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Genomic insight into iron acquisition by sulfate-reducing bacteria in microaerophilic environments

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Abstract Historically, sulfate-reducing bacteria (SRB) have been considered to be strict anaerobes, but reports in the past couple of decades indicate that SRB tolerate exposure to O_2 and can even grow in aerophilic environments. With the transition from anaerobic to microaerophilic conditions, the uptake of Fe(III) from the environment by SRB would become important. In evaluating the metabolic capability for the uptake of iron, the genomes of 26 SRB, representing eight families, were examined. All SRB reviewed carry genes (*feoA* and *feoB*) for the ferrous uptake system to transport Fe(II) across the plasma membrane into the cytoplasm. In addition, all of the SRB genomes examined have putative genes for a canonical ABC transporter that may transport ferric siderophore or ferric chelated species from the environment. Gram-negative SRB have additional machinery to import ferric siderophores and ferric chelated species since they have the TonB system that can work alongside any of the outer membrane porins annotated in the genome. Included in this review is the discussion that SRB may use the putative siderophore uptake system to import metals other than iron.

Keywords Sulfate-reducing bacteria (SRB) · Siderophores · *Desulfovibrio* · Microaerophilic · Metal recovery

Introduction

Sulfate-reducing bacteria (SRB) are broadly distributed throughout the biosphere and are an ecological and physiological group of microorganisms known for anaerobic growth, performing dissimilatory reduction of sulfates (Widdel et al. 2007). However, although commonly referred to as obligate anaerobes, SRB have systems to prevent cell death when exposed to O_2 (Barton and Fauque 2022). SRB grow at the oxic-anoxic interface in waste water biofilms (Santegoeds et al. 1998; Okabe et al. 2005), microbial mats (Canfield and Des Marais, 1991; Krekeler et al. 1997; Teske et al. 1998; Minz et al. 1999), lake sediments (Sass et al. 1997), marine environments and sediments (Jørgensen and Bak 1991; Teske et al. 1996), in marine intertidal zones (Smith et al. 2019), on the surface and inside cells of plant roots (Küsel et al. 1995; Blaabjerg

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and Finster 1998; Nielsen et al. 2001), on plastic substrates (Celis et al. 2009), carbon steel structures (Phan et al. 2021), in the oral cavity of humans (Campbell et al. 2013; da Silva et al. 2014) and the intestine of animals (Kushkevych 2017; Ran et al. 2019) including the human gut (Gibson et al. 1993; Jia et al. 2012). Iron (Fe) mainly exists in one of two readily inter-convertible redox states: the reduced ferrous form, Fe(II), and the oxidized ferric form, Fe(III). Although iron is an abundant metal on Earth, under oxidizing conditions, Fe(III) is extremely insoluble (10^{-18} M at pH 7.0) (Andrews et al. 2003), therefore, making it a limiting factor for bacterial growth (Neilands 1995). In order to counteract this limitation, under iron-restricted conditions, aerobic bacteria produce siderophores to sequester Fe(III) from the aerobic environment and facilitate iron uptake (Winkelmann 2001; Hider and Kong 2010). In general, each species of bacteria produces a siderophore with a unique molecular structure (Kramer et al. 2020).

At this time, the evaluation of ferric ion uptake in SRB has been limited to a report of siderophore production by *Desulfotomaculum acetoxidans* DSM 771 (Pado and Pawłowska-Ćwięk 2005), and the presence of siderophore genes in *Desulfovibrio vulgaris* Hildenborough (Bender et al. 2007) and uncultivated SRB (Osorio et al. 2008; Campbell et al. 2013). The purpose of this article is to shed light, from a genomic perspective, into this knowledge gap, unravelling the relation between SRB microaerophilic and their need for iron acquisition. The primary focuses of this review is on Gram-negative SRB because of their abundance in the environment. Sulfate-reducing archaea are not included in the review because their exposure to oxygen has received little attention. This review summarizes oxygen-dependent growth of SRB where the O_2 levels are microaerophilic (0.02%) or near atmospheric levels (18%), and reports on a genome search of 26 SRB, representing eight families, for annotated genes, which are appropriate for Fe(II) and Fe(III) uptake. Since SRB are often found in environments containing metals, the potential for other metal chelated ions uptake of by SRB is also discussed.

