Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Nutrient sulfur acquisition strategies employed by bacterial pathogens

Joshua M Lensmire and Neal D Hammer[1]

Pathogens have evolved elegant mechanisms to acquire essential nutrients from host environments. Sulfur is a requirement for bacterial growth and inorganic and organic sulfur-containing metabolites are abundant within the host–pathogen interface. A growing body of evidence suggests that pathogens are capable of scavenging both types of sulfur sources to fulfill the nutritional requirement. While therapeutic strategies focusing on inhibiting inorganic sulfate assimilation and cysteine synthesis show promise *in vitro, in vivo* efficacy maybe limited due to the diversity of host-derived sulfur sources and the fact that most pathogens are capable of acquiring multiple sources of sulfur.

Address
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA

Corresponding author: Hammer, Neal D (hammern2@msu.edu)
[1] Present address: 5163 Biomedical Physical Sciences, 567 Wilson Rd, East Lansing, MI 48824, USA.

Current Opinion in Microbiology 2019, **47**:52–58

This review comes from a themed issue on **Host-pathogen interactions: bacteria**

Edited by **Karen M Ottemann** and **Linda J Kenney**

For a complete overview see the Issue and the Editorial

Available online 7th December 2018

https://doi.org/10.1016/j.mib.2018.11.002

1369-5274/© 2018 Elsevier Ltd. All rights reserved.

Introduction

Pathogenic bacteria are adept at procuring essential nutrients from the host environment. Decades of research have revealed mechanisms by which bacterial pathogens acquire transition metals and carbon sources during infection. This review focuses on another nutritional requirement for bacterial growth, sulfur. Compared to our understanding of nutrient metal acquisition, how pathogens fulfill their sulfur requirement is less well understood.

Sulfur is a multivalent atom that conforms to a dynamic range of oxidation states (+6 to −2). This redox activity supports many aspects of eukaryotic and prokaryotic life [1]. For example, sulfur is incorporated in the amino acids cysteine (Cys) and methionine as well as several essential cofactors including but not limited to coenzyme A, coenzyme M, thiamine, lipoic acid, iron–sulfur [Fe–S] clusters, glutathione (GSH), and biotin [1]. The unique chemistry of these sulfur-containing compounds is essential for electron transfer and redox homeostasis. Humans and bacteria use sulfur-containing metabolites for many of the same functions; consequently, the molecules are readily available for pathogens during infection. For example, the host accumulates organosulfur compounds that contain sulfhydryl groups, or thiols, at high concentrations that function as antioxidants [2]. Numerous *in vitro* studies describe how bacteria acquire these organosulfur molecules for use as sulfur sources, and researchers are beginning to understand how these systems function during infection. This review will highlight these studies, define the sulfur sources available within the host, and present examples of pathogen sulfur acquisition strategies.

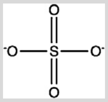
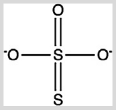
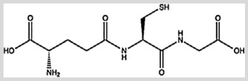
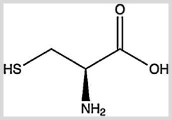
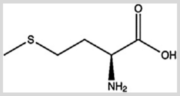
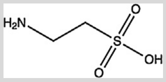
Inorganic sulfur assimilation

Inorganic sulfur compounds such as sulfate (SO_4^{2-}) play crucial roles in host physiology. Plasma levels of sulfate, the most highly oxidized form of sulfur (oxidation state of −6), can reach as high as 215.5 μM (Table 1) [3]. To use sulfate, bacteria first transport it into the cytoplasm, and subsequently reduce the molecule to an oxidation state of +2 via an energy intensive process involving a series of four enzymatic reactions and the input of eight electrons (Figure 1) [4]. Sulfate assimilation leads to production of sulfide which can be readily converted to Cys, a precursor to numerous sulfur-containing cofactors [2,5]. The first of four reactions leading to Cys begins with the enzyme ATP sulfurylase and the substrates sulfate and ATP [4]. The reaction produces adenosine phosphosulfate (APS) (Figure 1 #1) which is subsequently phosphorylated by APS kinase creating phosphoadenosine phosphosulfate (PAPS) (Figure 1 #2) [4]. PAPS is reduced by PAPS reductase yielding adenosine diphosphate (PAP) and sulfite (SO_3^{2-}) (Figure 1 #3) [4]. Sulfite is reduced to sulfide (S^{2-}) (Figure 1 #4) [4]. Ultimately, sulfide reacts with *O*-acetyl serine (OAS) via OAS sulfhydrylase (this enzyme is also called Cys synthase or OAS (thiol)-lyase) that produces Cys (Figure 1 #5) [4]. OAS is generated by serine *O*-acetyltransferase and the substrates serine and acetyl-coA (Figure 1 # 10) [4]. Reactions involved in the production of Cys from an inorganic sulfur molecule will be referred to as assimilation (Figure 1 #1–4).

