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REVIEW

Physiological roles of bacillithiol in intracellular metal processing

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Abstract Glutathione (GSH) is an abundantly produced low-molecular-weight (LMW) thiol in many organisms. However, a number of Gram-positive bacteria do not produce GSH, but instead produce bacillithiol (BSH) as one of the major LMW thiols. Similar to GSH, studies have found that BSH has various roles in the cell, including protection against hydrogen peroxide, hypochlorite and disulfide stress. BSH also participates in the detoxification of thiol-reactive antibiotics and the electrophilic metabolite methylglyoxal. Recently, a number of studies have highlighted additional roles for BSH in the processing of intracellular metals. Herein, we examine the potential functions of BSH in the biogenesis of Fe–S clusters, cytosolic metal buffering and the prevention of metal intoxication.

Keywords Low-molecular-weight thiols · Bacillithiol · Iron-sulfur cluster · Iron · Zinc · Manganese · Copper

Abbreviations

GSH	Glutathione
BSH	Bacillithiol
DIP	2,2-Dipyridyl

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Introduction

Non-proteinaceous low-molecular-weight (LMW) thiols play numerous functions in cellular physiology, but they are primarily recognized for maintaining a reduced intracellular environment and repairing oxidized proteins. Glutathione (GSH) is one of the most abundant and extensively studied LMW thiols (Fig. 1). Intracellular GSH is present at low millimolar concentrations and is maintained predominantly in its reduced form by GSH reductase (Fahey et al. 1978). GSH has roles in preventing osmotic and acidic stress, as well as protecting cells from intoxication by methylglyoxal, chlorine compounds and metal ions (Masip et al. 2006; Helbig et al. 2008a, b; Potter et al. 2012; Gutiérrez-Escobedo et al. 2013).

A number of Gram-positive bacteria do not produce GSH (Fahey et al. 1978; Fahey 2013). In *Actinomycetes*, mycothiol (MSH) serves as the most abundant LMW thiol (Fig. 1). Strains unable to produce MSH displayed increased sensitivities to oxidative and nitrosative stressors, alkylating agents and several antibiotics (reviewed in Newton et al. 2008).

In some *Firmicutes*, such as *Bacillus* and *Staphylococcus* species, bacillithiol (BSH) is a major LMW thiol together with coenzyme A and cysteine (Fig. 1) (Gaballa et al. 2010). The structure of BSH was elucidated in 2009 (Newton et al. 2009) and shortly thereafter the genes involved in its biosynthesis were identified (Gaballa et al. 2010). The first step in BSH biosynthesis is catalyzed by the glycosyltransferase BshA, which produces *N*-acetylglucosaminylmalate (GlcNAc-Mal) from UDP-*N*-acetylglucosamine and L-malate. BshB then removes the acetyl group to generate *N*-glucosaminemalate (GlcN-Mal). The last step is catalyzed by BshC, which adds cysteine to produce BSH. In characterized *Bacillus* spp., the accumulation of BSH

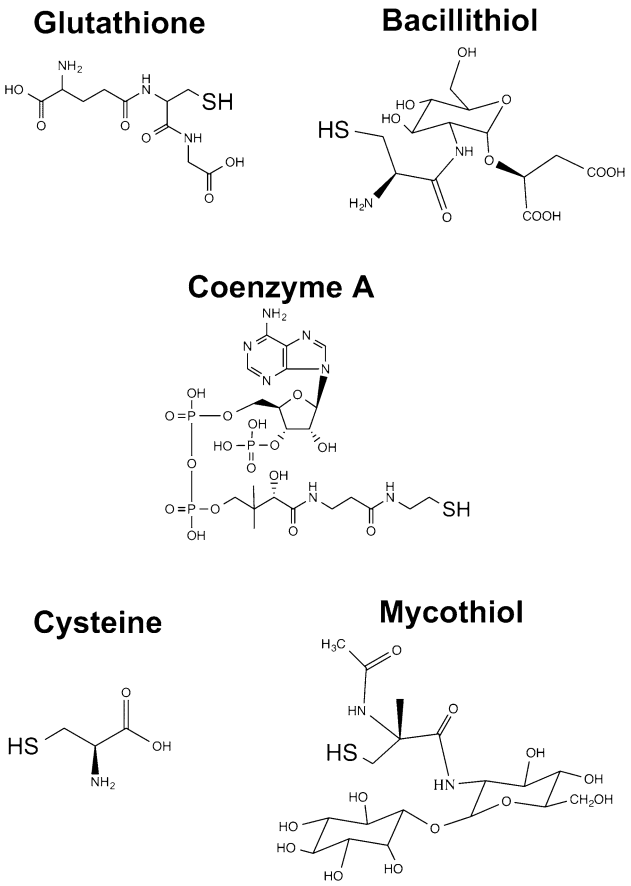

Fig. 1 Structures of the major low-molecular-weight thiols found in Bacteria. The structures of glutathione, mycothiol, bacillitiol, coenzyme A and cysteine are shown