Methods

SRB genomes analysis was made through search in each sequenced bacterial strain genome that is available at the Joint Genome Institute database (https://jgi.doe.gov/). To predict protein topology, namely transmembrane helices or beta sheets, signal peptide and membrane orientation, the information available at the Joint Genome Institute database was combined with information retrieved when submitting the protein primary sequence to the DeepTMHMM prediction website (https://dtu.biolib.com/DeepTMHMM), which was developed by the Technical University of Denmark (Hallgren et al. 2022). Primary protein sequence alignments were built with ClustalX 2.0 and Genedoc program was used for final figure preparation.

Oxygen respiration and microaerophilic growth of sulfate-reducing bacteria

Several SRB strains have been reported to oxidize H_2, formate, lactate, pyruvate, butyrate, ethanol, and acetate with O_2 reduction (Dannenberg et al. 1992). SRB tolerance to O_2 and its use as a terminal electron acceptor have been reviewed (Cypionka 2000; Sass and Cypionka 2007). *D. desulfuricans* strain CSN and *Desulfobulbus propionicus* have been reported to oxidize sulfite and sulfide to sulfate (Dannenberg et al. 1992). Using cells from anaerobic culture, several species of SRB display significant rates of O_2 respiration with H_2, lactate, pyruvate or endogenous polyglucose as electron donors (Table 1). Some species of SRB display reduction of O_2 at microaerophilic conditions, while others at atmospheric level. As presented in Table 1, the reduction of O_2 is characteristic of several different genera of SRB. The oxidation of internal reserves of polyglucose by whole cells is coupled to the reduction of O_2 by anaerobically grown *D. gigas* (Santos et al. 1993) and *D. salexigens* (van Niel et al. 1996). The greatest respiration rate with O_2 was reported for the SRB isolated from termites, *D. termitidis,* with 1570 nmol of O_2 reduced min^{-1} mg^{-1} of protein (Kuhnigk et al. 1996).

SRB have defense systems against oxygen stress and a constitutive respiratory systems for the reduction of O_2. To survive O_2 toxicity, *D. vulgaris* Hildenborough has a defense system which includes the following: superoxide dismutase, catalase, rubrerythrin, nigerythrin, thiol-specific peroxidases, hydroperoxidase, [Fe] hydrogenase, cytochrome *bd* oxygen reductase, and haem-copper oxygen reductase, which is a product of *cox* genes and rubredoxin:oxygen

Table 1 Microaerophillic SRB growth, their O_2 tolerance and respiration rates

Bacterial isolate	O_2 (%)	Respiration rate (nmol O_2 min^{-1} mg $protein^{-1}$)	Electron donor	References
Desulfovibrio desulfuricans CSN	4	250	H_2	Dilling and Cypionka (1990)
D. salexigens Mast1	Air saturated*	3–50	Polyglucose	van Niel et al. (1996)
D. salexigens Mast1	Air saturated	25	Pyruvate	van Niel and Gottschal (1998)
D. salexigens DSM 2638	Air saturated	20	Pyruvate	van Niel and Gottschal (1998)
D. gigas NCIMB 9332	Air saturated	40	Lactate	van Niel and Gottschal (1998)
D. desulfuricans ATCC 27,774	Air saturated	38	Lactate	van Niel and Gottschal (1998)
D. desulfuricans CSN	Air saturated	40	Lactate	van Niel and Gottschal (1998)
D. desulfuricans BH	Air saturated	16	Lactate	van Niel and Gottschal (1998)
D. oxyclinae P1B	5	n.d	Lactate**	Sigalevich and Cohen (2000)
D. oxyclinae N13	5	9	Lactate	Sass et al. (2002)
D. norvegicum Norway	5	4	Lactate	Sass et al. (2002)
Desulfomicrobium sp. Sal	5	4	Lactate	Sass et al. (2002)
Desulfomicrobium sp. AcI.2	5	38	Lactate	Sass et al. (2002)
Desulfobulbus sp. 86FS1	5	27	Lactate	Sass et al. (2002)
Desulfovibrio magneticus RS-1	~13%	128	Fumarate**	Lefèvre et al. (2016)