Sulfate is an established *in vivo* sulfur source for some pathogens (Table 1). A transposon-sequencing study examining *Acinetobacter baumannii* infection in the *Galleria mellonella* larvae infection model revealed that sulfate

Table 1

Evidence for pathogen acquisition and utilization of host-derived sulfur sources

Sulfur source	Concentration in host environments[a]	Example pathogens	Evidence[b]	References
Sulfate 	Plasma: 215.5 μM	*Mycobacterium tuberculosis*	*In vitro*	[7,8]
		Escherichia coli	*In vitro*	[4]
		Pseudomonas aeruginosa	*In vitro*	[57]
		Acinetobacter baumannii	*In vivo*	[6•]
		Salmonella enterica	*In vitro*	[4]
		Klebsiella aerogenes	*In vivo*	[28]
Thiosulfate 	Serum: 100.8 μM	*Escherichia coli*	*In vitro*	[4]
		Salmonella enterica	*In vitro*	[4]
		Staphylococcus aureus	*In vitro*	[11••]
		Clostridium difficile	*In vitro*	[12]
		Campylobacter jejuni	*In vitro*	[23]
Glutathione 	Plasma: 37.03 μM Intracellular: 1–10 mM	*Francisella tularensis*	*In vivo*	[36]
		Staphylococcus aureus	*In vitro*	[11••]
		Clostridium difficile	*In vitro*	[12]
		Bordetella pertussis	*In vitro*	[18]
		Neisseria meningitidis	*In vitro*	[58]
		Escherichia coli	*In vitro*	[59]
		Salmonella enterica	*In vitro*	[60]
		Haemophilus influenzae	*In vitro*	[61]
		Streptococcus mutans	*In vitro*	[62]
		Streptococcus pneumoniae	*In vivo*	[63]
Cysteine/cystine 	Cysteine serum: 33.5 μM Cystine serum: 62.9 μM	*Listeria monocytogenes*	*In vivo*	[22••]
		Staphylococcus aureus	*In vitro*	[11••]
		Campylobacter jejuni	*In vitro*	[23]
		Escherichia coli	*In vitro*	[13]
		Salmonella enterica	*In vitro*	[13]
		Streptococcus mutans	*in vitro*	[24]
Methionine 	Serum: 39.8 μM	*Klebsiella aerogenes*	*In vitro*	[13]
		Mycobacterium tuberculosis	*In vitro*	[13]
		Pseudomonas aeruginosa	*In vitro*	[13]
Taurine 	Plasma: 55.5 μM	*Enterobacteriaceae*	*In vitro*	[38]
		Staphylococcus aureus[c]	*In vitro*	[39]

[a] Plasma and serum levels of sulfur-containing metabolites were obtained from the following references: [2,3,34,64–66]. Metabolite concentrations in other tissues can be found using the Human Metabolome Database [67].

[b] '*In vitro*' indicates the cells proliferate in laboratory defined medium when the sulfur-containing metabolite is supplemented as the sole source of sulfur. '*In vivo*' denotes that mutant strains lacking sulfur source acquisition systems demonstrate virulence defects in an animal model of infection.

[c] Conflicting data demonstrating that taurine is not a viable sulfur source for *S. aureus* is reported in Refs. [39,40].

assimilatory enzymes sulfite reductase, PAPS reductase, serine *O*-acetyltransferase, and ATP sulfurylase are essential for infection [6•]. Sulfate assimilation is also key for *Mycobacterium tuberculosis* proliferation [7,8]. Researchers established that ATP sulfurylase (Figure 1 #1) and PAPS reductase (Figure 1 #3) are essential for *in vitro* growth using transposon site hybridization [8], and *M. tuberculosis* ATP sulfurylase mutants are impaired for macrophage infection [7]. These reports establish *M. tuberculosis* and *A. baumanii* as model pathogens for the study of sulfate assimilation during infection.

