**Characterization of H2S producing genes included in the search space**

For each gene considered, we stated 1. the main species the gene is found in (limited to gut inhabiting bacteria), 2. whether the downstream enzyme is dependent on pyridoxal 5’-phosphate (PLP) binding, and 3. the primary function of the gene with references.

* **Green** highlighted genes indicate that H2S output is central to the **primary** function of the gene and/or these genes are upregulated in the presence of cysteine.
* **Yellow** highlighted genes indicate that H2S output is a **secondary** function of the gene and/or is closely related to cysteine degradation. Most of these genes, apart from *malY*, are involved in some component of the transulfuration pathway and additionally exhibit cysteine desulfurase activity.
* **Red** highlighted genes indicate that the gene has been shown to produce H2S from cysteine, but the primary function of the gene is unrelated to H2S production and/or the gene does appear to be upregulated in the presence of cysteine. We refer to these genes as **erroneous** H2S producing genes.

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| ***gene abbreviation*** | **Full gene name** |

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| ***dcyD*** | **Cysteine desulfhydrase** |

* Main species:
	+ *Klebsiella pneumoniae (many strains)*
	+ *Escherichia coli (K-12)*
* UniProt Entry: <https://www.uniprot.org/uniprot/A6TB69>
* PLP-dependent: **YES**
* Primary function: catalyzes the the beta elimination of D-cysteine and other D-cysteine derivatives. Potentially useful for detoxification of D-cysteine. (1,2)

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| ***yhaOM* operon** | **Unnamed proteins** |

* Main species:
	+ *Escherichia coli (K-12)*
* PLP-dependent: **NO**
* Primary function: yhaO and yhaM are genes in the yhaOM operon, regulated by decR in E. coli (3). *yhaO* is likely a serine/cysteine transporter protein and *yhaM* has cysteine desulfurase activity (3). Of all the cysteine desulfurase active proteins in *E. coli, yhaM* has been proposed to be the primary cysteine desulfurase protein (4), as many of the other genes identified by Awona et al. (5,6) have other primary functions such as Fe-S complex formation in the case of *iscS*.

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| ***mgl*** | **Methionine gamma-lyase** |

* Main species:
	+ *Fusobacterium nucleatum*
	+ *Porphyromonas gingivalis*
	+ *Treponema denticola*
* PLP-dependent: **YES**
* Primary function: The above species are the most frequently encountered species in the human oral microbiome, and have been implicated in periodontitis (7). Due to the mucosal-degrading activity of methanethiol (CH3SH) and hydrogen sulfide (H2S), it is believed by some that *mgl* is actively expressed in the presence of methionine and cysteine and acts as a pathogenicity factor (8).

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| ***aspC* + *sseA*** | **Cysteine aminotransferase + 3-mercaptopyruvate sulfurtransferase** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ *aspC* found in *E. coli K-12*
	+ Many other species have *sseA* predicted based on homology including: *Yersinia spp., Salmonella spp., Brucella spp., Clostridium spp.* and *Klebsiella spp. (9)*
* PLP-dependent: *aspC* – **YES**; *sseA* – **NO**
* Primary function: Proposed by Shatalin et al. (9), H2S production by *sseA* leads to decreased antibiotic sensitivity. However, it appears that other modes of H2S production are more prominent in anerobic environments (10). This mechanism is more complicated, because *sseA* acts on 3-mercaptopyruvate, which is produced from cysteine by a cysteine aminotransferase, such as *aspC* (11).

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| ***metC*** | **Cystathionine beta-lyase** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ *Bacillus subtilis (168)*
* PLP-dependent: **YES**
* Primary function: Involved in the transulfuration pathway (cysteine to methionine interconversion). Specifically, *metC* reversibly converts cystathionine to homocysteine (5,12,13). Any cysteine desulfhydrase activity resulting in H2S production seems to be secondary to the primary function of cystathionine to homocysteine interconversion (3).

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| ***malY*** | **Cystathionine beta-lyase like; repressor of maltose regulon** |

* Main species:
	+ *Escherichia coli (K-12)*
* PLP-dependent: **YES**
* Primary function: *malY* is one of the few instances of a truly bi-functional protein on this list, serving as both a cystathionine beta-lyase (*metC*-like) and as a repressor of the maltose regulon responsible for the uptake and metabolism of maltose and maltodextrins (14,15). *malY* can recapitulate cystathionine beta-lyase activity in *metC* mutant strains of *E. coli*.

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| ***cysK*** | **Cysteine synthase A** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ *Bacillus subtilis (168)*
* PLP-dependent: **YES**
* Primary function: converts O-acetyl-serine + H2S -> acetate + cysteine (13), however, the pathway appears to be reversible, resulting in H2S production from cysteine (3,6). Additionally, appears to be crucial in contact dependent growth inhibition of competing bacteria (16).

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| ***cysM*** | **Cysteine synthase B** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ *Bacillus subtilis (168)*
* PLP-dependent: **YES**
* Primary function: converts O-acetyl-serine + H2S -> acetate + cysteine (13), however, the pathway appears to be reversible, resulting in H2S production from cysteine (3,6).

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| ***mccB*** | **Cystathionine gamma-lyase** |

* Main species:
	+ *Bacillus subtilis (168)*
* PLP-dependent: **YES**
* Primary function: Catalyzes the conversion of cystathionine -> cysteine. Can also catalyze a similar gamma-lyase reaction converting homocysteine + H2O -> H2S + 2-oxobutanoate + ammonia + pyruvate (13). Due to the potentially bifunctionality of this gene, it may be upgraded from erroneous to secondary production of H2S.

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| ***tnaA*** | **Tryptophanase** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ *Proteus vulgaris*
	+ *Shigella flexneri*
	+ *Klebsiella aerogenes*
* PLP-dependent: **YES**
* Primary function: Catalyzes the degradation of H2O + tryptophan -> indole + ammonia + pyruvate (17). *tnaA* has been shown to perform cysteine dehydratase activity under certain circumstances (5), but the upregulation of *tnaA* expression and activity could not be replicated in future studies (4). It is possible that the primary function of *tnaA* is tryptophan degradation and any cysteine desulhydrase activity is erroneous.

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| ***iscS*** | **Cysteine desulfurase** |

* Main species:
	+ *Escherichia coli (K-12)*
	+ (very common gene in all of life)
* PLP-dependent: **YES**
* Primary function: *iscS* is potentially the most clear-cut case of erroneous H2S production by gut bacteria. *iscS* is a master enzyme that transfers sulfur from cysteine to aid in Fe-S cluster assembly, a key structure in the formation of many proteins (18) (and many other references). However, it should be noted that the production of H2S mediated by *iscS* in E. coli can still prove to be quite substantial in anaerobic and high cysteine conditions (10).

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| ***mccA*** | **O-acetylserine dependent cystathionine beta-synthase** |

* Main species:
	+ *Bacillus subtilis (168)*
	+ *Staphylococcus aureus*
* PLP-dependent: **YES**
* Primary function: Converts O-acetylserine and homocysteine to cystathionine (13). Given the primary function of the enzyme, it appears that any cysteine desulfurase activity is either secondary or erroneous.

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