n.d. not determined

*Saturated = ~21%

**In the absence of SO_4^{2-} or $S_2O_3^{2-}$

oxidoreductase (Dolla et al. 2006). Similarly, *D. gigas* has two genes for superoxide dismutase with one designated as neelaredoxin, three genes for rubrerythrins, and single genes for catalase, peroxiredoxin and rubredoxin-like protein (Morais-Silva et al. 2014). Like *D. vulgaris* Hildenborough, other strains of *D. vulgaris* (RCH1, DP4 and Miyazaki), as well as *D. desulfuricans, D. magneticus, D. fructosovorans, D. piger* and *Desulfovibrio* sp. FW1012B, contain *cydAB* and *cox* genes (Lamrabet et al. 2011). Biochemical studies using the plasma membranes of *D. vulgaris* Hildenborough established the reduction of O_2 by the quinol *bd* oxidase encoded in *cydAB* genes and a unique cytochrome *c* oxidase of the *cc(o/b)o*$_3$ type in the *cox* gene system (Lamrabet et al. 2011). These two oxidases present different O_2 affinity, with a K_M of 300 nM for the *bd*-quinol oxidase and a K_M of 620 nM for the cytochrome *cc*(*o/b*)o_3 oxidase (Ramel et al. 2013).

There is a range of optimal O_2 concentrations that was found to support SRB growth. *D. desulfuricans* NCIB 8301 grows at an oxygen level of < 0.4% (Abdollahi and Wimpenny 1990), *D. desulfuricans* (strains Essex and CSN) grows at O_2 concentrations between 0.5 and 2%, while *Desulfobacterium autotrophicum* DSM grows at < 2% oxygen (Marschall et al. 1993). Growth of *D. vulgaris* Hildenborough occurs at 0.02% O_2, while *D. oxyclinae* strains P1B and N13, *D. norvegicum* strain Norway, *Desulfomicrobium* sp. strains Sal and Acl2, and *Desulfobulbus sp.* 86FS grow at 5% O_2. Higher levels of oxygen are tolerated by *D. magneticus* strain RS-1 and *D. desulfuricans* strain ATCC 27,774 at 12–14% and 18% O_2, respectively (see Table 2). The unique phenomenon of aerotaxis has been reported for several SRB including *D. vulgaris* Hildenborough (Johnson et al. 1997), *D. desulfuricans* strain DSM 9104 (Sass and Cypionka 2007) and *D. magneticus* RS-1 (Johnson et al. 1997; Lefèvre et al. 2016). Overall, SRB have a complex relation with O_2, some strains being able to tolerate oxygen levels close to air saturation, thus showing their capacity to adapt to changing environments defined by redox and oxygen gradients.

Metabolic requirement of SRB for iron

Iron in essential for many processes in bacteria, namely as a redox center in previously mentioned enzymes/proteins and respiratory complex systems, being assembled in mononuclear/binuclear Fe

Table 2 Microaerophilic growth of sulfate-reducing bacteria

Bacterial isolate	O_2	Culture system	References
Desulfovibrio termitidis	20–30 µM	1570 nmol of O_2 reduced min^{-1} mg^{-1} of protein	Kuhnigk et al. (1996)
D. salexigens	Air saturation (~20%)	Oxidizes polyglucose with O_2 as electron acceptor 3–50 nmol O_2 min^{-1} mg^{-1} protein	Van Niel et al. (1996)
D. vulgaris Hildenborough	0.02–0.04%	Growth formed a band in an oxygen gradient	Jonhson et al. (1997)
D. desulfiricans ATCC 27,774	18%	Cytochrome *c* and *b* content in aerobic grown and anaerobic grown cells was similar	Lobo et al. (2007)
D. magneticus RS-1	20–31 µM	Growth as a band at the oxic/anoxic interface	Lefèvre et al. (2016)