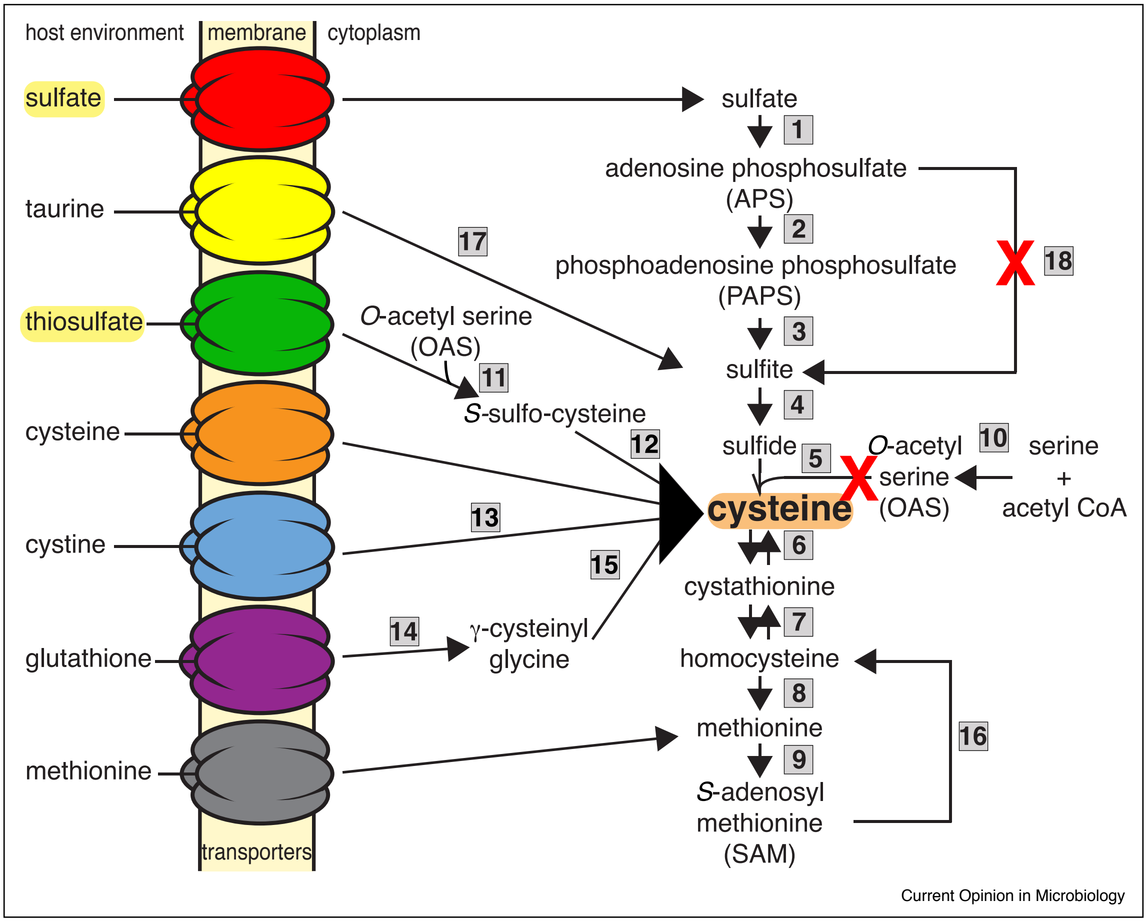
Thiosulfate

Thiosulfate ($S_2O_3^{2-}$) can be a substrate of OAS sulfhydrylase in certain species of bacteria (Table 1) leading directly to Cys production (Figure 1 #11, 12). Bacteria with thiosulfate assimilatory potential encode a specific OAS sulfhydrylase to generate *S*-sulfocysteine, which is subsequently reduced to Cys by an unknown mechanism (Figure 1 #12) [9]. Numerous pathogens including, *Salmonella enterica*, *Clostridium difficile*, and *Staphylococcus aureus* possess thiosulfate catabolic activity (Table 1) [10,11••,12]. Despite *in vitro* characterization of pathogen thiosulfate assimilation, *in vivo* acquisition of thiosulfate as a sulfur source during infection has not been demonstrated.

Organosulfur acquisition

The abundance of organosulfur metabolites in the host makes these potential sulfur sources attractive targets for pathogens. These organosulfur molecules include: Cys, oxidized Cys (referred to as cystine), glutathione (GSH),

Figure 1



Current Opinion in Microbiology

Overview of the enzymatic processes that support sulfur assimilation and integration of exogenous sulfur-containing metabolites. 1: ATP sulfurylase, 2: APS Kinase, 3: PAPS Reductase, 4: Sulfite Reductase, 5: Cysteine synthase or OAS sulfhydrylase (also referred to as *O*-acetylserine (thiol)-lyase), 6: cystathionine-gamma-synthase, 7: cystathionine beta synthase, 8: homocysteine *S*-methyltransferase, 9: *S*-adenosylmethionine synthetase, 10: serine *O*-acetyltransferase,11: Cysteine synthase (OAS sulfhydrylase *O*-acetylserine (thiol)-lyase) — in some species #5 and #11 are isozymes that catalyze a similar reaction but use different substrates, 12: unknown enzyme that converts *S*-sulfo-cysteine to cysteine, 13: Unknown reductase that reduces the disulfide bond in cystine,14: γ-glutamyl-transpeptidase, 15: peptidase, 16: SAM undergoes a methyl transfer reaction and becomes *S*-adenosyl homocysteine. SAH is subsequently converted to homocysteine by SAH hydrolase 17: α-ketoglutarate-dependent dioxygenase 18: APS reductase. The red 'X' indicates enzymes targeted for therapeutic intervention. Sulfur sources highlighted in yellow are inorganic sources of sulfur; The rest are organosulfur metabolites.

methionine, and taurine. An advantage of organosulfur utilization is that the molecules are typically more efficiently processed to Cys than inorganic sulfur, requiring only one or two enzymatic steps (Figure 1). In species that use both organic and inorganic sulfur sources, such as *Escherichia coli*, exogenous Cys is a competitive inhibitor of serine *O*-acetyltransferase (Figure 1 #10) [13]. Transcription of other sulfate assimilation genes is repressed by 0.5 mM cystine and various organosulfur molecules [14,15•]. These findings suggest that cells coordinate inorganic and organic sulfur source acquisition strategies. However, numerous pathogens including *Listeria monocytogenes*, *Clostridium perfringens*, *Legionella pneumophila*, *Bordetella pertussis*, *Neisseria gonorrhoeae*, *S. aureus*, and the streptococci are Cys auxotrophs due to incomplete sulfate assimilation pathways [11••,12,13,16–20]. *S. aureus*, for example, does not encode ATP sulfurylase, APS kinase, or PAPS reductase and is therefore unable to use sulfate as a source of sulfur. *S. aureus* is capable of consuming sulfide or thiosulfate as these inorganic sulfur sources can be converted to substrates for the OAS sulfhydrylases [11••]. Notably, a mutant strain that is limited in its ability to use sulfide or thiosulfate as sulfur sources via genetic inactivation of *cysM* is as virulent as WT [11••]. This result implies that *S. aureus* does not assimilate these inorganic molecules during infection and supports the hypothesis that the pathogen predominantly acquires organosulfur sources to proliferate within the host [11••]. In keeping with this hypothesis, the organosulfur sources Cys, cystine, and GSH are viable sources of sulfur for *S. aureus* [11••]. The fact that many pathogens seem to have lost, or never evolved, the ability to assimilate sulfate underscores the importance of organosulfur metabolites as potential sources of nutrient sulfur during infection.

