。在酸性矿山废水、沼泽湿地、土壤、淡水和海水环境、以及热液喷口处均发现有这类微生物的活动足迹[4-5]。近年来,以高通量测序技术与生物信息学技术为基础的现代分子生物学技术加速了学界对铁氧化微生物系统发生学、生态学以及铁氧化机理的理解,促进了其在地质学、地质生物学、环境科学、材料学和冶金学等研究领域中的应用。

正如 de Wit 和 Bouvier [6]、Baas-Becking[7]所提出的“Everything is everywhere but environment selects”,环境理化参数决定该环境中生物群落组成。同理,铁的赋存状态、溶解度和氧化还原电位等地球化学性质决定环境中铁的生物可利用性,从而决定相关环境中铁氧化菌的种类和丰度。本文从铁的地球化学性质出发,综合讨论了铁氧化微生物的多样性、铁氧化电子传递机制及其应用。

1 铁的地球化学特征

铁是地球上储量最多的金属元素,在地壳元素中,其丰度排名第四。据报道,沉积岩中铁的平均含量为 5% ~ 6%[8],含铁量最高的条带状铁建造(Banded Iron Formation,BIF)中铁含量可达 28%[9]。

铁的氧化还原电位介于 C、N、P、S 等元素之间,环境中铁元素常以氧化物、硫化物、碳酸盐矿物(菱铁矿)、磷酸盐矿物及硅酸盐矿物等形式赋存于岩石或土壤中[8,10]。自然环境中,铁多以 Fe(Ⅱ) 和 Fe(Ⅲ)存在,这两种价态铁的存在形态、分布和比例,很大程度上取决于环境的理化参数,如酸碱度、

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含氧量、氧化还原电位、有机和无机络合配体等[11-12]。由于分子氧在自然环境中普遍存在,大多数 Fe(Ⅱ)的氧化几乎都是由氧气介导的非生物氧化过程。例如,水体中存在大量分子氧时,游离态 Fe(Ⅱ)被迅速氧化(速率约 8.4 mg/min)为 Fe(Ⅲ)而沉淀。这也是为什么尽管地球上铁丰度较高,但陆地水体及海水却处于铁限制的地球化学背景的重要原因[13]。一般而言,分子氧介导的溶液态 Fe(Ⅱ)氧化的速率($-(d[\mathrm{Fe(II)}])/dt$)由氧浓度($[O_2]$)、亚铁浓度($[\mathrm{Fe(II)}]$)、质子浓度($[H^+]$)与温度等多个参数共同决定[5,14-15]:

$$-\frac{d[\mathrm{Fe(II)}]}{dt}=\frac{k\cdot[O_2]\cdot[\mathrm{Fe(II)}]}{[H^+]^2} \qquad (1)$$

式中,系数 k 为温度依赖型的速率常数,20 ℃时该速率常数为 3×10^{12} mol /(min·L)。

在中性富氧环境中,Fe(Ⅱ)可被迅速地氧化为 Fe(Ⅲ)。在中性微氧或缺氧环境中,Fe(Ⅱ)可长期较为稳定的以可溶离子态的形式存在,Fe(Ⅲ)则可与水发生水合作用形成水铁矿(FeOOH)而沉淀。在酸性环境中,Fe(Ⅱ)或 Fe(Ⅲ)常以可溶态的形式存在,Fe(Ⅱ)的氧化速度较慢(速率约 8.4×10^{-10} mg/min)[16]。但是,当环境中出现大量的有机或无机络合配体时,Fe(Ⅱ)或 Fe(Ⅲ)可通过与配体形成络合物而改变其溶解性。相对于中性富氧环境,中性微氧、中性缺氧或酸性环境中 Fe(Ⅱ)的非生物氧化过程较为缓慢,使得利用 Fe(Ⅱ)作为其电子供体的微生物能够在其中有效地生存和繁殖,这些微生物介导的铁氧化过程构成了此类环境中铁循环的重要部分[5,11,17]。

2　铁氧化微生物的多样性

基于微生物分类学,铁氧化微生物主要分布于古菌的广古菌门(Euryarchaeota)和泉古菌门(Crenarchaetoa),细菌的(Alpha-、Beta-、Gamma-、‘Zeta-’)变形菌门(Protobacteria)、放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)、绿菌门(Chlorobi)、嗜酸菌门(Acidophiles)和硝化螺菌门(Nitrospirae)[5]。依据铁氧化微生物的生存环境条件和代谢方式等特征,可分为嗜酸铁氧化菌(Acidophilic aerobic Fe(Ⅱ)-oxidizers)、中性微氧铁氧化菌(Neutrophilic microaerobic Fe(Ⅱ)-oxidizers)、中性厌氧光合铁氧化菌(Anaerobic phototrophic Fe(Ⅱ)-oxidizers)和中性厌氧硝酸盐还原铁氧化菌(Nitrate-reducing Fe(Ⅱ)-oxidizers)四类[3,17]。

