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FUNGAL-BACTERIAL INTERACTIONS SELECT FOR *Bacillus* COLONY MORPHOTYPE VARIANTS HARBORING MUTATIONS IN TWO-COMPONENT SYSTEMS CONTROLLING METABOLISM AND BIOFILM FORMATION

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We have previously reported that a strain of *Bacillus subtilis* isolated from onion rhizosphere undergoes a hereditary phenotypic variation after interaction with the fungus *Setophoma terrestris* (ST) in co-culture, a phenomenon that is manifested by a greater antagonistic capacity and the formation of robust biofilms. Through metabolomics analysis based on mass spectrometry, we observed differential profiles in *B. subtilis* before (pre-ST) and after (post-ST) interacting with the fungus, which revealed the paradoxical absence of surfactin and plipastatin in post-ST variants. Despite the absence of these classic antimicrobial lipopeptides, post-ST variants showed greater antifungal activity compared to pre-ST. Through comparative genomics we were able to determine that mutations in the ComQPXA quorum sensing system, especially in mutations in the *comA* gene, represent the genetic bases of the conversion to a post-ST variant. Here, we aim to evaluate the extent of this mutation-driven phenomenon by analyzing the short-term evolution of a closely related species, *B. mojavensis*, in co-culture with ST. An evolution experiment in co-culture was conducted to determine the emergence of *B. mojavensis* morphotypes as a result of the stress imposed by the interaction. At different times during the co-culture, samples of the bacterial colony were taken, and the emergence of different colony types was inspected on LB plates after plating aliquots of the sample obtained by the serial dilution method. After total CFU/ml counts and inspection/determination of morphotypes within the total colonies, the morphotypes were recovered and preserved at -80°C. In parallel, the *B. mojavensis* strain was allowed to evolve on an LB plate growing alone, without any applied stress other than nutrient depletion due to its growth. This condition, designated as the negative control, was treated in the same way as the co-culture condition. We observed that after co-culture with ST, *B. mojavensis* undergoes a phenotypic conversion leading to the emergence of rough colony morphotypes and the acquisition of traits reminiscent of the phenomenon described for *B. subtilis*. The phenotypic changes of the post-co-culture strains remain stable over time, so we decided to investigate possible mutations acquired during evolution in co-culture. For this, whole-genome

sequencing was performed on both the ancestral variant of *B. mojavensis* (never exposed to the fungus) and the post-ST variants obtained. We found that the different rough colony variants that emerged during interaction with ST harbor mutations in *degS* and *comP* genes, which are part of the DegS-DegU and ComQXPA two-component systems involved in metabolism, biofilm formation, and quorum sensing. Currently, our efforts focus on characterizing the impact of these mutations on key bacterial phenotypes to determine how stress imposed by an interaction can modulate bacterial processes that may be useful for biotechnological applications.

Palabras clave: ANTAGONISM - INTERSPECIES INTERACTION - SHORT TERM EVOLUTION - BIOFILM - METABOLISM