Cysteine (Cys) and cystine

Cys is a precursor for a number of sulfur-containing cofactors and many pathogens use it as a sulfur source (Table 1) [11••,17,21,22••,23]. Cystine is also a viable sulfur source for pathogens, but the disulfide bond between the two Cys molecules must be reduced (Figure 1 # 13, Table 1 [13,24]). Cys transporters have been difficult to identify due to the inherent redundancy of amino acid transporters [25]. Consequently, assessing the importance of Cys acquisition to pathogenesis has been a challenge. A possible exception is *L. monocytogenes*, a Cys auxotroph due to a complete lack of the sulfate assimilation pathway (Figure 1 #1–4) [21,26]. Mutation of the Cys-transport-associated protein CtaP, a putative substrate binding protein, reduces proliferation in a minimal defined medium [22••]. Supplementation of the medium with high concentrations of Cys restores growth of the mutant, providing evidence that CtaP functions in concert with a high affinity Cys importer [22••]. Consistent with this, a putative ABC transporter (*lmo0136-0137*) is encoded adjacent to *ctaP* [27]. Lmo0136-0137 is required for *L. monocytogenes* replication within Caco-2 cells and the *ctaP* mutant is attenuated in a murine model of systemic infection [22••,27]. CtaP also functions as a host cell adhesin, making it difficult to discern whether the virulence defect of the *ctaP* mutant is due to loss of a high affinity Cys importer, impaired host cell attachment, or both [22••]. Although additional biochemical validation of the CtaP-Lmo0136-0137 transport system is required, it is tempting to speculate that acquisition of Cys is a requirement for intracellular growth and pathogenicity of *L. monocytogenes*.

Methionine

The other proteinogenic sulfur-containing amino acid, methionine, is also an *in vitro* sulfur source for a subset of pathogens. To satisfy the sulfur requirement, methionine must undergo sequential recycling reactions that ultimately produce Cys [28]. The recycling pathway proceeds from *S*-adenosyl methionine, which becomes *S*-adenosyl homocysteine (SAH) after undergoing the methyl transfer reaction. SAH is converted to homocysteine (Figure 1 #9 and #16), and through a reverse transsulfuration reaction, homocysteine is converted to Cys (Figure 1 #7 and #6) [13,28,29]. *Klebsiella aerogenes, M. tuberculosis,* and *Pseudomonas aeruginosa* recycle methionine, enabling growth on methionine as a sole sulfur source [13]. Consistent with this, *M. tuberculosis* and *K. aerogenes* import methionine *in vitro* and *P. aeruginosa* encodes homologs of methionine transporters present in *E. coli* [28–31]. However, evidence that the pathogens utilize methionine as a source of sulfur during infection is lacking.

Glutathione (GSH)

GSH is a potent antioxidant in host and bacterial cells [2,32]. GSH contains a reactive thiol in the form of Cys, one of the three amino acids that comprise the tripeptide. A glutamate residue is bound to Cys via a unique γ-bond between the R group carboxyl of glutamate and the amino group of Cys. Glycine, the third amino acid, is covalently linked to Cys via a standard peptide bond. GSH functions as a low molecular weight thiol by fluctuating between reduced and oxidized (GSSG) states. In the oxidized state, a disulfide bond adjoins two GSH via the Cys thiols. Within eukaryotic cells, GSH levels range from 1 to 10 mM and are kept at a strict GSH:GSSG ratio [2,33]. On average, human plasma levels of GSH exceed GSSG nearly 30-fold (37.03 μM and 1.69 μM, respectively) (Table 1) [34]. The host spends considerable energy maintaining GSH levels greater than GSSG. This allows for maximal reactivity of the thiol groups to detoxify reactive oxygen species and other poisons [2,33].

GSH abundance makes it an ideal source of sulfur and numerous pathogens express transporters that scavenge the plentiful nutrient (Table 1). Upon import, liberation of Cys from GSH proceeds via γ−glutamyl-transpeptidase (Ggt) (Figure 1 #14), an enzyme that cleaves the γ-peptide bond releasing glutamate and producing γ-cysteinyl-glycine [2]. γ-cysteinyl-glycine is subsequently processed by peptidases that release Cys from glycine (Figure 1 #15) [2,35•]. The unique γ-peptidase activity of Ggt is required to initiate utilization of GSH as a sulfur source and a subset of pathogens encode Ggt (Table 1). For example, Ggt is required during *Francisella tularensis* infection as the enzyme cleaves host GSH to produce Cys, fulfilling the sulfur requirement, and driving intracellular proliferation [36].