varied from 2 to 5 mM (Sharma et al. 2013), depending on the growth conditions, while lower levels (~1 mM) were detected in *Staphylococcus aureus* (Posada et al. 2014).

The use of strains defective in BSH biosynthesis and biochemical analyses using chemically or chemoenzymatically synthesized BSH has yielded significant insight into the functions of this LMW thiol. Similar to GSH, BSH was found to protect against peroxide intoxication (Posada et al. 2014; Handtke et al. 2014), thiol/disulfide stress (Gaballa et al. 2013; Posada et al. 2014) and hypochlorite stress (Pöther et al. 2013; Chi et al. 2013). BSH has also been shown to be involved in the detoxification of the thiol-reactive antibiotics fosfomycin (Roberts et al. 2013) and rifamycin (Newton et al. 2012), as well as the electrophilic metabolite methylglyoxal (Chandrangsu et al. 2014).

Bacillithiol has a role in Fe–S cluster biogenesis

Recent studies have found that BSH has a role(s) in Fe–S cluster biogenesis. *S. aureus* strains lacking BSH displayed phenotypic abnormalities when cultured in chemically defined medium, which were corrected by (i) genetic complementation, (ii) supplementing the growth media with Fe, or (iii) supplementing the growth media with the amino acids leucine (Leu) and isoleucine (Ile) (Rosario-Cruz et al. 2015). A *Bacillus subtilis bshA* mutant strain displayed similar growth phenotypes when cultured in chemically defined media (Fang and Dos Santos 2015), which were corrected by supplementing the growth medium with (i) GlcNAc-Mal, (ii) BSSB, the oxidized form of BSH, (iii) casamino acids, or (iv) Fe (Fang and Dos Santos 2015). In all cases, the authors speculated that the growth phenotypes were the result of defects in biochemical pathways that require Fe- or Fe–S cluster-dependent enzymes for functionality.

LeuCD and IlvD are dehydratase enzymes that require Fe–S clusters for the biosynthesis of the branched-chain amino acids (BCAA) Leu and Ile, respectively. *S. aureus* and *B. subtilis* cells lacking BSH displayed decreased LeuCD and IlvD activities when compared to their respective wild-type (WT) strains (Fang and Dos Santos 2015; Rosario-Cruz et al. 2015). These findings may explain why limiting for either Leu or Ile in the growth media exacerbated the growth abnormalities of the BSH-deplete cells.

The decreased activities of Fe–S cluster-dependent enzymes in *B. subtilis* and *S. aureus* cells lacking BSH were not limited to enzymes involved in BCAA biosynthesis. The activities of the Fe–S cluster-dependent enzymes aconitase (AcnA) and glutamate synthase (GOGAT or GltBD) were also decreased in cell-free lysates of *bshA* mutants. Supplementing the culture medium with Fe increased the enzymatic activities of GOGAT, AcnA and LeuCD in a *B. subtilis bshA* mutant (Fang and Dos Santos 2015), but it had no observable effect on the activities of these enzymes in a *S. aureus bshA* mutant (Rosario-Cruz et al. 2015).

Reactive oxygen species (ROS) can damage Fe–S clusters resulting in decreased activities of enzymes that require these cofactors for catalysis (Jang and Imlay 2007). Two findings suggest that the decreased Fe–S enzyme activities were not the result of ROS damage in BSH-deplete cells. First, the addition of Fe(II) to cell-free lysates generated from either *B. subtilis* or *S. aureus bshA* mutant strains had no effect on AcnA enzyme activity, suggesting that the absence of BSH did not cause an increased abundance of repairable Fe–S clusters in AcnA (Fang and Dos Santos 2015; Rosario-Cruz et al. 2015). Second, the Leu- and Ile-dependent growth abnormalities of *S. aureus* BSH-deplete cells persisted when cultured in the absence of dioxygen (Rosario-Cruz et al. 2015).

