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Influence of biliary salts and of the *ramRA* locus on *Salmonella* Typhimurium invasion

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Bile represses *Salmonella* Typhimurium (ST) cell invasion, but it remains unclear which regulation pathway and particular bile components are involved. The *ramRA* global regulatory locus has been shown to regulate, in addition to multidrug efflux genes, invasion genes of the type III secretion system 1 (TTSS-1). Overexpression of *ramA*, either ectopic or due to *ramR* mutations is associated to decreased TTSS-1 genes expression, and to decreased invasion in some ST strains. As we recently showed that bile derepresses *ramA* transcription by inhibiting the binding of the RamR repressor to the *ramA* promoter, we investigated the involvement of *ramRA* in the bile-mediated repression of invasion.

We used qRT-PCR and intestinal epithelial cells invasion assays to test the effects of primary bile salts and their derivatives on invasion, at the gene expression (*ramA* and *hila*, the master activator of TTSS-1 genes) and phenotypic levels. Dependency of these effects on *ramRA* was tested using experimental mutants of this locus.

Individual bile salts repressed *hila* expression and cell invasion differentially, depending on their precise structure (hydroxylations, conjugation to taurine or glycine). For example, chenodeoxycholic acid-derived salts repressed invasion, whereas cholic acid-derived salts did not. Additionally, although bile increases *ramA* expression and *ramA* overexpression decreases TTSS-1 genes expression, results observed with the *ramRA* deletion mutant suggest that the *ramRA* locus has no major role in the repression of cell invasion by bile.