Taurine

The non-proteinogenic amino acid taurine is abundant in many mammalian tissues but is predominately found complexed to bile salts within the intestines [37]. Consequently, members of *Enterobacteriaceae* scavenge taurine for use as a sulfur source [38]. Taurine is a substrate for α-ketoglutarate-dependent dioxygenase, an enzyme that produces aminoacetaldehyde and sulfite (Figure 1 #17) which is then processed to sulfide by sulfite reductase (Figure 1 #4) [13]. It is unclear whether taurine is a viable sulfur source for non-enteric pathogens such as *S. aureus*. Published findings contradict the capacity of taurine to stimulate *S. aureus* growth in a sulfur deplete medium [39,40]. The fact that *S. aureus* lacks an α-ketoglutarate-dependent dioxygenase homologue supports the supposition that taurine is not a viable sulfur source for this pathogen [39].

Frontiers in pathogen nutrient sulfur acquisition

Mammals lack the ability to synthesize Cys from inorganic sulfur; consequently Cys and methionine are obtained from the diet [41••]. Therefore, bacterial sulfate assimilation and Cys synthesis have gained considerable interest as a potential therapeutic strategy to combat infection [42–45] (reviewed in Ref. [46]). An *in silico* screen identified inhibitors of both *Salmonella* OAS sulfhydrylase isozymes (Figure 1 #5 and #11) as potent *in vitro* antimicrobials [42]. OAS sulfhydrylase inhibitors are also being developed for the treatment of infections caused by *M. tuberculosis* (reviewed in Ref. [47]) and other bacterial pathogens [45,48–50].

The enzyme APS reductase, which functions in *M. tuberculosis* sulfate assimilation (Figure 1 #14), is also gaining interest as an antibacterial drug target [51]. APS reductase inhibitors result in significantly reduced levels of downstream sulfur-containing metabolites, and these compounds display bactericidal activity [51]. The *in vivo* efficacy of sulfate assimilation and Cys synthesis inhibitors has yet to be established. As discussed, most pathogens encode redundant nutrient sulfur procurement strategies that exploit host-derived organosulfur metabolites, so inhibiting sulfate assimilation may not be, ultimately, a successful strategy.

Evidence is mounting that some pathogens prefer organosulfur sources, and Cys auxotrophs are isolated from a surprising number of clinical samples derived from patients infected with bacterial species capable of sulfate assimilation [52–55]. Prominent examples are Uropathogenic *E. coli* (UPEC), as 1.5–2% of clinical isolates are Cys auxotrophs [55], and subsets of *Klebsiella* urinary tract infection isolates are also Cys auxotrophs [54]. Loss of Cys synthesis supports the hypothesis that during infection these pathogens have access to reservoirs of host Cys or Cys derivatives, obviating the need for endogenous Cys synthesis via sulfate assimilation. Urine contains a relatively high concentration of free Cys (65.8 μmol mmol^{-1} creatinine) [56], but Cys auxotrophs are also found among pathogens that colonize different host environments. Subpopulations of *Burkholderia cepacia* isolated from patients afflicted with cystic fibrosis and a subset of *Micrococcus* isolates, a skin commensal, are also Cys auxotrophs [52,53]. The prevalence of Cys auxotrophs and the diversity of sulfur sources available during infection suggests that pathogens will evade therapeutic interventions that exclusively target *de novo* Cys synthesis from inorganic sulfur sources. As the field of pathogen nutrient sulfur acquisition is just beginning to take shape, additional studies are needed to reveal the frequency of Cys auxotroph isolation in other notable bacterial diseases. These findings will define the therapeutic potential and efficacy of targeting *de novo* Cys synthesis and sulfate assimilation to treat bacterial infections.

Concluding remarks

Historically sulfur acquisition has been an understudied area in the field of pathogen nutrient acquisition. Reports described herein have considerably increased our knowledge of nutrient sulfur sources beyond sulfate by revealing that numerous pathogens that lack sulfate assimilation can use organosulfur sources. Future studies focused on defining sulfur source acquisition mechanisms and determining their importance in animal models of infection will elucidate novel avenues for the therapeutic intervention of increasingly antibiotic resistant pathogens.

Conflict of interest statement

Nothing declared.

Acknowledgements

We acknowledge Hammer laboratory members, Dr Victor DiRita (MSU), and Dr Chris Waters (MSU) for thoughtful and critical discussion of the manuscript. Funding was provided by Michigan State University and an American Heart Association Scientist Development Grant [16SDG30170026]. We apologize to colleagues whose work was not included due to space restrictions.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Beinert H: **A tribute to sulfur**. *Eur J Biochem* 2000, **267**:5657-5664.
2. Meister A, Anderson ME: **Glutathione**. *Annu Rev Biochem* 1983, **52**:711-760.
3. Hoppe B, Kemper MJ, Hvizd MG, Sailer DE, Langman CB: **Simultaneous determination of oxalate, citrate and sulfate in children's plasma with ion chromatography**. *Kidney Int* 1998, **53**:1348-1352.
4. Kredich NM: **Biosynthesis of cysteine**. In *Escherichia coli and Salmonella, Cellular and Molecular Biology.* Edited by Neidhardt. 1996:514-527.
5. Lill R, Mühlenhoff U: **Iron-sulfur protein biogenesis in eukaryotes: components and mechanisms**. *Annu Rev Cell Dev Biol* 2006, **22**:457-486.
6. • Gebhardt MJ, Gallagher LA, Jacobson RK, Usacheva EA, Peterson LR, Zurawski DV, Shuman HA: **Joint transcriptional control of virulence and resistance to antibiotic and environmental stress in *Acinetobacter baumannii***. *mBio* 2015, **6** e01660-15.