2.1　嗜酸铁氧化菌

嗜酸铁氧化菌通常生存于酸性生境(pH 为 1.0~4.0),如酸性浸出物、酸性矿山废水(AMD)、深海热液和热泉等富含铁、硫及其他金属元素的环境。嗜酸铁氧化菌主要归属于古菌的广古菌门和泉古菌门,以及细菌的(Alpha-、Beta-、Gamma-)变形菌门、放线菌门、厚壁菌门、嗜酸菌门和硝化螺菌门。(Alpha-、Beta-)变形菌门、嗜酸菌门和硝化螺菌门为革兰氏阴性菌,其余均为革兰氏阳性菌[18]。嗜酸铁氧化菌的生存多以单质 S 或 Fe(Ⅱ)为电子供体,以氧气(O_2)、硫酸盐(SO_4^{2-})或硝酸盐(NO_3^-)为电子受体,并以有机碳或无机碳为碳源。酸性环境中生物可利用的 Fe(Ⅱ)稳定存在,因此,铁氧化微生物可以轻松地与氧气介导的非生物氧化过程竞争获得 Fe(Ⅱ)。酸性铁氧化菌以 Fe(Ⅱ)为电子供体、氧气为电子受体的生长过程可表示为:

$$4Fe^{2+}+4H^{+}+O_2\rightarrow4Fe^{3+}+2H_2O \qquad (2)$$

第一株酸性铁氧化菌 *Acidithiobacillus ferrooxidans* 由 Colmer 及其同事于 1947 年分离[19]。目前已有包括 *Acidithiobacillus ferrooxidans*、*A. ferrivorans*、*Thiobacillus prosperus*、*Leptospirillum ferroxidans*、*L. ferriphilum* 等在内的数种酸性铁氧化菌被分离并得到进一步研究[18],其在环境修复和生物浸矿领域已有广泛地研究和应用[20-22]。

2.2　中性微氧铁氧化菌

中性微氧铁氧化菌同样可以 Fe(Ⅱ)为电子供体、O_2 为电子受体,并以有机碳或无机碳为碳源维持其生长。所不同的是,这类微生物通常分布于 O_2 浓度远低于空气(O_2<50 μmol/L)的土壤、氧化还原分层水体中的有氧-无氧交互界面、植物根际土壤、地下水渗流带、海底沉积物及其它氧气浓度较低的中性环境中。

目前已知的中性微氧铁氧化菌隶属于 Beta-变形杆菌纲和‘Zeta-’变形杆菌纲,前者主要存在于淡水环境[23-24],后者主要存在于海水环境[10,25],它们可形成微生物席(microbial-mat)以营造适宜的生长化学梯度[10,26-28],提高其生存能力。这类微生物胞外具有螺旋柄状(twisted stalks)、发瓣状或鞘状结构的典型特征[29-30],该特征已成为识别这类微生物的重要标志[27,31]。在氧浓度较低的中性及近中性条件下,Fe(Ⅱ)的非生物氧化速率较低,微生物介导的铁氧化过程处于主导地位。淡水环境培养实验表

明,微氧铁氧化菌对低氧浓度环境中铁的氧化贡献达 40% ~ 50%,最高甚至可达 75%[26-27,32]。以化能自养微氧铁氧化菌为例,其介导的 Fe(Ⅱ)氧化过程可表示为:

$$4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3\downarrow + 8H^+ \quad (3)$$

中性微氧铁氧化菌 *Gallionlla ferruginea* 最早于 1836 年被 Ehrenberg 发现,直到 20 世纪才首次获得纯化分离[33]。改进的微生物培养方法提出后[34],如包括 *Gallionella*、*Leptothrix*、*Mariprofundus* 和 *Ghiorsea* 菌目在内的多种铁氧化微生物获得分离[10]。目前,中性微氧铁氧化菌的研究主要集中在分离纯化新菌、探究铁氧化电子传递路径以及作为地质微生物探索其在早期或现代地球环境中的作用等方向[10,27]。

