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## **EXPLORING THE RANGE OF GTA-MEDIATED GENE TRANSFER IN ALPHA-PROTEOBACTERIA**

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The exchange of genetic information by horizontal gene transfer (HGT) accompanies and shapes the evolution of prokaryotes. While some bacteria are eventually able to take up pieces of DNA by natural transformation, sometimes bacterial genomic DNA is carried along with mobile genetic elements like plasmids or phage genomes and transferred by dedicated mechanisms such as conjugation or transduction, respectively. Gene transfer agents (GTAs) are specialized phage-like particles that pack and transfer genomic DNA fragments that are shorter than their own genome, from a GTA-producer host cell to a recipient cell through a process that combines features of transduction and natural transformation. GTAs have been described to mediate an effective mechanism of horizontal gene transfer within bacterial populations, i.e., in species of Rhodobacterales and Caulobacterales. However, the host range of the GTAs remains so far unexplored. Thus, the direct contribution of GTA-mediated HGT to the evolution of bacterial communities in their natural environments is unknown. Three major requirements have been described for a bacterium to be able to act as a recipient of DNA transferred by a GTA particle. These are i) to have a surface polysaccharide that allows proper adsorption of the GTA to the bacterial surface, ii) to have a functional natural DNA-uptake system, iii) to have a functional homologous recombination system to stably integrate newly acquired DNA into the genome. To elucidate the potential role of GTAs in disseminating genetic information beyond producer species, we analyzed the presence of GTA-recipient traits within the alpha-proteobacterial phylogeny. The ubiquitous distribution of such genetic and phenotypic features suggest that their absence might not be the limiting factor to transfer success. Therefore, may interspecific GTA-mediated gene transfer occur when regions of DNA with high sequence identity are shared between a GTA donor and a potential recipient cell? In this work, we present the conception of a gene transfer assay designed to assess the breadth of GTAs' transfer capabilities, using genetically modified GTA-producing bacterial strains that generate GTA particles carrying an antibiotic resistance marker flanked by DNA sequences that are homologous with potential alpha-proteobacterial recipient species' genomes. We provide details on how the GTA-donor mutant strains were constructed, and on the optimization of the experimental conditions for evaluating gene transfer.

Palabras clave: Horizontal Gene Transfer – GTAs – alpha-proteobacteria