centers, FeS cluster and cytochromes. Glass et al. (2018), using inductively coupled plasma mass spectrometry (ICP-MS) and Synchrotron Radiation X-Ray Fluorescence Spectrometry (SXRF), found high levels of Fe (~22 mM) in SRB *Desulfococcus multivorans*, revealing the high amount of iron needed by SRB to sustain growth. A hallmark characteristic of SRB is the constitutive production of the siroheme-containing dissimilatory sulfite reductase and multiheme cytochromes *c* where the number of hemes per molecule can be one, two, four, eight, nine or sixteen (Moura et al. 1991). Additionally, cytochromes *b*, *o* and *d* are found in SRB enzymes for instance in cytochrome oxidase (*cc*/(*o*/*b*)o_3) (Lamrabet et al. 2011) and quinol oxidase (*bd*) (Lemos et al. 2001). Cytochromes *b* are essential electron carriers across the inner memebrane for fumarate reductase (Guan et al. 2018) and respiratory complexes containing FeS clusters, such as DsrMKJOP and QmoABC complexes (Pereira et al. 2011). Additionally, SRB produce ferritin and bacterioferritin (Romão et al. 2000; Macedo et al. 2002, 2003). Unique to the SRB bacterioferritin is the presence of 12 Fe-coproporphyrin III groups that are between each pair of protein subunits. In SRB, bacterioferretin is used to poise cytoplasmic redox potential to defend against oxygen toxicity and not for iron storage (Figueiredo et al. 2012).

A considerable amount of energy is used for iron metabolism as evidenced by *D. ferrophilus* strain IS5 having 95 genes for cytochrome production (Deng and Okamoto 2017; Deng et al. 2018). For growth of SRB under anaerobic conditions, iron uptake is made by specific ferrous transporter system (FeoAB), which was first reported for *Escherichia coli* by Hantke (1987), and this iron transporter is widely distributed in bacteria growing under anaerobic conditions (Lau et al. 2016). As reviewed by Cartron et al. (2006), several secondary uptake systems for ferrous transport are present in bacteria, and it is assumed that SRB also use alternate Fe(II) transporters. However, in an oxygen atmosphere, the Fe(II) level is less than the threshold of the Feo system (Niessen and Soppa 2020), and aerobic bacteria have developed a specialized system for uptake of insoluble Fe(III).

Ferric uptake regulator and iron homeostasis

Iron uptake in bacteria is commonly regulated by the Ferric Uptake Regulatory (Fur) protein, where, under adequate iron levels, Fur and free ferrous Fe function as the corepressor preventing transcription of iron uptake genes. Under iron-limited conditions, the transcription of iron uptake genes is not blocked by the Fur repressor protein, because free Fe(II) does not saturate the Fur repressor (Andrews et al. 2003). *D. vulgaris* Hildenborough possesses three Fur regulator paralogs and these include DVU0942 (Fur for iron homeostasis), DVU3095 (Peroxide operon regulator—PerR—for oxidative stress response), and DVU1340 (Zinc Uptake Regulator—Zur—for zinc homeostasis) (Hemme and Wall 2004). The Fur/Zur/PerR family of transcriptional regulators is found in many of the Deltaproteobacteria (Rodionov et al. 2004). In *D. vulgaris* Hildenborough, there are several examples where iron metabolizing proteins are regulated independent of the Fur systems, and these include: expression of a putative siderophore uptake system (DVU0650 to DVU0646), production of bacterioferritin (DVU1397), ferritin (DVU1568), [Fe] hydrogenase (DVU1771) and ferredoxin II (DVU0305) (Bender et al. 2007). While iron homeostasis in bacteria commonly is attributed to regulation

where Fe(II)-binds to the Fur repressor (Hantke and Braun 2000), some bacteria employ Diphtheria toxin repressor (DtxR) as an Fe(II)-response regulator (Hantke and Braun 2000), while others use a two-component regulatory system (Quatrini et al. 2005). However, molecular analysis indicates that *D. vulgaris* Hildenborough does not produce DtxR proteins or Fe(III)-specific histidine kinases, since appropriate response regulators have not been identified to date (Bender et al. 2007).