2.3　中性厌氧光合铁氧化菌

中性厌氧光合铁氧化菌可定殖于淡水、海水、沼泽湿地和土壤沉积物等富含 Fe(Ⅱ)的浅表层透光环境,是重要的初级生产者。环境中厌氧光合铁氧化菌包括 Gamma-变形菌纲中的紫色硫细菌(Purple Sulfur Bacteria,PSB)、Alpha-变形菌纲中的紫色非硫细菌(Purple Non-Sulfur Bacteria,PNSB)和绿菌门中的绿硫菌(Green Sulfur Bacteria,GSB)[4,10]。中性厌氧光合铁氧化微生物代谢方式具有多样性,除 Fe(Ⅱ)外,还可利用 H_2 或 H_2S 作为电子供体,以有机碳或无机碳作为电子受体进行生长繁殖[4,35]。厌氧光合铁氧化菌是厌氧环境中重要的初级生产者[36],其以光为能源、以 Fe(Ⅱ)作为电子供体、以碳酸盐或二氧化碳作为电子受体营化能自养生长时的代谢过程可表示如下。

$$4Fe^{2+} + HCO_3^- + 10H_2O + \text{light}(h\nu) \rightarrow \langle CH_2O\rangle + 4Fe(OH)_3\downarrow + 7H^+ \quad (4)$$

环境中的中性厌氧光合铁氧化微生物的首次分离获得是由 Widdel 等[37]在 20 世纪末期完成的。近 30 年已经在多种环境中分离纯化出众多的厌氧光合铁氧化菌[4],包括 PSB 中的 *Thiodycton* sp. F4,PNSB 中的 *Rhodobacter ferrooxidans* SW2[38]、*Rhodopseudomonas palustris* TIE-1、*Rodovulum iodosum* 和 *Rodovulum robiginosum* 等,和属于 GSB 的 *Chlorobium ferrooxidans* KoFox、*Chlorobium phaeoferrooxidans* 和 *Chlorobium* sp. N1 等。研究证明,中性厌氧光合铁养化微生物对不同的 Fe(Ⅱ)浓度、盐度、温度和光照强度具有较强的适应性和耐受性[4,39-42],因此它能够在更广阔的生态位上生存,可能表明铁氧化微生物是早期地球上潜在的生命形式。该类微生物是研究地球早期生命与环境演化的重要模型[43-46]。

2.4　中性厌氧硝酸盐还原铁氧还菌

中性厌氧硝酸盐还原铁氧化菌在黑暗和光照环境中均可生存,在环境中的分布较为广泛,常存在于淡水湖泊底泥、土壤沉积物、高盐环境、海底沉积物和热液喷口等[47-51]。中性厌氧硝酸盐还原铁氧化微生物在 Alpha-、Beta-、Gamma-、Delta-变形菌门和放线菌门,以及古菌(如 *Ferroglobus placidus*[52])中均有所分布[4]。中性厌氧硝酸盐还原铁氧化菌是营化能厌氧(lithotrophic)生长的微生物,可利用亚铁或有机物(如乙酸盐)作为电子供体,利用硝酸盐作为电子受体进行生长繁殖。厌氧硝酸盐还原铁氧化菌在氧化 Fe(Ⅱ)的同时耦合 NO_3^- 还原的过程可以表示为[4]:

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3\downarrow + N_2\uparrow + 18H^+ \quad (5)$$

该式产物中,除产生 N_2 外,还可产生 NO、N_2O 等温室气体[4]。

20 世纪 90 年代,环境中多株硝酸盐还原铁氧化菌和富集培养物陆续获得分离[52-54]。依据硝酸盐还原微生物介导铁氧化方式的不同,其可以被分为三类[3-4,10]。第一类,仅以 Fe(Ⅱ)为电子供体产生能量,以无机碳为碳源(营固碳作用)维持细胞生长的自养型(Autotroph)硝酸盐还原铁氧化菌。迄今为止,从环境中分离获得的自养硝酸盐还原铁氧化菌均为富集培养物且只有三份:淡水沉积物中分离到的富集培养物 KS(Enrichment culture KS)[55]、富集培养物 BP(Enrichment culture BP)[56]和一株从地下水中富集到的纯培养物(未命名)[57]。第二类,以 Fe(Ⅱ)为电子供体,同时需要额外添加有机物(如乙酸盐)维持细胞生长的混合营养型(Mixtroph)硝酸盐还原铁氧化菌。第三类,需添加有机物作为电子供体、碳源和能源,微生物不直接氧化 Fe(Ⅱ),而是以异养反硝化过程产生的活性氮中间产物(如 NO_2^-、N_2O、NO 等)实现 Fe(Ⅱ)间接氧化的化能反硝化菌(Chemodenitrifiers,如大肠杆菌)。而对于目前分离纯化获得的厌氧硝酸盐还原铁氧化菌单菌株则多介于第二类和第三类,两类微生物诱导铁矿化时往往会导致其细胞结壳、生长受阻[4,58]。目前对其的研究包括探究铁氧化机理及环境效应[4,56],以及作为地质微生物研究其在早期地球环境演化中的作用等[59-60]。