An elegant study that establishes the importance of sulfate assimilation inA. *baumannii* infection of *G. mellonella* larvae.

7. Fontán P, Aris V, Ghanny S, Soteropoulos P, Smith I: **Global transcriptional profile of *Mycobacterium tuberculosis* during THP-1 human macrophage infection**. *Infect Immun* 2008, **76**:717-725.
8. Sassetti CM, Boyd DH, Rubin EJ: **Comprehensive identification of conditionally essential genes in mycobacteria**. *Proc Natl Acad Sci U S A* 2001, **98**:12712-12717.
9. Nakamura T, Iwahashi H, Eguchi Y: **Enzymatic proof for the identity of the S-sulfocysteine synthase and cysteine synthase B of *Salmonella typhimurium***. *J Bacteriol* 1984, **158**:1122-1127.
10. Nakamura T, Kon Y, Iwahashi H, Eguchi Y: **Evidence that thiosulfate assimilation by *Salmonella typhimurium* is catalyzed by cysteine synthase B**. *J Bacteriol* 1983, **156**:656-662.

11. Lithgow JK, Hayhurst EJ, Cohen G, Aharonowitz Y, Foster SJ:
•• **Role of a cysteine synthase in *Staphylococcus aureus***. *J Bacteriol* 2004, **186**:1579-1590.
An important study that is one of the first to explore sulfur assimilation beyond sulfate in*S. aureus*. This publication expanded our knowledge of *S. aureus* sulfur source utilization

12. Dubois T, Dancer-Thibonnier M, Monot M, Hamiot A, Bouillaut L, Soutourina O, Martin-Verstraete I, Dupuy B: **Control of *Clostridium difficile* physiopathology in response to cysteine availability**. *Infect Immun* 2016, **84**:2389-2405.

13. Guédon E, Martin-Verstraete I: **Cysteine metabolism and its regulation in bacteria**. In *Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering.* Edited by Wendisch VF. Springer Berlin Heidelberg; 2007:195-218.

14. Kredich N: **Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. Effects of growth on varying sulfur sources and O-acetyl-L-serine on gene expression**. *J Biol Chem* 1971, **246**:3474-3484.

15. Ostrowski J, Kredich NM: **In vitro interactions of CysB protein**
• **with the *cysJIH* promoter of *Salmonella typhimurium*: inhibitory effects of sulfide**. *J Bacteriol* 1990, **172**:779-785.
Notable findings that demonstrate serine*O*-acetyltransferase is responsive to the levels of cysteine in the cell by a negative feedback loop.

16. André G, Haudecoeur E, Monot M, Ohtani K, Shimizu T, Dupuy B, Martin-Verstraete I: **Global regulation of gene expression in response to cysteine availability in *Clostridium perfringens***. *BMC Microbiol* 2010, **10**:234.

17. Ewann F, Hoffman PS: **Cysteine metabolism in *Legionella pneumophila*: characterization of an L-cystine-utilizing mutant**. *Appl Environ Microbiol* 2006, **72**:3993-4000.

18. Stenson TH, Patton AK, Weiss AA: **Reduced glutathione is required for pertussis toxin secretion by *Bordetella pertussis***. *Infect Immun* 2003, **71**:1316-1320.

19. Stainer DW, Scholte MJ: **A simple chemically defined medium for the production of phase I *Bordetella pertussis***. *J Gen Microbiol* 1971, **63**:211-220.

20. Catlin BW: **Nutritional requirements and auxotyping**. In *The Gonococcus.* Edited by Roberts RB. 1977.

21. Schär J, Stoll R, Schauer K, Loeffler DIM, Eylert E, Joseph B, Eisenreich W, Fuchs TM, Goebel W: **Pyruvate carboxylase plays a crucial role in carbon metabolism of extra- and intracellularly replicating *Listeria monocytogenes***. *J Bacteriol* 2010, **192**:1774-1784.

22. Xayarath B, Marquis H, Port GC, Freitag NE: ***Listeria***
•• ***monocytogenes* CtaP is a multifunctional cysteine transport-associated protein required for bacterial pathogenesis**. *Mol Microbiol* 2009, **74**:956-973.
A prominent study that establishes the importance of cysteine import to the pathogenesis of*L. monocytogenes* by investigating virulence of a cysteine transport associated protein mutant.

23. Vorwerk H, Mohr J, Huber C, Wensel O, Schmidt-Hohagen K, Gripp E, Josenhans C, Schomburg D, Eisenreich W, Hofreuter D: **Utilization of host-derived cysteine-containing peptides overcomes the restricted sulphur metabolism of *Campylobacter jejuni***. *Mol Microbiol* 2014, **93**:1224-1245.

24. Kim J, Senadheera DB, Lévesque CM, Cvitkovitch DG: **TcyR regulates L-cystine uptake via the TcyABC transporter in *Streptococcus mutans***. *FEMS Microbiol Lett* 2012, **328**:114-121.

25. Burguière P, Auger S, Hullo M-F, Danchin A, Martin-Verstraete I: **Three different systems participate in L-cystine uptake in *Bacillus subtilis***. *J Bacteriol* 2004, **186**:4875-4884.

26. Tsai H-N, Hodgson DA: **Development of a synthetic minimal medium for *Listeria monocytogenes***. *Appl Environ Microbiol* 2003, **69**:6943-6945.