In *S. aureus*, Fe–S clusters are synthesized on the SufBCD scaffold before being transferred to Fe–S cluster carrier molecules, which then traffic the cofactors to target

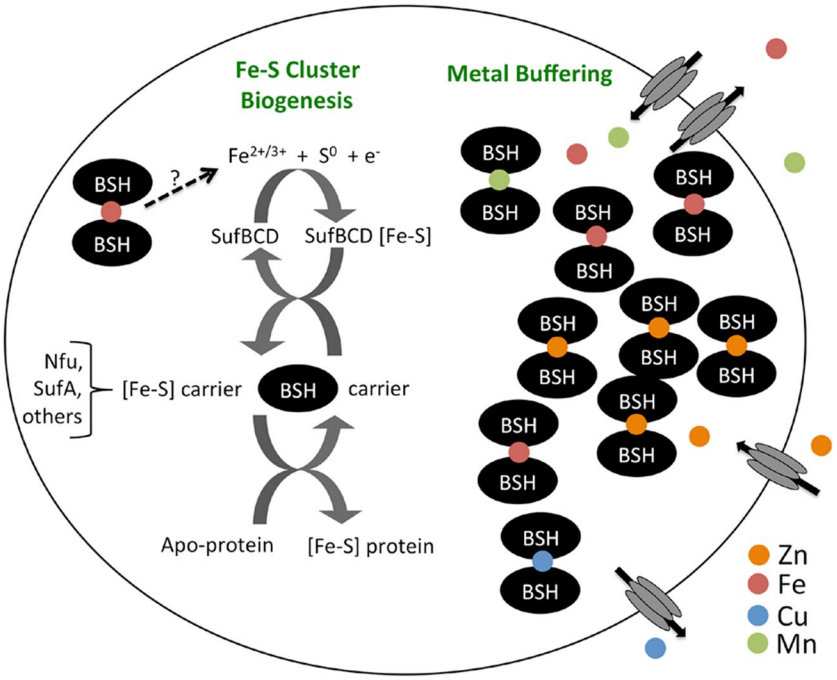

Fig. 2 Working model for the roles of bacillithiol (BSH) in Fe–S cluster biogenesis and intracellular metal ion buffering. BSH is proposed to have a role in the carriage of Fe–S clusters. BSH was shown to act as a Zn buffer in vivo and additional data suggest that BSH also has roles in the processing of alternate metals such as Fe, Cu and Mn, but further experimentation is necessary to determine whether BSH also acts as a buffer for these metals

proteins. Efforts in creating strains with mutations in the *sufCDSUB* operon have been unsuccessful (Bae et al. 2004; Chaudhuri et al. 2009; Fey et al. 2013; Valentino et al. 2014, Mashruwala et al. 2015), suggesting that these gene products are essential and that Suf is the sole Fe–S cluster biosynthetic machinery system in *S. aureus*. Genetic and biochemical analysis by our group supports the hypotheses that the *S. aureus* Nfu (Mashruwala et al. 2015) and SufA (Rosario-Cruz et al. 2015) proteins are Fe–S cluster carriers. Strains lacking both Nfu and BSH had exacerbated growth defects, including glutamate/glutamine auxotrophies and displayed AcnA and GOGAT enzymatic activities that were significantly lower than strains lacking only Nfu or BSH (Rosario-Cruz et al. 2015). These results further support the hypothesis that BSH has a role in Fe–S cluster biogenesis and suggest that BSH functions independently of Nfu. Importantly, overexpression of *nfu* or *sufA* allowed for partial or full recovery of the growth phenotypes and enzymatic defects of the *bshA* mutant strain (Rosario-Cruz et al. 2015), providing genetic evidence that the role of BSH in Fe–S cluster biogenesis has functional overlap with Fe–S cluster carriers (Fig. 2).

In *B. subtilis*, the SufBCD system is also predicted to be the sole Fe–S cluster biosynthetic system (Albrecht et al. 2010). In a *B. subtilis bshA* mutant strain, SufC accumulated to lower levels than in the WT, but SufB accumulated to similar levels in both strains (Fang and Dos Santos 2015). SufC is required for Suf function and decreased Fe–S cluster synthesis could account for the decreased Fe–S cluster-dependent enzyme activity observed in the *B. subtilis* BSH-deplete cells. The addition of the Fe chelator 2,2-dipyridyl (DIP) increased SufC expression in BSH-deplete cells, while AcnA activity was slightly lowered and GOGAT activity was unaffected. While the addition of Fe(II) corrected both the growth phenotypes and decreased Fe–S cluster-dependent enzyme activities of BSH-deplete cells, the effect of Fe addition on SufC accumulation was not reported.