Genomic review for iron transporters in SRB

The export of siderophores in Gram-negative bacteria is frequently found to be associated with the three types of transport systems, namely: the ABC superfamily, the resistance-nodulation-division (RND) superfamily and the major facilitator superfamily (MFS) (Alav et al. 2021). A putative transporter for the siderophore enterobactin (*fepC*, DVU0648) was reported for *Desulfovibrio vulgaris* Hildenborough and is annotated as an iron compound ABC transporter (Bender et al. 2007). The siderophore enterobactin is considered an archetype for microbial Fe(III) transport (Raymond et al. 2003).

In *Escherichia coli,* the protein encoded by *fepC* is a ferric-enterobactin specific plasma membrane permease (Ozenberger et al. 1987). In our study, genes homologous to DVU0648 were found in all SRB genomes (Table 3), and all of these genes predict a cytoplasmic ATP binding protein, which is in the same operon as an outside substrate binding protein and a permease with 7, 8, or 10 transmembrane helices. The DVU0648 segment was compared to the genome of *E. coli* and was found to be homologous to the *fhuC/fhuD/fhuB* gene cluster or the *fceE/fceD/fceC* gene cluster, with some structural differences. This suggests that DVU0648 must be part of the machinery for the transport of chemical species across the inner membrane that can be present in any bacteria and can be, or not, specific for iron.

Bender et al. (2007) also reported in the genome of *D. vulgaris* Hildenborough a cluster of 12 genes, which includes: ABC transporters (DVU2380, DVU2384 to DVU2387), the biopolymer transporter (TolRQ; DVU2388 to DVU2389) and a transmembrane transporter for polymers across the membrane (TonB DVU2390 and TonB receptor DVU2383). In our survey of SRB genomes, the TonB system of proteins is present in all the Gram-negative SRB genomes, and absent in the genome of Gram-positive SRB of the *Peptococcaceae* family. The *tonB-exbB-exbD* genes are conserved in all the chosen SRB, with at least one copy, but there can be three copies in the same genome. TonB genes listed in Table 3 encode a proline rich protein with one transmembrane helix in the N-terminal domain (TonB), an integral membrane protein with three transmembrane helices (ExbB), and a periplasm-facing protein with only one transmembrane helix on its N-terminal domain (ExbD).

Additionally, in *D. vulgaris* Hildenborough, the DVU0799 gene encodes an outer membrane pore-forming protein with a structure of 18 transmembrane beta sheets organized in barrel shape, and permeability properties characteristic of bacterial porins (Zeng et al. 2017). An additional porin with a general diffusion channel encoded on DVU0797 has been reported for *D. vulgaris* Hildenborough along with porins in minor abundance encoded by genes DVU0273 and DVU0371 (Walian et al. 2012). The Omp-DP porin, which has a monomeric structure was isolated from *D. piger* outer membrane, and appears to be non-specific for the chemical species that transport, with an exclusion limit for transmembrane solute movement of approximately 300 Da (Avidan et al. 2008). Homologous of the DVU0799 porin were found in *D. oxyclinae, D. thermophilum and D. baculatum,* while homologus of the *E. coli* putative gene that encodes the FhuE – a rhodotorulic acid/coprogen transporter, with 22 predicted transmembrane beta sheets can be identified in the rest of the analysed genomes (see Table 3). However, the *E. coli fhuF* coding sequence, which encodes a cytoplasmic ferric-siderophore reductase active against coprogen, ferrichrome, and ferrioxamine B seems to be absent, suggesting that an alternative systems for ferric-siderophores reduction have to exist in SRB.