27. Schauer K, Geginat G, Liang C, Goebel W, Dandekar T, Fuchs TM: **Deciphering the intracellular metabolism of *Listeria monocytogenes* by mutant screening and modelling**. *BMC Genomics* 2010, **11**:573.

28. Seiflein TA, Lawrence JG: **Methionine-to-cysteine recycling in *Klebsiella aerogenes***. *J Bacteriol* 2001, **183**:336-346.

29. Figge RM: **Methionine biosynthesis in *Escherichia coli* and *Corynebacterium glutamicum***. In *Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering.* Edited by Wendisch VF. Springer Berlin Heidelberg; 2007:163-193.

30. Wheeler PR, Coldham NG, Keating L, Gordon SV, Wooff EE, Parish T, Hewinson RG: **Functional demonstration of reverse transsulfuration in the *Mycobacterium tuberculosis* complex reveals that methionine is the preferred sulfur source for pathogenic Mycobacteria**. *J Biol Chem* 2005, **280**:8069-8078.

31. Zhang Z, Feige JN, Chang AB, Anderson IJ, Brodianski VM, Vitreschak AG, Gelfand MS, Saier MH: **A transporter of *Escherichia coli* specific for L- and D-methionine is the prototype for a new family within the ABC superfamily**. *Arch Microbiol* 2003, **180**:88-100.

32. Fahey RC, Brown WC, Adams WB, Worsham MB: **Occurrence of glutathione in bacteria**. *J Bacteriol* 1978, **133**:1126-1129.

33. Masip L, Veeravalli K, Gerogiou G: **The many faces of glutathione in bacteria**. *Antioxid Redox Signal* 2006, **8**:753-762.

34. Tavazzi B, Lazzarino G, Leone P, Amorini AM, Bellia F, Janson CG, Di Pietro V, Ceccarelli L, Donzelli S, Francis JS *et al.*: **Simultaneous high performance liquid chromatographic separation of purines, pyrimidines, N-acetylated amino acids, and dicarboxylic acids for the chemical diagnosis of inborn errors of metabolism**. *Clin Biochem* 2005, **38**:997-1008.

35. Suzuki H, Kamatani S, Kim ES, Kumagai H: **Aminopeptidases A,**
• **B, and N and dipeptidase D are the four cysteinylglycinases of *Escherichia coli* K-12**. *J Bacteriol* 2001, **183**:1489-1490.
This study establishes the importance of GSH degradation to proliferation of*F. tularensis*. The paper reveals a *ggt* mutant is attenuated in a murine model of infection

36. Alkhuder K, Meibom KL, Dubail I, Dupuis M, Charbit A: **Glutathione provides a source of cysteine essential for intracellular multiplication of *Francisella tularensis***. *PLoS Pathog* 2009, **5** e1000284.

37. Sjovall J: **Dietary glycine and taurine on bile acid conjugation in man. Bile acids and steroids 75**. *Exp Biol Med* 1959, **100**:676-678.

38. Cowie DB, Bolton ET, Sands MK: **Sulfur metabolism in *Escherichia coli.* II. Competitive utilization of labeled and nonlabeled sulfur compounds**. *J Bacteriol* 1951, **62**:63-74.

39. Soutourina O, Poupel O, Coppée J-Y, Danchin A, Msadek T, Martin-Verstraete I: **CymR, the master regulator of cysteine metabolism in *Staphylococcus aureus*, controls host sulphur source utilization and plays a role in biofilm formation**. *Mol Microbiol* 2009, **73**:194-211.

40. Smiley DW, Wilkinson BJ: **Survey of taurine uptake and metabolism in *Staphylococcus aureus***. *J Gen Microbiol* 1983, **129**:2421-2428.

41. Schwartz NB, Lyle S, Ozeran JD, Li H, Deyrup A, Ng K, Westley J:
•• **Sulfate activation and transport in mammals: system components and mechanisms**. *Chem Biol Interact* 1998, **109**:143-151.
An intriguing study that developed chemical inhibitors of OAS sulfhydrylase displaying*in vitro* growth inhibition.

42. Spyrakis F, Singh R, Cozzini P, Campanini B, Salsi E, Felici P, Raboni S, Benedetti P, Cruciani G, Kellogg GE *et al.*: **Isozyme-specific ligands for O-acetylserine sulfhydrylase, a novel antibiotic target**. *PLoS One* 2013, **8**:e77558.

43. Mazumder M, Gourinath S: **Structure-based design of inhibitors of the crucial cysteine biosynthetic pathway enzyme O-acetyl serine sulfhydrylase**. *Curr Top Med Chem* 2016, **16**:948-959.

44. Benoni R, Pertinhez TA, Spyrakis F, Davalli S, Pellegrino S, Paredi G, Pezzotti A, Bettati S, Campanini B, Mozzarelli A: **Structural insight into the interaction of O-acetylserine sulfhydrylase with competitive, peptidic inhibitors by saturation transfer difference-NMR**. *FEBS Lett* 2016, **590**:943-953.