BSH has roles in metal buffering

Metal ions such as zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) are required for the functionality of a number of metalloproteins and, therefore, it is thought that all cells require one or more of these metals in trace amounts. The use of these metals has both favorable and unfavorable consequences; the metals aid in the proper function of metabolism, yet elevated intracellular metal accumulation can result in intoxication. As a result, bacteria may use different mechanisms to prevent metal intoxication including the use of (i) transcriptional regulators that sense and respond to changes in intracellular metal concentrations, (ii) proteins that export metal ions from the cytosol, (iii) molecules that chelate or sequester metals, and (iv) molecules that chaperone or traffic metals to their target proteins within the cell.

Studies have found that BSH plays a role in metal ion buffering and, thereby, protects cells from metal ion intoxication (Fig. 2). Ma et al. discovered that BSH functions in

buffering the intracellular Zn pool in *B. subtilis* (Ma et al. 2014). The authors found that BSH bound Zn(II) with high affinity in vitro, predominantly in a 2:1 ratio, and most likely using both the thiolate and carboxylate moieties as ligands. The authors also noted that the thiol group of BSH could coordinate Co(II). Challenging *B. subtilis* with Zn(II) led to decreased accumulation of Zn in the *bshC* mutant strain when compared to the WT, which was most likely due to increased expression of the CadA and CzcD metal efflux systems. Moreover, the absence of BSH resulted in increased sensitivity to Zn(II) and Cd(II) in strains defective in the export of these metals. Nearly, ~2/3 of the intracellular Zn was found to associate with LMW molecules in the WT strain. The authors proposed that the presence of BSH allows for increased accumulation of Zn and the absence of BSH reduces the Zn buffering capacity of the cell.

The authors also noted that the expression of *cadA* was increased after diamide treatment, which causes disulfide stress. Data presented suggest that upon depletion of the reduced form of BSH, Zn is released from the LMW pool, which binds to CzrA leading to *cadA* derepression. The authors also showed that the addition of BSH facilitated the removal of Zn from the CzrA in vitro, and propose this as a possible mechanism by which BSH facilitates Zn dissociation from CzrA in vivo upon the transition from Zn excess to normal conditions. These findings highlight the importance of BSH in the integration of the cellular networks involved in Zn homeostasis and thiol metabolism.

Bacillus subtilis and *S. aureus* strains lacking BSH display sensitivity to various metals including Cu(I), Cu(II), Cr(IV), Cd(II) and As(III) (Rajkarnikar et al. 2013; Ma et al. 2014; Fang and Dos Santos 2015). Ma et al. found that the expression of the *cadA* and *czcD* genes, which aid in Cd efflux, was not induced in the BSH-deplete strain upon Cd(II) treatment, suggesting that BSH is required for the induction of these systems under Cd stress. The authors hypothesize that Cd mobilizes Zn from BSH, which can then metalate CzrA resulting in derepression of the *cadA* and *czcD* genes. A *bshC* mutant has a lower Zn buffering capacity and, therefore, the amount of Zn released under Cd(II) treatment was presumably lower than that of the WT strain, resulting in decreased *cadA* and *czcD* derepression. Similarly, As(III) sensitivity was observed in the *bshC* mutant strain and a lower level of induction of the arsenic resistance system (*arsR*) occurred in cells lacking BSH when compared to the WT strain. The authors hypothesized that As(III) mobilization within the cell might be limited in the absence of BSH, thereby affecting As(III) loading into ArsR resulting in decreased expression of *arsR*. Alternatively, it was postulated that Zn released from BSH upon treatment with As(III) might somehow facilitate AsrR dissociation from the *arsR* promoter (Ma et al. 2014).

