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Metal compounds in simulated food environments impact the frequency of horizontal gene transfer

Dayanne Maria da Silva Coimbra[1], Micaela Guidotti Takeuchi[1], Fernanda Raghiante[1], Carolyne Ferreira Dumont[1], Ana Laura Martins Ferreira[1], Letícia Roberta Martins Costa[1], Rosanne Aparecida Capanema[1], Deborah Araújo Policarpo[1], Luma Gonçalves Júlio[1], Rodrigo Profeta[1], Lívia de Moraes Bomediano Camilo[1], Wendel Guerra[1], Lígia Nunes de Morais Ribeiro[1], Daise Aparecida Rossi[1], Roberta Torres de Melo[1]

[1.] Universidade Federal de Uberlândia, Uberlândia/Minas Gerais, Brasil

Horizontal gene transfer (THG) impact and the combination of inhibitors against the mobile genetic elements transfer highlight a new, viable and promising strategy to minimize the high risks related to bacterial microevolution. Antibiotic resistance plasmids acquisition via gene recombination remains poorly understood in wild ecosystems and industrial environments. Our proposal aimed to provide new insights into the relationship between innovative copper-based metal compounds on conjugation frequency (CF) in the presence of co-products mimicking the conditions of the animal food industry chicken juice (CJ) and whey (SL), hotspots enhancing bacterial identification, conjugation between *Salmonella* Heidelberg (SH) and *Escherichia coli* J53 AzR (EC) and mitigating measures to reduce this process. Transconjugants complete genome analysis was carried out to verify the interference of the coproducts in CF and the use of crude copper complex (DRI12) and nanostructured in lipid carrier (NLCDRI12) as mitigators. The plasmids passage with resistance genes was evaluated and syntenic analysis was used to visualize the exchange or gene blocks deletion. The coproducts presence stimulated CF, but this was reduced in DRI-12 presence. In SL presence, DRI-12 allowed all plasmids carrying resistance genes to pass through and, in the similarity analysis, interfered with exchanges and collinearity regions deletions. Compared to DRI-12, NLCDRI12 did not reduce the transconjugants numbers, but it did prevent the IncC and IncX1_1 plasmids passage (resistance to sulfonamides and tetracyclines). There was an enhancing effect of SL and an inhibiting effect of NLCDRI12 on the plasmids TH. DRI12 and NLCDR12 had distinct impacts on the plasmid transfer control and of ampicillin-resistant transconjugants frequency in challenging environments with SL and CJ presence. The CFs in all DRI12 treatments were significantly reduced from 5.94 to 2.32 logUFC/mL in the coproducts absence, 6.40 to 1.47 in SL presence and 6.52 to 2.04 in of CJ presence, maintaining consistency with the rearrangement and deletion identified in the syntenic analysis. NLCDRI12 did not significantly reduce the transconjugants numbers (4.67 logUFC/mL without coproducts; 6.17 associated with SL and 5.01 with CJ) when compared to DRI12. In the NLCDRI12 + SL and NLCDRI12 + CJ treatments, we obtained syntenic authenticity of the HS plasmid, consistent with a high number of transconjugants expression. The syntenic rearrangement identification in the NLCDRI12 alone treatment justifies the significantly lower count when compared to NLCDRI12 + SL (difference of 1.5 logUFC/mL). Whole genome sequencing allowed us to unravel the challenges of using the two different compounds and their interactions with the bacteria, highlighting the impact of the SL coproduct in stimulating the resistance genes transfer, even in the metal compounds presence, which can mitigate CF and amplifying factors in the selection and spread of resistant strains, except when associated with modulators such as the presence of SL. Our results highlight the multifactorial interactions complexity between antimicrobials, the

environment and the bacterial resistance mechanisms, reinforcing the need for an integrated approach to tackle the global challenge of resistance.

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