3 微生物铁氧化机制及其电子传递路径

20 世纪末，DNA 测序、基因芯片和质谱等技术的推广应用深化了学界对环境中微生物介导的铁氧化过程的认识[18,35,61-62]。在四类铁氧化微生物中均发现潜在的铁氧化酶 Cyc2 的存在，表明 Cyc2 可能是研究环境中微生物介导的铁氧化过程的重要生物标志物。对微生物铁氧化机制的研究发现，Fe(Ⅱ)氧化多发生于外膜细胞色素（图 1），这与微生物生理学及能量学相符[12]。从生理学及能量学角度讲，若 Fe(Ⅱ)在微生物胞内或周质中被氧化，将消耗微生物额外的能量。微生物需要将 Fe(Ⅲ)运出胞外，以避免其在胞内或周质中沉淀而导致细胞死亡[4,12]。一般而言，微生物铁氧化电子传递路径为 Fe(Ⅱ) → 外膜细胞色素 → 周质蛋白 → 内膜（膜结合细胞色素以及所有终端细胞质膜氧化酶 → 电子受体（O_2、NO_3^-、H_2O 等），且符合 Ingledew 提出的电子分叉原理[12,18,63-64]：微生物从 Fe(Ⅱ)获得的电子除顺氧化还原电位（Downhill）流向电子受体外，部分还将逆氧化还原电位（Uphill）传输至 NADH 脱氢酶（Complex Ⅰ）以产生 NADH 用于固碳或其他生命活动，逆氧化还原电位的传递需要耗费相当的能量才能顺利进行[12,65]。

3.1 酸性条件下微生物铁氧化机制及电子传递路径

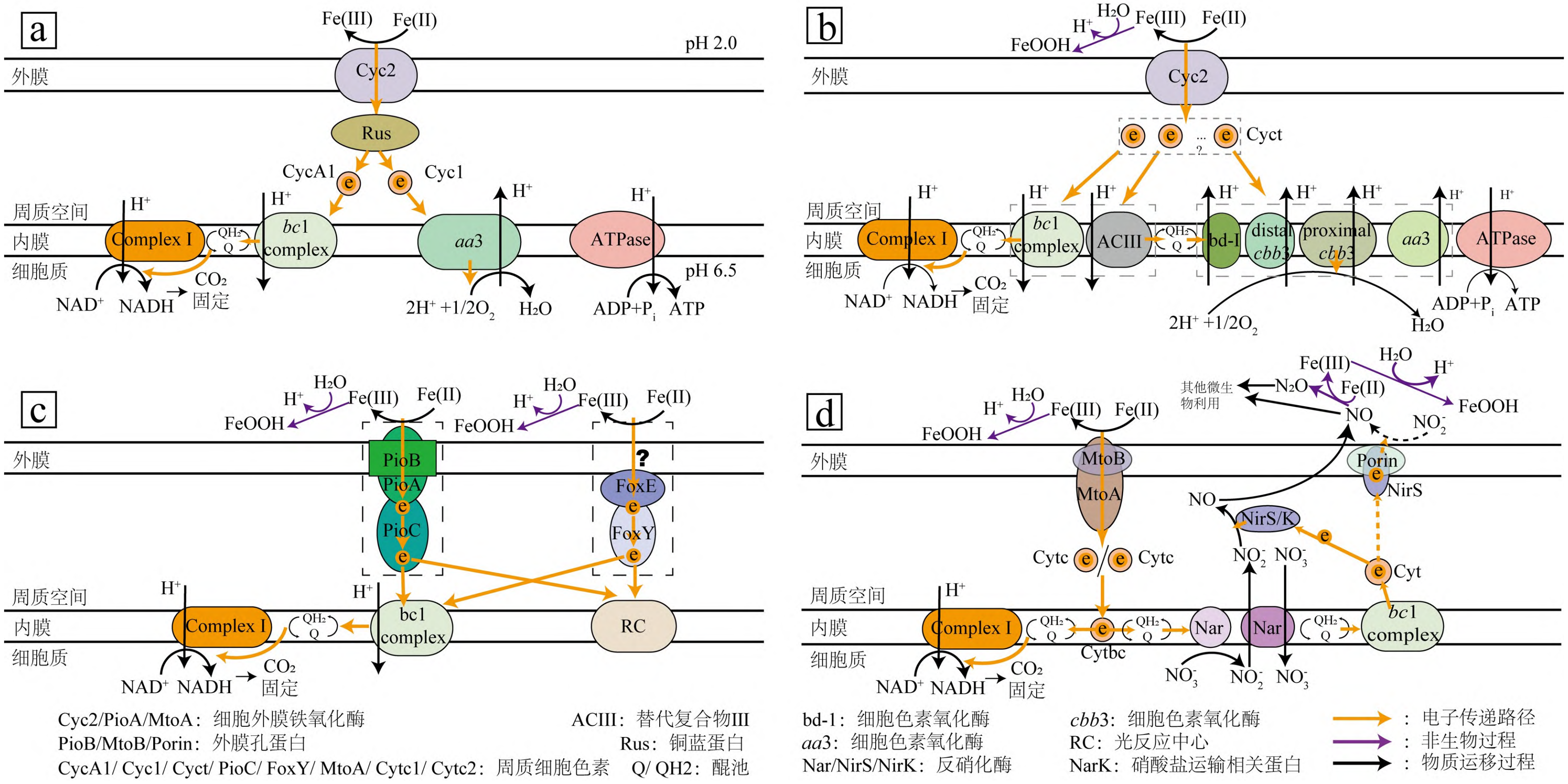
酸性条件下，微生物需要面对细胞内外巨大的质子浓度差，而该类微生物通过在细胞膜上的 ATP 合酶（ATPase）可利用这一质子浓度差产生 ATP 以维持其生命活动。目前，酸性铁氧化菌研究涉及到的关键铁氧化酶主要有 Cyc 2[20,69]、Cyc 572[70-71]、Cyt b573[72]、Sulfocyanin[73]和 FoxCD[74-75]等，它们均位于细胞外膜[65]。以现阶段研究较多的酸性铁氧化菌 *Acidithiobacillus ferrooxidans* 为例[20]（图 1a），电子在细胞色素 Cyc2 从 Fe(Ⅱ)顺氧化还原电位传递到 O_2 的氧化过程为：Fe(Ⅱ) → Cyc2 → Rus → Cyc1 → Cyt aa3 → O_2[20,66]；电子从 Fe(Ⅱ)逆氧化还原电位传递至醌池以合成 NADH 的还原过程为：Fe(Ⅱ) → Cyc2 → Rusticyanin → Cyc A1 → bc_1 → Complex Ⅰ → NADH[20,66]；*Acidithiobacillus ferrooxidans* 细胞通过调节铜蓝蛋白（Rusticyanin）分支上的电子流，实现产生 NAD^+ 以还原无机碳及还原 O_2 二者间的平衡[12]。