Biosynthesis of the enterobactin iron transport system in *Escherichia coli* has been extensively characterized, and EntS is a protein localized in the plasma membrane used to export enterobactin (Furrer et al. 2002). In this study, several strains of SRB shown an *entS,* and its presence would indicate that these SRB can have the ability to produce this siderophore (see Table 3). Primary sequence alignment of the EntS sequences identified in SRB genomes, with the *E. coli* EntS sequence, show the conservation of the four characteristic domains (see supplementary Fig. S1),

Table 3 SRB genome analysis on Fe(II) uptake system and Fe(III)/Fe(III)-siderophores uptake and efflux system

		Fe(II) uptake system [a]	ABC transporter	*tonB* [b]	*entS*	Outer membrane porin [c]
Desulfovibrionaceae						
Desulfovibrio vulgaris Hildenborough	DVU	2571	0648	2390/3101		0799*
Desulfovibrio desulfuricans ATCC 27,774	Ddes_	0646	0989	0860/1461		0429
Desulfovibrio alaskensis G20	Dde_	2669	3105	3632		1600
Desulfovibrio oxyclinae DSM 11,498	B149DRAFT_	02,475	02,820	00,834	00,686	00,152*
Desulfobacteraceae						
Desulfatibacillum alkenivorans DSM 16,219	EJ43DRAFT_	03,359	03,849	00,509/01788	02,192	04,110
Desulfatirhabdium butyrativorans DSM 18,734	G492DRAFT_	00,965	03,541	02,106		02,593
Desulfobacter curvatus DSM 3379	B147DRAFT_	02,948	04,093	03,585/02102/03890	03,908	04,663
Desulfococcus oleovorans Hxd3	Dole_	0344	1651	2211/1735		1859
Desulfobacterium autotrophicum HRM2	HRM2_	42,720	02,330	30,570	37,980	08,940
Desulfomicrobiaceae						
Desulfomicrobium escambiense DSM 10,707	G394DRAFT_	01,536	01,668	03,099	00,975	00,258
Desulfomicrobium norvegicum DSM 1741	Ga0104486_	101,760	101,458	101,175		104,268
Desulfomicrobium thermophilum DSM 16,697	BR00DRAFT_	0639	0685	1606		1407*
Desulfomicrobium orale DSM 12,838	Ga0133307_		111,097	11,421/111718		111,726
Desulfomicrobiumbaculatum X, DSM 4028	Dbac_	0012	1712	0587		0310*
Desulfohalobiaceae						
Desulfohalobium retbaense HR100	Dret_	0316	2149	1586/0914/1657		0028
Desulfonatronospira thiodismutans ASO3-1	DthioDRAFT_		1786	2505		1492
Desulfonatronovibrio hydrogenovorans DSM 9292	P771DRAFT_	2770	1262	2795		2261
Desulfobulbaceae						
Desulfobulbus japonicus DSM 18,378	G493DRAFT_	00,247	04,671	03,848/02776/04374		04,154
Desulfobulbus mediterraneus DSM 13,871	G494DRAFT_	02,034	03,547	02,793/02348	02,417	00,252
Desulfobulbus propionicus 1pr3, DSM 2032	Despr_	1856	3027	1507		3104
Desulfobulbus oralis 041	Ga0169735_	11,944	111,434	11,709		112,454
Desulfobulbus sp. Tol-SR	Ga0063341_	105,589	1,039,150	1,039,105		100,863

Table 3 (continued)

		Fe(II) uptake system [a]	ABC transporter	*tonB* [b]	*entS*	Outer membrane porin [c]
Sytrophobacteraceae						
Syntrophobacter fumaroxidans MPOB	Sfum_	2493	2133	0754		2744
Peptococcaceae						
Desulfotomaculum acetoxidans	Dtox_	1654/1657	4183		1613	
Desulfotomaculum ferrireducens GSS09	Ga0198639_	11,632	111,136		11,417	
Desulfotomaculum reducens MI-1	Dred_	0648	2646		1272	
Nitrospirae						
Thermodesulfovibrio yellowstonii DSM 11,347	THEYE_	A0966	A1080	A0957	A1311	A1182

[a]*feoB* locus tag from *feoA-feoB* (Ferrous iron transport protein)

[b]*tonb* locus tag form *tonB-exbB-exbD* (tonB—Inner membrane receptors)

[c]Outer membrane porin-like with predicted 22 transmembrane beta sheets

[*]Outer membrane porin-like with predicted 18 transmembrane beta sheets, homologous to DVU0799

and supports the idea that some SRB may produce siderophores such as enterobactin (see Fig. 1).

