Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

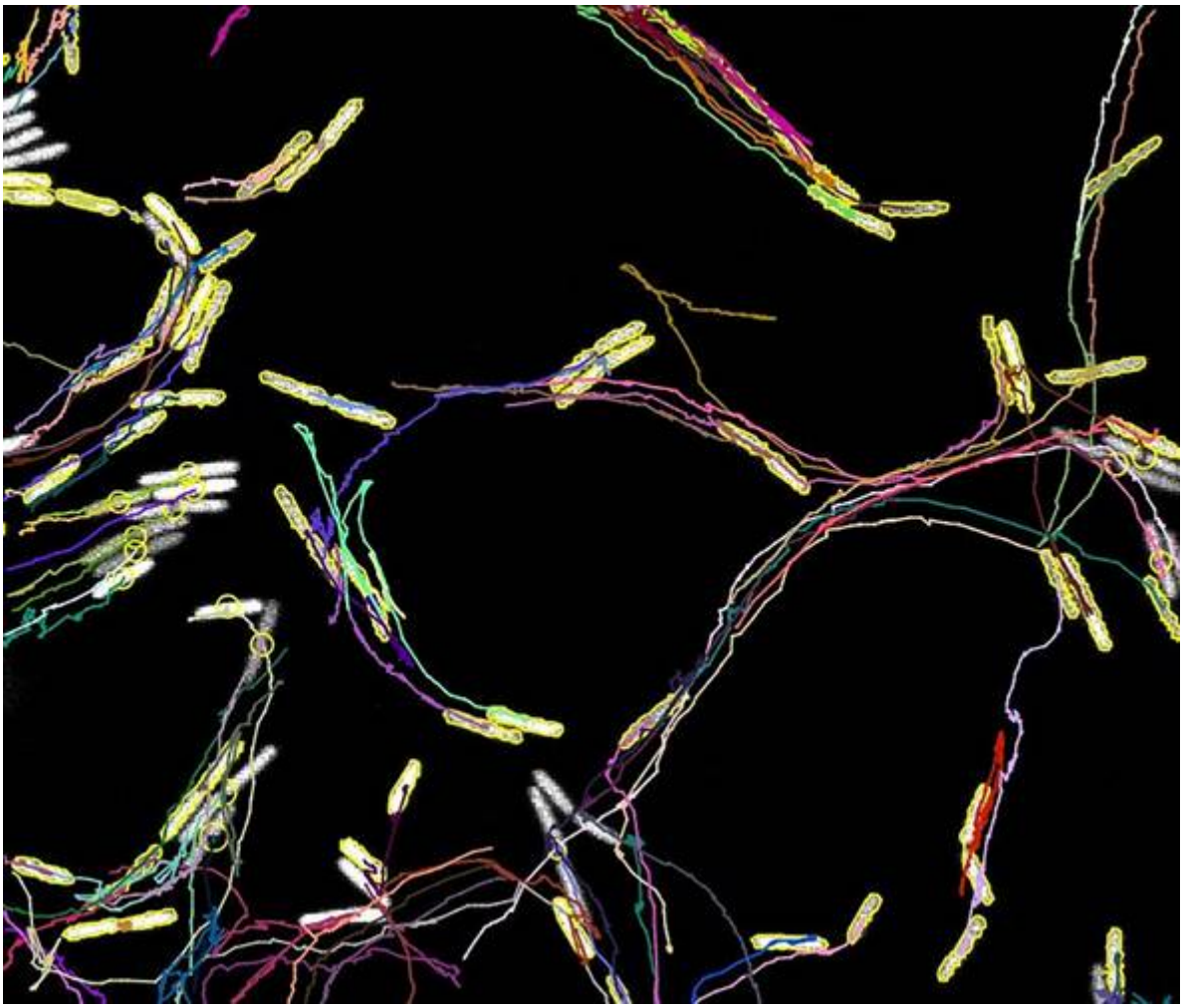

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CRISPR-CAS MEDIATED BASE EDITING IN PHYTOPATHOGENIC BACTERIA Xanthomonas vesicatoria

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Xanthomonas spp. are gram-negative phytopathogenic bacteria that affect a wide variety of different crops worldwide. Among them, *Xanthomonas vesicatoria* (Xv) infects tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.), causing bacterial spot. This disease is the reason for substantial yield loss leading to great economic loss. Current management of this disease relies on copper-based bactericides, which are only partially effective and pose environmental concerns. Xanthan, the main exopolysaccharide (EPS) produced by *Xanthomonas* spp., plays an important role in bacterial virulence, influencing factors such as motility, biofilm formation, resistance to stress agents, colonization and survival in the plant. The production of xanthan is regulated by the *gum* operon in which gumB is the first gene that codes for an outer membrane protein. In other *Xanthomonas* species, *gumB* mutants were unable to polymerize and export xanthan. We therefore believe that the same effect will be observed when editing *gumB* in Xv. The genomic modifications in phytopathogenic bacteria as Xv by traditional methods are laborious and time-consuming. Instead, the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR associated proteins) based genome editing technology has become increasingly important in prokaryotic research over the years, but is rarely used in phytopathogenic strains. In this study we used a cytosine base editor (CBE) mediated by CRISPR-Cas to edit the *gumB* gene in Xv strain BNM 208, to obtain a xanthan-deficient Xv strain. The editing strategy lies on the use of CBE to generate a C>T base change causing a newly premature stop codon through the design of an specific RNA guide (gRNA) to edit gumB. Here, we construct the editing vector and transform BNM 208 in order to obtain the edited strain, ?gumB. We further characterized virulence associated phenotypic traits as EPS production (precipitation of xanthan), biofilm formation (by violet cristal technique) and swarming motility (in a semisolid medium). The ? *gumB* strain lacks the capacity for xanthan production, swarming motility and

biofilm formation. We find interesting the study of mutant strains deficient in the function of different virulence factors being crucial for understanding bacterial virulence factors, pathogenesis and colonization mechanisms. This will help us identify action targets that will lead to development of a disease management alternative in the future. Moreover, the use of CRISPR technology in *Xanthomonas* spp., and even in phytopathogenic bacteria is still scarce. This is the first strain of Xv to be edited using CRISPR-Cas mediated technologies.

Palabras clave: Xanthomonas - cytosine base editor - CRISPR