3.2 中性微好氧微生物铁氧化机制及电子传递路径

中性铁氧化菌为避免细胞结壳，Fe(Ⅱ)极有可能是在细胞外而不是细胞内或周质中被氧化[76]。研究发现，几乎所有已分离的微氧铁氧化菌的基因组中都存在编码细胞色素与孔蛋白的融合体——Cyc2 的基因[61,67,76-79]，而且在培养环境富含铁时，该基因维持较高的表达水平，并随着铁含量增加其表达量上调[67]。最新研究证明，Cyc2 是介导胞外铁氧化过程的关键铁氧化酶[80]，它们可能是指示环境中铁氧化过程发生的重要生物标志物[10]。以近年研究较完善的中性微氧铁氧化菌‘*Zeta*-’变形菌纲的电子传递过程为例[25,67]（图 1b），其顺氧化还原电位电子传递的氧化过程为：Fe(Ⅱ) → Cyc2 → Cyc1（或 Cyt） → Cyt cbb_3（或 Cyt aa_3，Cyt bd-1） → O_2；逆氧化还原电位的还原过程为：Fe(Ⅲ) → Cyc 2 → bc_1（或 ACIII） Complex → Complex Ⅰ → NADH[67]。与酸性铁氧化菌对比，该类菌或可通过调节氧化过程中 Cyc1（或 Cyt）处的电子流实现 NAD^+ 及 O_2 二者还原和氧化间的平衡[67]。

3.3 中性厌氧光合微生物铁氧化机制及电子传递路径

中性厌氧条件下，对光合铁氧化微生物介导的铁氧化电子传递过程的研究较少，主要集中在 *Rhodopseudomonas palustris* TIE-1 和 *Rhodobacter ferrooxidans* SW2 两株菌上[4,10]。涉及到铁氧化的关键基因是 *Rhodopseudomonas palustris* TIE-1 的 *pio* 操纵子[81-82]和 *Rhodobacter ferrooxidans* SW2 中的 *fox* 操纵子[83]，二者不同源。*pio* 操纵子的三个基因分别编码的蛋白为：周质十血红素 C 型细胞色素的 PioA、外膜桶状蛋白 PioB 和周质高电势铁硫簇蛋白 PioC，其中 PioA 嵌入 PioB 构成一个嵌合体[81,83]。*fox* 操纵子的三个基因分别编码蛋白：周质十血红素 C 型细胞色素（也是潜在的铁氧化蛋白）FoxE[84-85]、推测的醌蛋白 FoxY 和推测的周质电子传递蛋白 FoxZ[86]，对蛋白 FoxZ 的功能和定位目前尚不清楚，尚需进一步研究。*Rhodopseudomonas palustris* TIE-1 和 *Rhodobacter ferrooxidans* SW2 的光合铁氧化过程[4]（图 1c），顺氧化还原电位的电子传递途径为：Fe(Ⅱ) → PioA/PioB（FoxE）→ PioC/PoxY → RC；逆氧化还原电位的电子传递途径为：Fe(Ⅱ) → PioA/PioB（FoxE）→ PioC/ FoxY → bc_1 Complex→ Complex Ⅰ → NADH。事实上，厌氧光合微生物其铁氧化方式灵活多样，单一的电子传递模式无法囊括



