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MICROBIOLOGICAL IMAGE

Endophytic microorganisms *Agrobacterium tumefaciens* 6N2 and *Meyerozyma guilliermondii* 6N serve as models for the study of microbial interactions in colony biofilms

Los microorganismos endofíticos *Agrobacterium tumefaciens* 6N2 y *Meyerozyma guilliermondii* 6N sirven como modelos para el estudio de las interacciones microbianas en *biofilms* en colonia

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We analyzed the morphology of colonies of endophytic bacteria and yeasts by fluorescence. *Agrobacterium tumefaciens* 6N2 (formerly 197MX) is a non-pathogenic isolate from sugarcane with features of relevance for microbial interactions[1]. *Meyerozyma guilliermondii* 6N was obtained from the same host[3].

A. tumefaciens 6N2 was tagged with *gfp*.AAV-a, allowing the detection of cells actively expressing the fluorescence[2]. GFP-tagged 6N2 and *M. guilliermondii* 6N were cultured at 30 °C in nutrient broth until late exponential phase[1]. Cell densities were adjusted to ∼0.1 OD_{600nm}, and spot inoculated on nutrient agar (NA) and YPD agar plates[5], as pure or mixed cultures (proportion 1:1). Plates were incubated at 30 °C for 72 h; colonies were imbibed with 10 μl of 25 μM Calcofluor White M2R (CW) that binds chitin and cellulose, and observed with a 4× objective lens under a Zeiss SteREO Lumar.V12 Stereomicroscope with GFP and DAPI filters.

While 6N2 colonies exhibited a wrinkled surface on YPD agar (Fig. 1A and C), they were smooth and convex on NA (Fig. 2A and C). Under interaction conditions 6N2 showed singular patterns: 6N2 was located at the edges and the center on YPD agar (Fig. 1D), and was evenly distributed on NA (Fig. 2D). In both media 6N seemed to be located on top of the bacterial growth (Fig. 1B and B). 6N development was granular with lobate borders on YPD agar (Fig. 1B and F), and was smooth and dotted on NA (Fig. 2B and F). Noteworthy, 6N2 was not stained with CW (Fig. 1E and E). Beyond the effect of the interactions on the host plant, the images show

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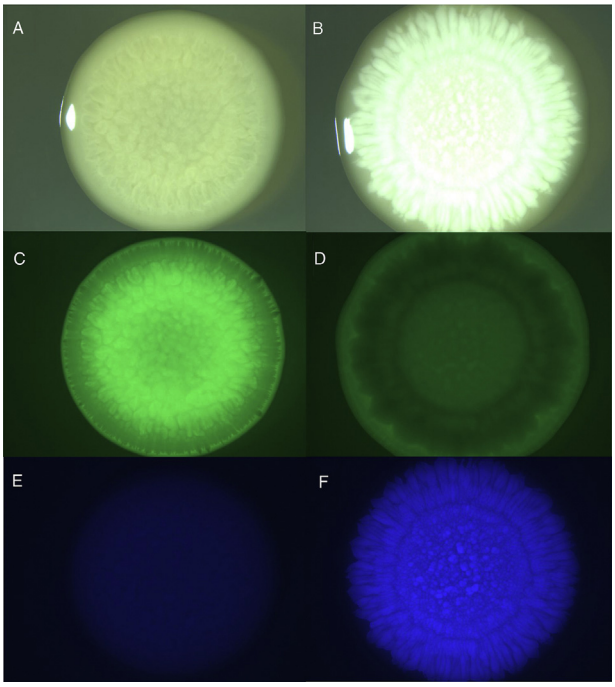

Figure 1 Colony biofilms of *A. tumefaciens* 6N2 and *M. guilliermondii* 6N growing on YPD agar. A, C and E: pure colony of 6N2 observed with visible illumination, GFP filter and DAPI filter, respectively. B, D and F: colony of mixed cultures of 6N2 and 6N observed with visible illumination, GFP filter and DAPI filter, respectively.

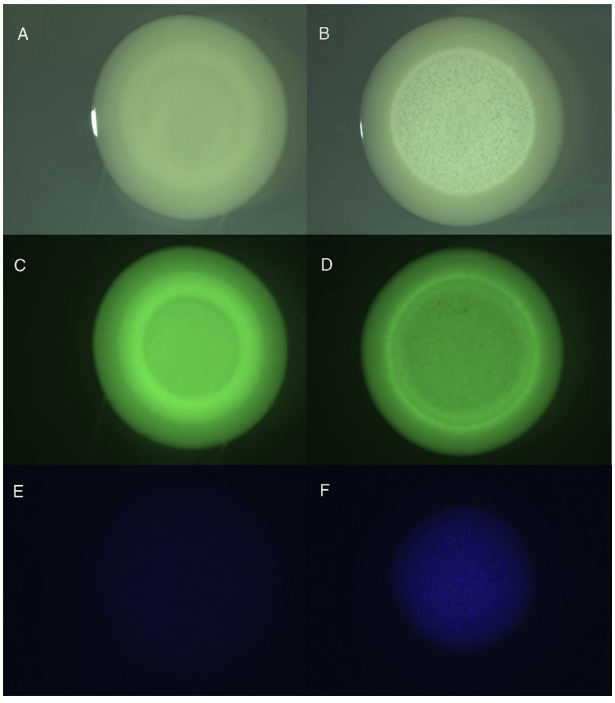

Figure 2 Colony biofilms of *A. tumefaciens* 6N2 and *M. guilliermondii* 6N growing on NA. A, C and E: pure colony of 6N2 observed with visible illumination, GFP filter and DAPI filter, respectively. B, D and F: colony of mixed cultures of 6N2 and 6N observed with visible illumination, GFP filter and DAPI filter, respectively.

the potential of the strains and the techniques for the study of interactions in a colony biofilm, even if the true location of each strain requires other techniques (*e.g.*, confocal microscopy)[4].

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Conflict of interest

The authors declare that they have no conflicts of interest.

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