BSH also appears to have a role in Fe homeostasis. When cultured in a chemically defined medium, *S. aureus* BSH-deplete cells had increased transcription of genes necessary for Fe uptake, which are under the transcriptional control of the ferric uptake regulator (Fur) (Rosario-Cruz et al. 2015). This result suggests that BSH depletion leads to non-metalated Fur and derepression of genes involved in Fe acquisition. Further analysis found that the overall Fe load in the WT and BSH-deplete cells was statistically indistinguishable. Moreover, the growth phenotypes displayed by the *bshA* mutant were corrected by supplementing the growth medium with Fe and exacerbated by the addition of DIP (Rosario-Cruz et al. 2015 and Z. Rosario-Cruz and J.M. Boyd, unpublished results). As previously mentioned, the growth phenotypes were also suppressed by overexpression of *sufA*, which encodes for an A-type protein (Vinella et al. 2009; Rosario-Cruz et al. 2015). In addition to their roles as Fe–S cluster carriers, A-type proteins bind Fe(II) and Fe(III) in vitro (Ding and Clark 2004; Mapolelo et al. 2012) and evidence supports a role for these proteins in Fe donation for Fe–S cluster synthesis in vivo (Ding et al. 2004; Landry et al. 2013). Perhaps increasing the concentration of SufA improves the growth of BSH-deplete cells by increasing bioavailable Fe for processes such as Fe–S cluster synthesis.

Bacillus subtilis cells lacking BSH had a slight, but statistically significant decrease in total cellular Fe (Fang and Dos Santos 2015). These strains also displayed decreased activities of the mononuclear Fe-dependent enzymes threonine dehydrogenase and quercetin 2,3-dioxygenase. Supplementing the growth media with Fe(II) corrected the growth abnormalities of the *B. subtilis* BSH-deplete cells and increased Fe–S cluster-dependent enzyme activity. The addition of Fe(II) to cell-free lysates did not alter the activities of mononuclear Fe-dependent enzymes, but the effect of Fe supplementation to the growth medium on their activities was not reported. The addition of DIP increased the expression of SufC in BSH-deplete cells and, thereby, possibly increased the output of Suf. In the WT strain, DIP treatment did not increase SufC expression providing further evidence that BSH has a role in Fe homeostasis.

Bacillus subtilis BSH-deplete cells had a slight increase in intracellular Mn when compared to the WT strain (Fang and Dos Santos 2015). Upon challenge with paraquat, which results in superoxide stress, both the WT and *bshA* mutant accumulated Mn, but the accumulation was greater in the BSH-deplete cells. Mn accumulation occurred concomitant with increased Mn-dependent superoxide dismutase (Sod) activity. In *Escherichia coli*, Mn aids in combating oxidative stress by replacing Fe in mononuclear Fe enzymes, thereby maintaining protein functionality by shifting towards a Mn-centered metabolism (Sobota and Imlay 2011). In addition, free intracellular Mn can

eliminate superoxide stress in *B. subtilis* by a yet uncharacterized mechanism (Inaoka et al. 1999). This may explain why the *B. subtilis* WT and BSH-deficient cells have increased levels of both labile Mn and protein-associated Mn under paraquat stress (Fang and Dos Santos 2015). Why BSH-deplete cells have increased basal levels of Mn and increased Mn uptake upon paraquat treatment remains unknown.

Both *S. aureus* and *B. subtilis* cells lacking BSH are sensitive to Cu(II) intoxication (Rajkarnikar et al. 2013). *B. subtilis bshA* mutants are also sensitive to Cu(I) intoxication (Fang and Dos Santos 2015). Copper toxicity could be, in part, a result of a Cu-catalyzed Fenton-type reaction resulting in the production of hydroxyl radicals and DNA damage (Gunther et al. 1995). In *E. coli,* copper intoxication does not appear to be a result of increased DNA damage (Macomber et al. 2007), but Cu does inactivate Fe–S cluster-dependent enzymes (Macomber and Imlay 2009). Consistent with these findings, Cu(I) treatment affected AcnA and GltBD activities in the *B. subtilis* WT strain. However, the activities of these enzymes were unaffected by Cu(I) challenge in the *bshA* mutant strain (Fang and Dos Santos 2015). We have also noted that *S. aureus* BSH-deplete cells display increased generation times and lower growth yields when Cu(II) is provided in the growth media, but these phenotypes persist in the absence of dioxygen (Z. Rosario-Cruz and J.M. Boyd, unpublished results). These findings suggest that the *S. aureus* Cu(II)-dependent growth defects are not the result of ROS accumulation.