a为酸性条件下*Acidithiobacillus ferrooxidans*的铁氧化电子传递路径模式图，改自Quatrini等[66]；b为中性微氧条件下Zeta-变形菌纲的铁氧化电子传递路径模式图，改自McAllister等[67]；c为中性厌氧条件下光合微生物*Rhodopseudomonas palustris* TIE-1和*Rhodobacter ferrooxidans* SW2的铁氧化电子传递模式图，改自Bryce等[4]；d为中性厌氧条件下富集培养物Culture KS中的*Gallionellaceae* sp. 的铁氧化电子传递路径模式图，改自He等[68]和Bryce等[4]。

图1　微生物铁氧化机制及其电子传递路径

Fig.1　Schematics of the current hypotheses on the mechanism of Fe(II) oxidation

所有厌氧光合铁氧化微生物的铁氧化过程[4]。例如,近期对远洋环境中分离的厌氧光合铁氧化菌 *Chlorobium phaeoferrooxidans* 的基因组研究发现,其中存在编码外膜细胞色素 Cyc2 的相关基因[87-88],而细胞色素 Cyc2 在其他几类铁氧化微生物中均存在,表明这几类铁氧化微生物间存在着某种潜在的联系。对环境中厌氧光合铁氧化微生物机理及铁氧化电子传递路径有待进一步研究。

3.4 中性厌氧硝酸盐还原微生物铁氧化机制及电子传递路径

近几年,关于硝酸盐还原的铁氧化微生物的富集培养物的研究报道较多,对富集培养物 Culture KS 的基因组地研究发现,其中存在编码铁氧化酶 Cyc2、细胞色素 C-孔蛋白复合物 MtoAB 和细胞色素 C-孔蛋白复合物 PcoAB 基因的同系物,它们可能均为铁氧化酶[68,89]。该类细胞的铁氧化途径是,从 Fe(Ⅱ)处获得的电子经由外膜蛋白-周质蛋白和内膜相关蛋白,进行固碳以及硝酸盐还原等相关细胞生命活动。以化能自养硝酸盐还原铁氧化菌 *Gallionellaceae* sp. 富集培养物为例(图1d),其顺氧化还原电位还原硝酸盐的电子传递途径氧化过程为 Fe(Ⅱ) → MtoAB → Cytc1/Cytc2 → Cytbc → Nar → NO_3^- → bc_1 → Cytc → NirS/K → NO_2^-;逆氧化还原电位产生还原力固碳的还原过程为 Fe(Ⅱ) → MtoAB → Cytc1/Cytc2 →bc1 complex → Complex Ⅰ → NADH。需要说明的是,以有机物作为共底物的混合营养型硝酸盐还原铁氧化菌的亚铁氧化电子传递机制可能更为复杂。事实上,这类硝酸盐还原铁氧化菌是否能进行亚铁的酶催化氧化仍存争议[58,90-95]。迄今为止,还未发现任何直接证据证明混合营养型硝酸盐还原铁氧化菌中存在铁氧化酶,这类菌的铁氧化机制仍需进一步的研究。

