Carbon starvation genes mediate the cross-talk between metabolism and pathogenesis of *Salmonella* Typhimurium

Salmonella enterica serovar Typhimurium (*S*. Typhimurium) can infect a wide range of host animals to cause diseases like gastroenteritis and typhoid fever. Therefore it serves as an ideal model organism to study *Salmonella* pathogenesis. The adaptability of *Salmonella* to diverse environmental conditions is due to its ability to utilize a plethora of nutrients. While various carbon and nitrogen sources are supplied by the host, the role of peptides as alternate nutrient source for *Salmonella* is not clearly defined. These peptides are presented by the host as antimicrobial peptides, but can serve as nutrients too, once taken up by peptide transporters and digested by the pathogen. The importance of peptide transporters is also reported for alternate functions such as quorum sensing, competence, chemotaxis and virulence. The ABC transporters of peptides are well studied in *Salmonella*, whereas little is known about the putative peptide transporter family named as carbon starvation genes which lack ATP binding site. Two carbon starvation (*cst*) genes, *cstA* and *yjiY*, are the only genes known to belong to this group of peptide transporters in *Salmonella*. *cstA* was previously reported to be required for virulence of *Salmonella* in *C. elegans.*

To establish the role of *cst* genes in the metabolism and pathogenesis of *S*. Typhimurium, the knockout strains for the genes *cstA* and *yjiY* in *S*. Typhimurium, denoted as Δ*cstA* and Δ*yjiY*, were generated. The metabolic capacity of these mutants was checked by phenotype microarray revealing that *cst* knockout strains were compromised in peptide metabolism and Δ*yjiY* strain showed remarkable difference from the wild type in the ability to utilize a few peptides. Fluorescent peptide uptake assay showed reduced uptake of specific dipeptides by Δ*yjiY* strain. Thus, *cst* genes contribute to metabolism of *Salmonella* by transporting specific peptides.

Upon infecting *C. elegans*, Δ*cstA* was unable to colonize the intestine of the worm verifying the reported role of *cstA*. However, in mammalian model systems, Δ*yjiY*, but not Δ*cstA*, was unable to invade various types of host cells, attributed to defective adhesion of Δ*yjiY* because of lack of flagella. The *in vivo* significance of *yjiY* was established when Δ*yjiY* showed decreased colonization of mouse gut. Transcriptome analysis showed upregulation of the virulence factor *mgtC* in Δ*yjiY*, which led to better proliferation of Δ*yjiY* inside macrophages. The expression of *mgtC* is induced in the absence of proline suggesting that *yjiY* might be involved in transporting proline rich peptides. Therefore, both *cst* genes are required for the virulence of *Salmonella*, but in different host systems.

When biofilm forming ability of the wild type and *cst* mutant strains was tested *in vitro*, only Δ*yjiY* strain was unable to form biofilm. Confocal microscopy and assessment of rdar morphotype revealed that Δ*yjiY* strain lacked extracellular polymeric substance (EPS) production, which is an essential component of biofilm matrix. The mechanism behind was found to be the downregulation in the expression of the biofilm master regulator gene, *csgD* that controls EPS biosynthesis in *Salmonella*. Conclusively, *yjiY* is required for EPS biosynthesis and hence biofilm formation in *Salmonella*.