Conclusions and future directions

The discovery of BSH as one of the major LMW thiols in some Gram-positive bacteria is relatively recent and many questions about its physiological roles remain unanswered. Genetic studies suggest that the role of BSH in Fe–S cluster biogenesis has functional overlap with Fe–S cluster carrier molecules, but the exact role of BSH in Fe–S cluster biogenesis is currently unknown. GSH has been shown to act as a ligand for Fe–S cluster binding in vitro (Qi et al. 2012). GSH molecules can also act as ligands for Fe–S cluster binding in conjunction with monothiol glutaredoxins (Feng et al. 2006). In both scenarios, it is thought that GSH participates as an Fe–S cluster carrier in vivo. Further genetic and biochemical studies are necessary to determine whether BSH, like GSH, can ligate an Fe–S cluster and/or ligate an Fe–S cluster in conjunction with a protein partner. It will then be necessary to examine whether BSH or the protein-BSH complex can transfer the bound Fe–S clusters to apo-proteins.

BSH can bind Zn(II) and Co(II) in vitro and data suggest that it serves as an intracellular Zn buffer in vivo (Ma et al. 2014). Studies by others in the field imply that BSH also has roles in the processing of alternate metals, such as Fe, Mn, As, Cd and Cu, but it is currently unknown if BSH directly ligates these metals. Additional physiological and biochemical studies will be necessary to determine if BSH can ligate these metals and to uncover additional role(s) for BSH in metal ion homeostasis and potentially detoxification. It will also be interesting to learn more about the role(s) of BSH in altering the affinities of ArsR and CzrA for DNA.

Bacteria face a number of challenges in their environments including metal limitation. Not surprisingly, human pathogenic bacteria face metal limitation within our bodies (reviewed in Hood and Skaar 2012). Additionally, human macrophages bombard phagocytized bacteria with Cu, which disrupts Fe–S cluster biogenesis, to aid in bacterial clearance (Johnson et al. 2015). Pathogenic bacteria, such as *S. aureus,* are notorious for their ability to adapt to various stress environments, allowing them to successfully cause a wide range of infections (Tuchscherr and Löffler 2015). Having a better understanding of the role of gene products, metabolites and/or regulatory proteins required to cope with stressful conditions during host infection will aid in identifying new antimicrobial targets. In some bacterial pathogens, such as *S. aureus,* BSH may play various roles during the infection process such as serving as an intracellular reservoir of metals, thereby allowing functionality of metal-dependent proteins during periods of metal starvation. BSH may also serve as a buffering agent to prevent the detrimental effects of metal-induced intoxication. In summary, not only does BSH have roles in combating oxidative stress and detoxification of thiol-reactive antibiotics, but it also participates in Fe–S cluster biogenesis and metal homeostasis, which are processes required for successful bacterial colonization and infection. Moreover, BSH is absent in mammals, making BSH biosynthesis an attractive antimicrobial target. Future studies using models of infection will be necessary to elucidate the roles of BSH in microbial pathogenesis.

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References

Albrecht AG, Netz DJA, Miethke M et al (2010) SufU is an essential iron-sulfur cluster scaffold protein in *Bacillus subtilis*. J Bacteriol 192:1643–1651. doi:10.1128/JB.01536-09

Bae T, Banger AK, Wallace A et al (2004) *Staphylococcus aureus* virulence genes identified by bursa aurealis mutagenesis and nematode killing. Proc Natl Acad Sci U S A 101:12312–12317. doi:10.1073/pnas.0404728101

Chandrangsu P, Dusi R, Hamilton CJ, Helmann JD (2014) Methylglyoxal resistance in *Bacillus subtilis:* contributions of bacillithiol-dependent and independent pathways. Mol Microbiol 91:706–715. doi:10.1111/mmi.12489

Chaudhuri RR, Allen AG, Owen PJ et al (2009) Comprehensive identification of essential *Staphylococcus aureus* genes using transposon-mediated differential hybridisation (TMDH). BMC Genom 10:291. doi:10.1186/1471-2164-10-291

Chi BK, Roberts AA, Huyen TTT et al (2013) S-bacillithiolation protects conserved and essential proteins against hypochlorite stress in *Firmicutes* bacteria. Antioxid Redox Signal 18:1273–1295. doi:10.1089/ars.2012.4686

Ding H, Clark RJ (2004) Characterization of iron binding in IscA, an ancient iron-sulphur cluster assembly protein. Biochem J 379:433–440. doi:10.1042/BJ20031702