45. Salsi E, Bayden AS, Spyrakis F, Amadasi A, Campanini B, Bettati S, Dodatko T, Cozzini P, Kellogg GE, Cook PF *et al.*: **Design of O-acetylserine sulfhydrylase inhibitors by mimicking nature**. *J Med Chem* 2010, **53**:345-356.
46. Campanini B, Pieroni M, Raboni S, Bettati S, Benoni R, Pecchini C, Costantino G, Mozzarelli A: **Inhibitors of the sulfur assimilation pathway in bacterial pathogens as enhancers of antibiotic therapy**. *Curr Med Chem* 2015, **22**:187-213.
47. Schnell R, Sriram D, Schneider G: **Pyridoxal-phosphate dependent mycobacterial cysteine synthases: structure, mechanism and potential as drug targets**. *Biochim Biophys Acta* 2015, **1854**:1175-1183.
48. Amori L, Katkevica S, Bruno A, Campanini B, Felici P, Mozzarelli A, Costantino G: **Design and synthesis of trans-2-substituted-cyclopropane-1-carboxylic acids as the first non-natural small molecule inhibitors of O-acetylserine sulfhydrylase**. *MedChemComm* 2012, **3**:1111.
49. Brunner K, Steiner EM, Reshma RS, Sriram D, Schnell R, Schneider G: **Profiling of in vitro activities of urea-based inhibitors against cysteine synthases from *Mycobacterium tuberculosis***. *Bioorg Med Chem Lett* 2017, **27**:4582-4587.
50. Brunner K, Maric S, Reshma RS, Almqvist H, Seashore-Ludlow B, Gustavsson A-L, Poyraz Ö, Yogeeswari P, Lundbäck T, Vallin M *et al.*: **Inhibitors of the cysteine synthase CysM with antibacterial potency against dormant *Mycobacterium tuberculosis***. *J Med Chem* 2016, **59**:6848-6859.
51. Palde PB, Bhaskar A, Pedró Rosa LE, Madoux F, Chase P, Gupta V, Spicer T, Scampavia L, Singh A, Carroll KS: **First-in-class inhibitors of sulfur metabolism with bactericidal activity against non-replicating *M. tuberculosis***. *ACS Chem Biol* 2016, **11**:172-184.
52. Barth AL, Pitt TL: **Auxotrophy of *Burkholderia (Pseudomonas) cepacia* from cystic fibrosis patients**. *J Clin Microbiol* 1995, **33**:2192-2194.
53. Farrior JW, Kloos WE: **Sulfur amino acid auxotrophy in *Micrococcus* species isolated from human skin**. *Can J Microbiol* 1976, **22**:1680-1690.
54. McIver CJ, Tapsall JW: **Characteristics of cysteine-requiring strains of *Klebsiella* isolated from urinary tract infections**. *J Med Microbiol* 1988, **26**:211-215.
55. Gibreel TM, Sifaw Ghenghesh K: **Cysteine-dependent uropathogens: isolation, identification and susceptibility to antimicrobial agents**. *Jamahiriya Med J* 2002, **2**:52-54.
56. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P *et al.*: **The human urine metabolome**. *PLoS One* 2013, **8**:e73076.
57. Robinson CV, Elkins MR, Bialkowski KM, Thornton DJ, Kertesz MA: **Desulfurization of mucin by *Pseudomonas aeruginosa*: influence of sulfate in the lungs of cystic fibrosis patients**. *J Med Microbiol* 2012, **61**:1644-1653.
58. Takahashi H, Hirose K, Watanabe H: **Necessity of meningococcal gamma-glutamyl aminopeptidase for *Neisseria meningitidis* growth in rat cerebrospinal fluid (CSF) and CSF-like medium**. *J Bacteriol* 2004, **186**:244-247.
59. Suzuki H, Koyanagi T, Izuka S, Onishi A, Kumagai H: **The *yliA, -B, -C*, and *-D* genes of *Escherichia coli* K-12 encode a novel glutathione importer with an ATP-binding cassette**. *J Bacteriol* 2005, **187**:5861-5867.
60. Wang Z, Zhang M, Shi X, Xiang Q: **Purification and characterization of an ATPase GsiA from *Salmonella enterica***. *Biomed Res Int* 2017, **2017** 3076091.
61. Vergauwen B, Elegheert J, Dansercoer A, Devreese B, Savvides SN: **Glutathione import in *Haemophilus influenzae* Rd is primed by the periplasmic heme-binding protein HbpA**. *Proc Natl Acad Sci U S A* 2010, **107**:13270-13275.
62. Vergauwen B, Verstraete K, Senadheera DB, Dansercoer A, Cvitkovitch DG, Guédon E, Savvides SN: **Molecular and structural basis of glutathione import in Gram-positive bacteria via GshT and the cystine ABC importer TcyBC of *Streptococcus mutans***. *Mol Microbiol* 2013, **89**:288-303.
63. Potter AJ, Trappetti C, Paton JC: ***Streptococcus pneumoniae* uses glutathione to defend against oxidative stress and metal ion toxicity**. *J Bacteriol* 2012, **194**:6248-6254.
64. Ivankovich AD, Braverman B, Stephens TS, Shulman M, Heyman HJ: **Sodium thiosulfate disposition in humans relation to sodium nitroprusside toxicity**. *Anesthesiology: J Am Soc Anesthesiol* 1983, **58**:11-17.
65. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B *et al.*: **The human serum metabolome**. *PLoS One* 2011, **6**:e16957.
66. Cynober LA: **Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance**. *Nutrition* 2002, **18**:761-766.
67. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S *et al.*: **HMDB: the human metabolome database**. *Nucleic Acids Res* 2007, **35**:D521-D526.