mBio 2020 vol.11 N3

Linearized siderophore products secreted via macab efflux pump protect salmonella enterica serovar typhimurium from oxidative stress

Bogomolnaya L.M., Tilvawala R., Elfenbein J.R., Cirillo J.D., Andrews-Polymenis H.L.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2020 Bogomolnaya et al. Nontyphoidal salmonellae (NTS) are exposed to reactive oxygen species (ROS) during their residency in the gut. To survive oxidative stress encountered during infection, salmonellae employ several mechanisms. One of these mechanisms involves the multidrug efflux pump MacAB, although the natural substrate of this pump has not been identified. MacAB homologs in pseudomonads secrete products of nonribosomal peptide synthesis (NRPS). In Salmonella enterica serovar Typhimu-rium, the siderophore enterobactin is produced by NRPS in response to iron starva-tion and this molecule can be processed into salmochelin and several linear metab-olites. We found that Salmonella mutants lacking the key NRPS enzyme EntF are sensitive to peroxide mediated killing and cannot detoxify extracellular H2O2. More-over, EntF and MacAB function in a common pathway to promote survival of Salmonella during oxidative stress. We further demonstrated that S. Typhimurium secretes siderophores in iron-rich media when peroxide is present and that these MacAB-secreted metabolites participate in protection of bacteria against H2O2. We showed that secretion of anti-H2O2 molecules is independent of the presence of the known siderophore efflux pumps EntS and IroC, well-described efflux systems involved in secretion of enterobactin and salmochelin. Both salmochelin and enterobactin are dispensable for S. Typhimurium protection against ROS; however, linear metabolites of enterobactin produced by esterases IroE and Fes are needed for bacterial survival in peroxide-containing media. We determined that linearized enterobactin trimer protects S. Typhimurium against peroxide-mediated killing in a MacAB-dependent fashion. Thus, we suggest that linearized enterobactin trimer is a natural substrate of MacAB and that its purpose is to detoxify extracellular reactive oxygen species. IMPORTANCE Nontyphoidal Salmonella bacteria induce a classic inflammatory diarrhea by eliciting a large influx of neutrophils, producing a robust oxidative burst. Despite substantial progress understanding the benefits to the host of the inflammatory response to Salmonella, little is known regarding how Salmonella can simultane-ously resist the damaging effects of the oxidative burst. The multidrug efflux pump MacAB is important for survival of oxidative stress both in vitro and during infection. We describe a new pathway used by Salmonella Typhimurium to detoxify extracellular reactive oxygen species using a multidrug efflux pump (MacAB) to secrete a linear siderophore, a metabolite of enterobactin. The natural substrates of many multi-drug efflux pumps are unknown, and functional roles of the linear metabolites of enterobactin are unknown. We bring two novel discoveries together to highlight an important mechanism used by Salmonella to survive under the oxidative stress con-ditions that this organism encounters during the classic inflammatory diarrhea that it also induces.

http://dx.doi.org/10.1128/mBio.00528-20

Keywords

MacAB, Multidrug efflux pump, Salmonella, Siderophores

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