Ding H, Clark RJ, Ding B (2004) IscA mediates iron delivery for assembly of iron-sulfur clusters in IscU under the limited accessible free iron conditions. J Biol Chem 279:37499–37504. doi:10.1074/jbc.M404533200

Fahey RC (2013) Glutathione analogs in prokaryotes. Biochim Biophys Acta 1830:3182–3198. doi:10.1016/j.bbagen.2012.10.006

Fahey RC, Brown WC, Adams WB, Worsham MB (1978) Occurrence of glutathione in bacteria. J Bacteriol 133:1126–1129

Fang Z, Dos Santos PC (2015) Protective role of bacillithiol in superoxide stress and Fe–S metabolism in *Bacillus subtilis*. Microbiologyopen. doi:10.1002/mbo3.267

Feng Y, Zhong N, Rouhier N et al (2006) Structural insight into poplar glutaredoxin C1 with a bridging iron-sulfur cluster at the active site. Biochemistry 45:7998–8008. doi:10.1021/bi060444t

Fey PD, Endres JL, Yajjala VK et al (2013) A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. mBio 4:e00537-12. doi:10.1128/mBio.00537-12

Gaballa A, Newton GL, Antelmann H et al (2010) Biosynthesis and functions of bacillithiol, a major low-molecular-weight thiol in *Bacilli*. Proc Natl Acad Sci U S A 107:6482–6486. doi:10.1073/pnas.1000928107

Gaballa A, Chi BK, Roberts AA et al (2013) Redox regulation in *Bacillus subtilis*: the bacilliredoxins BrxA (YphP) and BrxB (YqiW) function in de-bacillithiolation of S-bacillithiolated OhrR and MetE. Antioxid Redox Signal 21:357–367. doi:10.1089/ars.2013.5327

Gunther MR, Hanna PM, Mason RP, Cohen MS (1995) Hydroxyl radical formation from cuprous ion and hydrogen peroxide: a spin-trapping study. Arch Biochem Biophys 316:515–522. doi:10.1006/abbi.1995.1068

Gutiérrez-Escobedo G, Orta-Zavalza E, Castaño I, De Las Peñas A (2013) Role of glutathione in the oxidative stress response in the fungal pathogen *Candidaglabrata*. Curr Genet 59:91–106. doi:10.1007/s00294-013-0390-1

Handtke S, Schroeter R, Jürgen B et al (2014) *Bacillus pumilus* reveals a remarkably high resistance to hydrogen peroxide provoked oxidative stress. PLoS One 9:e85625. doi:10.1371/journal.pone.0085625

Helbig K, Bleuel C, Krauss GJ, Nies DH (2008a) Glutathione and transition-metal homeostasis in *Escherichia coli*. J Bacteriol 190:5431–5438. doi:10.1128/JB.00271-08

Helbig K, Grosse C, Nies DH (2008b) Cadmium toxicity in glutathione mutants of *Escherichia coli*. J Bacteriol 190:5439–5454. doi:10.1128/JB.00272-08

Hood MI, Skaar EP (2012) Nutritional immunity: transition metals at the pathogen-host interface. Nat Rev Microbiol 10:525–537. doi:10.1038/nrmicro2836

Inaoka T, Matsumura Y, Tsuchido T (1999) SodA and manganese are essential for resistance to oxidative stress in growing and sporulating cells of *Bacillus subtilis*. J Bacteriol 181:1939–1943

Jang S, Imlay JA (2007) Micromolar intracellular hydrogen peroxide disrupts metabolism by damaging iron-sulfur enzymes. J Biol Chem 282:929–937. doi:10.1074/jbc.M607646200

Johnson MDL, Kehl-Fie TE, Klein R et al (2015) Role of copper efflux in pneumococcal pathogenesis and resistance to macrophage-mediated immune clearance. Infect Immun 83:1684–1694. doi:10.1128/IAI.03015-14

Landry AP, Cheng Z, Ding H (2013) Iron binding activity is essential for the function of IscA in iron-sulphur cluster biogenesis. Dalton Trans 42:3100–3106. doi:10.1039/c2dt32000b

Ma Z, Chandrangsu P, Helmann TC et al (2014) Bacillithiol is a major buffer of the labile zinc pool in *Bacillus subtilis*. Mol Microbiol 94:756–770. doi:10.1111/mmi.12794