Iron homeostasis was evaluated using genome analysis of uncultivated acidophilic SRB that are classified as members of a new deltaproteobacterial order *Candidatus* Acidulodesulfobacterales (Tan et al. 2019). These acidophilic SRB contained the *mntH* gene, which has a primary function of Mn(II)

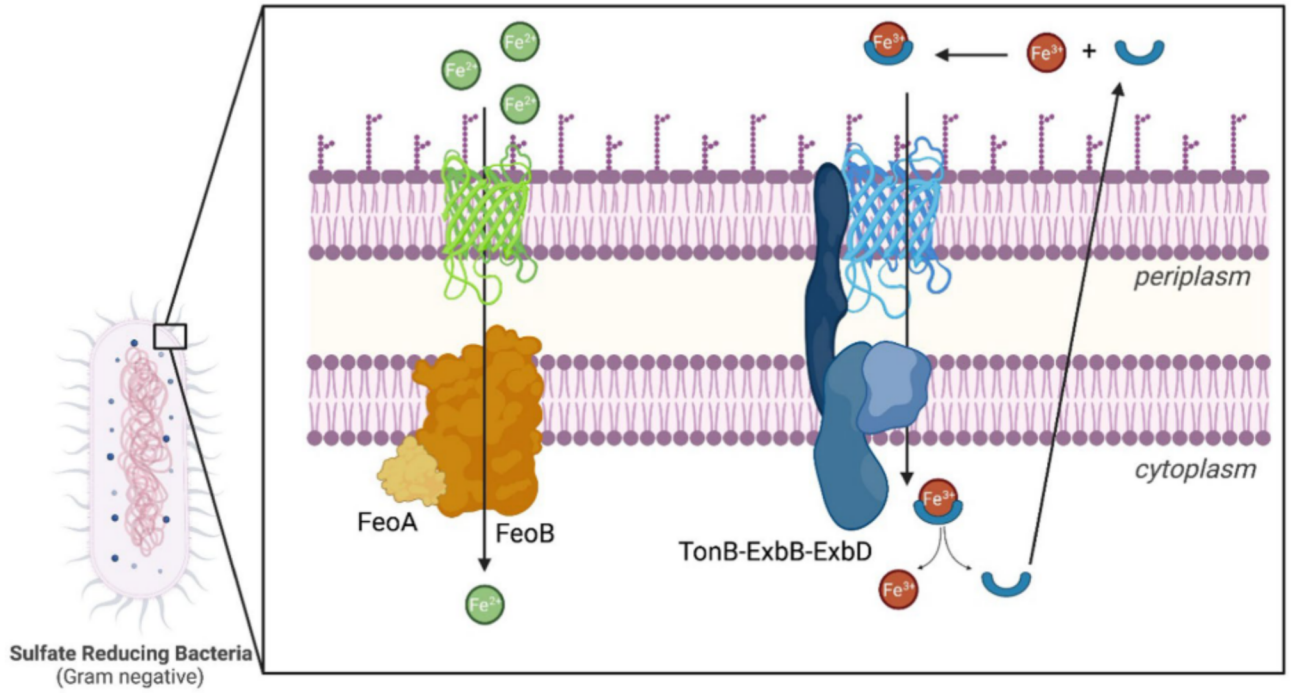

Fig. 1 Conserved iron transport systems present in Gram-negative SRB, based on its genome analysis

uptake, and is a secondary Fe(II) transporter (Osorio et al. 2008). Also present are the four TonB-dependent outer membrane Fe(III) siderophore transporters groups, which are characteristic of the CirA-linear (catecholate siderophore receptor) and FepA-cyclic (outer membrane active transporter) catecholate-type siderophores (see supplemental information in Osorio et al. (2008)). In iron limited environments, siderophore production and uptake by acidophilic SRB could support growth by sequestering iron from insoluble minerals in the environment (Osorio et al. 2008).