4 铁氧化微生物的应用

4.1 铁氧化微生物在地球早期研究的作用

4.1.1 铁氧化微生物是探究早期生命演化的重要模式生物

在地球诞生至大氧化事件(46~25 亿年)的大部分时间里,海水总体上被低氧(低于十万分之一现今大气氧浓度)、富铁($(0.4\sim1.2)\times10^{-4}$ mol/L)、贫硫的还原性环境所主导[96-100]。约 32~29 亿年前,产氧光合微生物的出现使得大气氧含量逐步升高,直到约 24~23 亿年前,以大气氧含量跃升为标志的大氧化事件的发生[101-102]使得海水表层逐渐被氧化。研究表明富含亚铁的铁化海洋可能在地质历史时期一直存在[103-104]甚至短暂的主导海水化学条件[99,105]。事实上,当下环境中仍然存在少量类似的铁化水体[47,106-108]。地质时期长时间存在的富铁水体为铁氧化菌的活动提供了丰富的生态位和演化空间[105]。由铁氧化菌和铁还原菌共同驱动的铁循环可能是地球早期主要的生物地球化学循环,进一步带动了环境中 C、N、O、S 等元素的地球化学循环[109-110]。现代环境中铁氧化微生物分布广泛、门类众多、在各个生境中均有所发现[4-5,17],其极有可能是地球早期铁氧化微生物环境适应的结果[44,60],因此现代环境中的铁氧化微生物是研究地球早期生命演化的重要模式生物[59]。

4.1.2 微生物介导的铁氧化是解释前寒武条带状铁建造的成因的重要模型

环境中铁氧化微生物的研究为揭示地球早期沉积的条带状铁建造(BIF)的成因提供了一个较为合理的解释。条带状铁建造广泛分布于太古宙与元古宙的多个时期,在新太古代和古元古代的沉积达到巅峰(27~24 亿年)[111-112]。在对铁氧化微生物开展研究前,对条带状铁建造成因的解释主要为非生物作用,如热液成因、紫外线对铁的氧化作用[111]、紫外线光解水产生氧气导致铁氧化[113],以及部分生物作用,即产氧光合生物产生的溶解氧进行的化学氧化[114]。然而上述解释均被证明不能或不足以满足地球早期 BIF 形成所需的三价铁沉积速率[9,110,113]。随着更多环境中铁氧化微生物的不断发现和分离,以及研究的不断推进,铁氧化微生物可作为解释前寒武 BIF 形成的重要模型[9,110]:(1)产氧光合作用前,地球仍处于缺氧富铁贫硫的环境,厌氧光合铁氧化微生物和厌氧硝酸盐还原铁氧化微生物在早期地球铁的氧化沉积可能扮演着重要角色[39,113,115-116];(2)产氧光合作用出现后,随着大气和海洋的逐渐氧化,微氧铁氧化菌在这种微氧条件下可能极大的促进了海水铁的氧化和沉积[27,93]。

4.2 铁氧化微生物在现代生产生活中的应用

4.2.1 铁氧化微生物在氮、磷、硫以及重金属去除中的应用

NO_3^-、PO_4^{3-}、SO_4^{2-} 以及重(类)金属等是现代环

境中较为常见的污染物,除运用已有的物理和化学等污染修复手段,利用铁氧化微生物进行生物修复也是解决这些问题的重要策略[117]。在环境重金属的去除方面,与人工合成的吸附剂相比较,铁氧化微生物矿化作用产生的(羟基)铁氧化物对重金属具有更好的吸附性能,且能通过共沉淀作用最大限度的去除环境中的重金属[118-121]。因此铁氧化微生物有望成为土壤或水体环境中重金属污染生物修复的重要材料。Katsoyiannis 等[122]发现微生物铁氧化产物能够有效吸附去除饮用水中的砷,Ahoranta 等[123]利用铁氧化微生物产生的黄钾铁矾实现对酸性溶液中砷和铁的有效去除,表明铁氧化微生物物在水处理中的广泛应用前景。在环境磷去除方面,铁氧化微生物介导的成矿过程对磷酸盐具有较好的共沉淀和吸附去除效能[32,119],可能是潜在的除磷方法。例如,Buliauskaitė 等[124]在 *Leptothrix* sp. 和 *Gallionella* sp. 对溶液中磷的去除实验中,发现其具有较好的磷去除能力。在硫酸盐或硝酸盐污染修复方面,铁氧化菌还被用于地下水和工业废水的硝酸盐与硫酸盐等常规污染物质的生物去除[58,125-127]。Jakus 等[57]发现从含黄铁矿的岩溶地区分离到的厌氧硝酸盐还原铁氧化菌富集培养物在贫有机质环境中良好的自养生长和硝酸盐还原能力,这一发现表明该富集培养物在工业废水或岩溶地区地下水硝酸盐污染去除的良好应用前景。值得注意的是,以上铁氧化菌在环境污染的生物修复研究仍停留在实验阶段,其离在环境中大规模应用仍有差距。