Macomber L, Imlay JA (2009) The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci U S A 106:8344–8349. doi:10.1073/pnas.0812808106

Macomber L, Rensing C, Imlay JA (2007) Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*. J Bacteriol 189:1616–1626. doi:10.1128/JB.01357-06

Mapolelo DT, Zhang B, Naik SG et al (2012) Spectroscopic and functional characterization of iron-sulfur cluster-bound forms of *Azotobactervinelandii* (Nif)IscA. Biochemistry 51:8071–8084. doi:10.1021/bi3006658

Mashruwala AA, Pang YY, Rosario-Cruz Z et al (2015) Nfu facilitates the maturation of iron-sulfur proteins and participates in virulence in *Staphylococcus aureus*. Mol Microbiol 95:383–409. doi:10.1111/mmi.12860

Masip L, Veeravalli K, Georgiou G (2006) The many faces of glutathione in bacteria. Antioxid Redox Signal 8:753–762. doi:10.1089/ars.2006.8.753

Newton GL, Buchmeier N, Fahey RC (2008) Biosynthesis and functions of mycothiol, the unique protective thiol of *Actinobacteria*. Microbiol Mol Biol Rev 72:471–494. doi:10.1128/MMBR.00008-08

Newton GL, Rawat M, La Clair JJ et al (2009) Bacillithiol is an antioxidant thiol produced in Bacilli. Nat Chem Biol 5:625–627. doi:10.1038/nchembio.189

Newton GL, Fahey RC, Rawat M (2012) Detoxification of toxins by bacillithiol in *Staphylococcus aureus*. Microbiology 158:1117–1126. doi:10.1099/mic.0.055715-0

Posada AC, Kolar SL, Dusi RG et al (2014) The importance of bacillithiol in the oxidative stress response of *Staphylococcus aureus*. Infect Immun 82:316–332. doi:10.1128/IAI.01074-13

Pöther D-C, Gierok P, Harms M et al (2013) Distribution and infection-related functions of bacillithiol in *Staphylococcus aureus*. Int J Med Microbiol 303:114–123. doi:10.1016/j.ijmm.2013.01.003

Potter AJ, Trappetti C, Paton JC (2012) *Streptococcus pneumoniae* uses glutathione to defend against oxidative stress and metal ion toxicity. J Bacteriol 194:6248–6254. doi:10.1128/JB.01393-12

Qi W, Li J, Chain CY et al (2012) Glutathione complexed Fe–S centers. J Am Chem Soc 134:10745–10748. doi:10.1021/ja302186j

Rajkarnikar A, Strankman A, Duran S et al (2013) Analysis of mutants disrupted in bacillithiol metabolism in *Staphylococcus aureus*. Biochem Biophys 436:128–133. doi:10.1016/j.bbrc.2013.04.027

Roberts AA, Sharma SV, Strankman A et al (2013) Mechanistic studies of FosB: a divalent-metal-dependent bacillithiol-S-transferase that mediates fosfomycin resistance in *Staphylococcus aureus*. Biochem J 451:69–79

Rosario-Cruz Z, Chahal HK, Mike LA et al (2015) Bacillithiol has a role in Fe–S cluster biogenesis in *Staphylococcus aureus*. Mol Microbiol. doi:10.1111/mmi.13115

Sharma SV, Arbach M, Roberts AA et al (2013) Biophysical features of bacillithiol, the glutathione surrogate of *Bacillus subtilis* and

other Firmicutes. ChemBioChem 14:2160–2168. doi:10.1002/cbic.201300404

Sobota JM, Imlay JA (2011) Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichiacoli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. Proc Natl Acad Sci U S A 108:5402–5407. doi:10.1073/pnas.1100410108

Tuchscherr L, Löffler B (2015) *Staphylococcus aureus* dynamically adapts global regulators and virulence factor expression in the course from acute to chronic infection. Curr Genet. doi:10.1007/s00294-015-0503-0

Valentino MD, Foulston L, Sadaka A, et al. (2014) Genes contributing to *Staphylococcus aureus* fitnessin abscess- and infection-related ecologies. mBio 5:e01729–14. doi: 10.1128/mBio.01729-14

Vinella D, Brochier-Armanet C, Loiseau L et al (2009) Iron-sulfur (Fe/S) protein biogenesis: phylogenomic and genetic studies of A-type carriers. PLoS Genet 5:e1000497. doi:10.1371/journal.pgen.1000497