Single-cell genomic amplicons of SRB isolated from the human oral cavity revealed the presence of *Desulfobulbus* sp. strain Dsb1-5 and *Desulfovibrio* sp. strain Dsv1 belonging to Deltaproteobacteria (Campbell et al. 2013). Both SRB strains contained putative virulence factors associated with iron metabolism, which included the following: (i) FeoAB, ferrous iron uptake, (ii) TonB-dependent receptor, (iii) ABC-type ferric-siderophore transport system, and (iv) Fur family ferric uptake regulator. The presence of a putative siderophore transport system would be important for these bacteria, because lactoferrin is the iron source in the oral environment of the host (Wang et al. 2012). Since siderophores have a greater affinity for Fe(III) than lactoferrin, bacteria can use siderophores to sequester Fe(III) from lactoferrin (Schryvers and Stojiljkovic 1999).

An interesting feature of iron uptake using siderophores is the availability of ferrisiderophores to all bacteria in the environment. It was proposed that *D. vulgaris* Hildenborough could import iron carried on siderophores produced by other species of bacteria in the environment (Bender et al. 2007). An iron chelating system has been reported in bacteria that do not produce siderophores, these microbes would utilize siderophores secreted by other bacteria in the immediate community (Butaitė et al. 2017). This stealing of ferrisiderophores may be important for SRB in oxic layers of mats, biofilms and above anaerobic muds.

Siderophore-driven metal recovery

Siderophores may be used for the recovery of metals from secondary resources such as metal-rich industrial effluents and metal slags (Zhang et al. 2020). Certain industrial effluents are polymetallic (Staicu et al. 2021), therefore the selective recovery of metals with high market value using high-affinity siderophores receives increasing attention (Staicu and Stolz 2021). This approach is particularly relevant in the geopolitical context where a handful of countries have control over key critical raw materials such as metals needed for energy transition (Pommeret et al. 2022).

Although SRB generate a low amount of chemical energy using sulfates as terminal electron acceptors in anaerobic respiration (Barton and Fauque 2009; Staicu and Barton 2021; Staicu et al. 2022), they are ubiquitous in nature, and therefore they represent an exceptional group of microbes for isolation of novel siderophore-producing SRB strains, with industrial relevance. In addition, owing to their unique systems for Fe and other metals uptake, SRB are an interesting ecological group deserving further investigation. Several studies have looked into the recovery of metals from low grade ores and metal solutions using siderophores (Hofmann et al. 2021; Williamson et al. 2021). Currently, this recovery strategy is still at a low technology readiness level, requiring the testing of siderophores against real matrices and then its implementation at a full-scale level. Nevertheless, in the context of future scenarios of metal scarcity their recovery and reuse from waste matrices and secondary resources will gain major importance.

Conclusions

Our search shows that SRB carry the genes for ferrous iron uptake and all SRB examined have a canonical ABC-type transporter that can translocate ferric siderophores or ferric chelated species from the environment, along with similar molecules. Summarized in Fig. 1 are some of the components from the iron transport in SRB. Gram-negative SRB have additional machinery to import ferric siderophores and ferric chelated species since they have the TonB system that can work alongside outer membrane porins. This supports the idea that SRB can transport ferric siderophores that are produce by other bacteria. In order for SRB to use siderophores produced by other bacteria, mixed cultures would need to be present in the same environment, and systems to study this would include mats, biofilms and aerobic/anaerobic aquatic interfaces. The absence of homologous genes for a ferric-siderophore reductase (*fhuf*) leads us to

believe that SRB have alternative reductive paths to release iron from the imported ferric iron chelated forms. Gram-negative SRB generally are cytochrome-rich bacteria, some of them soluble in the periplasm with low midpoint redox potentials and exposed heme co-factor that can chemically produce Fe(II), which could then enter the cytoplasm trough the Feo system. The siderophore-assisted recovery of metals from primary and secondary sources may become a solid technological solution in the future, therefore SRB, owing to their diversity and ecological relevance, would be an ideal pool for isolating industrially-relevant siderophores and metallophores.

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