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BIOLOGICALLY ACTIVE SUBSTANCES THAT BLOCK THE CONJUGATION PROCESS IN BACTERIA

Annotation: Horizontal gene transfer (HGT) in food matrices has been investigated under conditions that favor gene exchange. However, the major challenge lies in determining the specific conditions pertaining to the adapted microbial pairs associated with the food matrix. HGT is primarily responsible for enhancing the microbial repertoire for the evolution and spread of antimicrobial resistance and is a major target for controlling pathogens of public health concern in food ecosystems.

Key words: bacterial conjugation, blaTEM, chicken juice, nanostructured lipid carriers, whey.

Antimicrobial resistance (AMR) is one of the main global threats today, and recent reports estimate that it directly caused 1.27 million deaths in 2019. In Brazil, the National Health Surveillance Agency, through the Evaluation of National Indicators of Health Care-Related Infections (HAIs) and Microbial Resistance (MR), in the year 2021, estimated that Escherichia coli resistance to different classes of beta-lactams can reach rates of 47.5% to cephalosporins, 16.5% to carbapenems in hospital settings, and 74.28% and 62.85% to ampicillin and amoxicillin, respectively, in samples from humans and animals. Antimicrobial tolerance levels may increase through gene exchange between the two important pathogens.

The presence of blaTEM genes β-lactamases related to ampicillin resistance has been reported in isolates of the family Enterobacteriaceae for more than 80.9% of the samples tested and in different environments . The blaTEM gene is one of the most common variants of genes conferring beta-lactamase resistance to bacteria such as E. coli and Klebsiella pneumoniae, as well as other Gram-negative bacteria. Its spread is facilitated by horizontal gene transfer (HGT), such as bacterial conjugation, a feature universally conserved among bacteria. Resistance mediated by the blaTEM gene stands out for its involvement in food matrices in the dissemination and microevolution of antibiotic-resistant bacteria. It presents itself as a major challenge in the control of Gram-negative bacteria

Typhimurium, ampicillin resistance rates exceed 75%. In response to this health challenge , research is focused on finding actions that reinforce key interventions in combating antibiotic-resistant microorganisms and the mechanisms via which resistance genes are spread . In addition, monitoring AMR in E. coli is already a mandatory indicator to verify the occurrence of bacterial phenotypes resistant to clinically important antibiotics such as carbapenems, colistin, and beta-lactams in the environment and in products of animal origin in Europe. Horizontal gene transfer is a major contributor to increased diversity in the microbial genomic repertoire, and conjugation is one of the most common mechanisms for transferring antibiotic resistance plasmids. This process is characterized by the dynamics of direct contact and assembly of the type IV secretion system (T4SS) of a donor bacterium and DNA transfer to a recipient bacterium . The overall rate of conjugation is determined not only by the type of plasmid that can be transferred but also by the cell physiology of the strains forming the conjugation pair, as well as the environmental conditions and the availability of energy, which directly influence the efficiency of conjugation and the subsequent growth of the transconjugants

Although extensively investigated for different factors, approaches to inhibit bacterial conjugation are still understudied. Thus, potential targets settle on the conjugative elements and the membrane assembly system of plasmid-encoded conjugation. Therefore, the approach that aims to associate anti-conjugative compounds with already demonstrated microbial properties to prevent the spread of resistance without acting as a growth inhibitor is of interest .

Essential oils (EOs) are known for their antimicrobial properties that, in association with nanoparticles, have advantages in overcoming limitations of use in food, such as odor, besides increasing their efficacy and stability, promoting greater solubility and bioavailability. They are also natural and sustainable and can be incorporated into the food industry. Nanoparticles can also improve the targeted delivery of EOs to specific tissues or cells . Promising work on the use of essential oil-loaded nanoparticles for the control of pathogenic microorganisms is already underway, and researchers have already demonstrated that turmeric-loaded lipid nanocarriers (NLCs) can effectively control wild-type microorganisms such as Pseudomonas aeruginosa . However, little is known about the action of nanoparticles carrying bioactive compounds in gene transfer processes and the use of new molecules that can prevent or decrease bacterial conjugation. Understanding the dynamics of HGT allows for explaining the complexity of some modulating factors of the transfer, and in vitro measurements of this process generate effective answers about the transfer rates among microorganisms.

Furthermore, the central objective of this work was to evaluate the action of chicken juice and whey coproducts on the frequency of bacterial conjugation of the blaTEM gene and the use of sage and olibanum NLCs as inhibitors of gene transfer.

Conjugation in a liquid medium was chosen for the tests based on the conjugation rates obtained in a previous study . We used the pair of microorganisms for which we determined an increase of more than 2.5 to 3.5 orders of magnitude in the percentage of frequency of conjugation in the presence of CJ and SL when compared to the traditional process (without the presence of coproducts). The pre-established 3-h period for evaluating the conjugation process represents the shift flow configurations present in animal product industries. The presence of the coproduct's whey and chicken exudate in this interval increased the gene transfer by 0.4 and 0.5 log CFU/mL ($p = 0.013$), respectively. The use of NpS had the greatest impact, with expressive inhibition results in all treatments, and reduced the conjugation efficiency by up to 124-fold when animal-origin coproducts were added. The NpO showed more effective results in the traditional conjugation model (reduction of four orders of magnitude) and the presence of CJ (reduction of 9 orders of magnitude). Moreover, it is essential to note that SL promoted a higher increase in conjugation frequency when compared to CJ, resulting in a greater challenge for inhibition with NCL, which was reduced by three orders of magnitude.

Bacterial conjugation events occur in various environments and are ubiquitous . They can also occur in bacterial communities present even in animal products, such as fresh milk and chicken meat, and the genes can potentially be transferred during food consumption . Mimicking the food production chain in this study, with the use of CJ or SL coproducts, evidenced the construction of a model in this area associated with environmental conditions that more accurately represent the extrinsic factors that can influence HGT, such as microbial mobility, fluid movements, and the microenvironment that can disturb the stabilization of the mating pair , depending on the junction between receiver and donor through the pilus. The gradual process of DNA transfer in a liquid medium and under agitation ensured efficient recombination rates when incubated for 3 h, equivalent to approximately one-third of a work shift in the food industry. The frequency of conjugation is generally higher in environments under agitation compared to static environments, probably due to the increased number of collisions between cells. Two nanostructured lipid carriers associated with sage and olibanum essential oils were evaluated to act as conjugation inhibitors. Coproducts of animal origin, namely whey and chicken juice, potentiated horizontal gene transfer. As a mitigating measure, the use of a nanocarrier associated with essential oils of sage and olibanum promoted a significant reduction in conjugation frequency. The inhibitory effect of sage nanoparticles and olibanum is a promising alternative to mitigate the microevolution of resistance gene transfer in the food industry. It motivates us to conduct further studies with these molecules to control microorganisms of public health importance.

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