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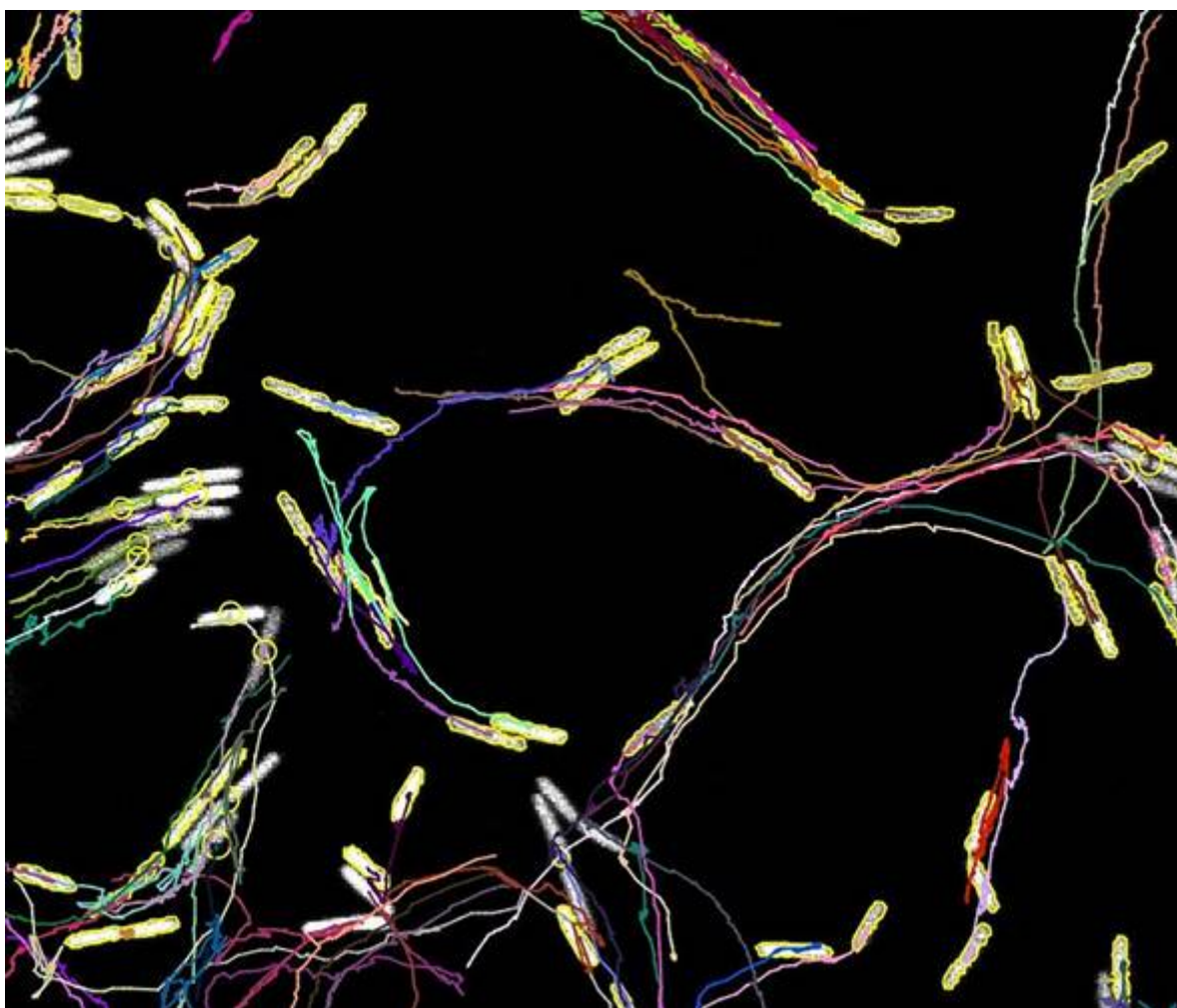


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## **CHARACTERIZATION OF MERCURY RESISTANCE LOCI ENCODED IN A MULTI-DRUG RESISTANCE PLASMID FROM A *Salmonella* CLINICAL ISOLATE.**

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Mercury (Hg) is a toxic metal that severely affects human health and biota diversity. Bacteria evolved mechanisms to detect and resist this pollutant, either in its ionized form ( $\text{Hg}^{2+}$ ) or when covalently bounded with organic compounds (organomercurials). Detoxification requires the entry of these toxic species into the cytoplasm through transporters and, if required, the release of  $\text{Hg}^{2+}$  by the action of an organomercurial lyase. Then  $\text{Hg}^{2+}$  is reduced to  $\text{Hg}^0$ , which is less harmful and volatilizes allowing its elimination. Resistance determinants are encoded in a single operon associated with the sensor/regulator protein MerR, which detects the ion in the cytoplasm and activates its transcription. These loci are commonly encoded in plasmids that also contain multiple resistance determinants to other metal(loid)s and antibiotics, and mobile genetic elements. Given the problem of the dissemination and co-selection of antimicrobial resistance, recently our laboratory initiated the characterization of one of these plasmids present in different clinical isolates of *Salmonella Typhimurium*. This plasmid contains not one but two Hg resistance loci, *mer1* and *mer2*, and the strains that carry them are at least 16 times more resistant to  $\text{Hg}^{2+}$  than the wild strain. To evaluate the contribution of each locus to Hg tolerance we cloned them in compatible plasmids and introduced individually as well as in pairs in *S. Typhimurium* and in *Escherichia coli*. The presence of either *mer1* or *mer2* increased the resistance to  $\text{HgCl}_2$ , although *mer1* was the main contributor. This dominance was even evident in cells carrying both loci. Although *mer1* and *mer2* count with transporters to uptake organomercurial compounds, only *mer2* encoding the organomercurial lyase MerB provided resistance to the organic form. No contribution of *mer1* to organomercurial tolerance was observed even when both loci are present in the cells. In view of these results, the regulatory particularities of these loci when present in the same cell were investigated. To do this, we cloned in different plasmids the MerR regulator proteins, MerR1 and MerR2, and the promoter region of each operon (*Pmer1* and *Pmer2*) upstream the lacZ reporter gene. Using  $\beta$ -galactosidase activity assays we analyzed the ability of each regulatory protein to modulate the expression of its associated operon and the possibility of cross talk between them. The results obtained indicate that both MerR1 and MerR2 efficiently control transcription from both operons, although a greater activation capacity for its associated operon was observed. The ability of each MerR regulator to repress its own expression along with the transcription of its paralog, as well as the role of other regulatory

determinants present in these loci, is under current investigation. These findings are not only important to better understand mercury resistance in bacteria and to develop bioremediation tools for environmental care, but are also relevant for health.

Palabras clave: Mercury resistance - Salmonella Typhimurium - MerR - transcriptional regulation