4.2.2　铁氧化微生物在合成新材料领域中的应用

微生物介导的生物矿化产物已在储能材料领域中展现出较好的应用前景。前人发现革兰氏阴性硝酸盐还原铁氧化菌 *Acidovorax* sp. 的生物矿化产物 γ-FeOOH 经处理后具有良好的储能性能。在该菌生物矿化过程中,铁氧化物(γ-FeOOH)被限制在细菌细胞壁的的两层膜之间。经高温加热处理后,γ-FeOOH 被转化为具有细菌形态的中空的多孔贝壳状结构的赤铁矿(α-Fe_2O_3)纳米晶体。这些晶体在与锂反应时表现出更强的充放电的电化学可逆性。与相同尺寸的非织构化 α-Fe_2O_3 颗粒相比,其具有更高的反应速率。这种细菌诱导的生物矿化合成策略为电化学能量存储提供了一种高效和可扩展的方法[128]。此外,由趋磁细菌生物矿化作用所产生的磁铁矿纳米颗粒,由于其较窄的尺寸分布、统一的形态结构和较低的毒性,以及含有可防止颗粒外溢的生物膜等独特性质,使其在新材料领域颇受关注[129]。近期研究表明,*Acidithiobacillus ferrooxidans* 具有与趋磁细菌类似的功能,预示其在该领域潜在的应用价值[130-131]。

4.2.3　铁氧化微生物在生物浸矿中的应用

与非生物冶金技术相比,生物浸矿技术具有低能耗、低成本和低环境危害性等特点,在金属回收方面具有明显优势。铁氧化微生物在生物浸矿领域有着较大的应用潜力,嗜酸铁氧化菌 *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* 等或其混合培养物已被用于从金属贫矿[132、工厂二次废弃物[133]和电子废弃物(e-waste)[134-140]等回收贵金属的相关研究。利用酸性铁氧化菌进行生物浸矿的研究主要集中于提升贵金属浸出效率[138,140-141],例如 Wei 等[138]应用直流电场促进 *Acidithiobacillus ferrooxidans* 的生长,将电子废弃物中的 Cu 的 100%浸出时间由 5 天缩短至 3 天,大幅提升了铜的浸出效率。也有研究致力于使用分子生物学技术实现贵金属元素选择性回收,Jung 等[142]利用基因编辑技术将多组氨酸(polyhistidine)附加在 *Acidithiobacillus ferrooxidans* 上的两个内源重组表达蛋白上,发现细胞对钴和铜的结合能力显著增强,且对钴有明显的结合偏好,从而使得混和金属溶液中的贵金属选择性生物浸出成为可能。

5　小结

迄今为止,对环境中的铁氧化微生物物种和生态多样性、铁氧化机制和环境影响的研究已经取得了较大的进展[3,10]。研究表明,铁氧化微生物在酸性到中性环境中均有所分布,既有细菌也有古菌,既可作为研究地球早期生命演化以及前寒武条带状铁建造成因的微生物模型,也可作为环境生物修复、新材料研发以及生物冶金的重要生物资源。然而,环境中铁氧化微生物从细胞材料、代谢机理和地球化学效应等方面的研究尚有待完善。

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Advances in Microbial Iron Oxidation and Its Application

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Abstract: Iron ranks fourth in abundance among crustal elements and can be taken up by microbial assimilation as a biologically essential element or as an electron source in microbial metabolism. Microbially mediated iron oxidation is an important part of the iron cycle and thus drives the biogeochemical cycling of biogenic elements such as C, N, O and S in the environment, which is strongly associated with the emission or storage of carbon and nitrogen, the greenhouse effect, and fate of nutrients and toxic metals or metalloids. The microorganisms involved in Fe(Ⅱ) oxidation can be classified into four groups based on their growth environment and electron acceptor status: acidophilic Fe(Ⅱ) oxidizers, neutral microaerobic Fe(Ⅱ) oxidizers, neutral anaerobic photosynthetic Fe(Ⅱ) oxidizers and neutral anaerobic nitrate reducing Fe(Ⅱ) oxidizers. A summary of the proposed mechanisms of electron transport in microorganisms involved in iron oxidation shows that they all share a common paradigm, that is, ferrous iron is oxidised on outer membrane cytochromes and the electrons obtained from ferrous iron are transmitted from the extracellular membrane through the periplasmic electron transport proteins to various proteins in the inner cell membrane for carbon sequestration and/or reduction of electron acceptors. The study of microorganisms involved in iron oxidation in modern environments has been widely applied to the evolution of early life on Earth, the bioremediation of environmental pollution as well as the synthesis of new materials and bioleaching. This paper reviews the microbial phylogenetic types and electron transfer mechanisms that mediate iron oxidation processes and describes their applications in geology, environment, materials and metallurgy. A great deal of work remains to be done on the isolation and identification of iron-oxidizing bacteria, the detailed analysis of iron-oxidizing metabolic pathways and their geochemical effects, the establishment and improvement of the iron-oxidizing microbiome database, and the application of iron oxidation in environmental pollution.

Key words: iron oxidizers; electron transfer mechanisms; early evolution of life; bioremediation