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Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts

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ABSTRACT

The inactivation of quorum sensing signals, a phenomenon known as quorum quenching, has been described in diverse microorganisms, though it remains almost unexplored in yeasts. Beyond the well-known properties of these microorganisms for the industry or as eukaryotic models, the role of yeasts in soil or in the inner tissues of a plant is largely unknown. In this report, the wider survey of quorum quenching activities in yeasts isolated from Antarctic soil and the inner tissues of sugarcane, a tropical crop, is presented. Results show that, independently of their niche, quorum quenching activities are broadly present in unicellular fungi. Although yeasts showing a broad range of quorum quenching activity are present in the two niches, at the same time specific AHL inactivation profiles can also be found. Furthermore, yeasts from both sampling sites show quorum quenching activities compatible with lactonase-like and acylase-like inactivations of AHLs. Interestingly, the characterization of *Rhodotorula mucilaginosa* 7Apo1 showed that the presence of a particular AHL does not interfere with the quenching of a second molecule. Evidence suggests that yeasts could play a role in the modulation of the quorum sensing activity of bacteria. The relationship among phylogeny, sampling sites and yeast quorum quenching activities of the isolates is analyzed.

Introduction

The role of yeasts in soils and in plants has remained relatively unexplored, in comparison with their bacterial counterparts. Antarctic soil is an environment that remains relatively pristine with a very low anthropic impact. Despite its oligotrophic characteristics and the adverse environment conditions, Antarctic soil mycobiota is highly diverse (for

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a recent review see Hassan et al. 2016, and references therein). Several reports shed light on the rich diversity and the biotechnological potential of unicellular fungi obtained from that continent (Lawley et al. 2004; Rovati et al. 2013; Vishniac 2006). In contrast, very little research has been performed on endophytic yeasts. Sugarcane is a crop from tropical and subtropical regions that represents a niche with opposite characteristics to those found in Antarctic soils. Its culture requires plentiful sunshine and high water supplies, and the inner tissues are rich in nutrients (Romero et al. 2009), which allow a broad diversity of endophytic microorganisms. A recent report described that the endophytic community of sugarcane is highly diverse for fungi and bacteria (De Souza et al. 2016). It is probable that non-motile microorganisms, e. g. yeasts, gain access to the inner tissues of the plant by stomata or wounds where they could modify the host physiology.

In soil, as endophytes or in almost any niche, yeasts are not expected to be found as pure cultures but as mixed populations with bacteria and other organisms playing a wide range of beneficial or detrimental interactions. Those based on quorum sensing systems have only been explored in *Candida albicans* and *Pseudomonas aeruginosa*, two opportunistic microorganisms commonly found in cystic fibrosis patients (De Sordi & Mühlschlegel 2009). These regulatory mechanisms are chemical communication systems that influence a plethora of phenotypes related to microbial interactions improving, in general, the fitness, the colonization of a particular niche and the utilization of a substrate in a cell-density dependent manner (Fuqua et al. 1996). In Gram-negative bacteria, the most characterized systems are those utilizing *N*-acyl homoserine lactones (AHLs) as signaling molecules. AHLs are composed of a homoserine lactone ring esterified with an acyl chain from 4 to 18 carbons in length. The third position in the chain can be fully reduced or oxidized with a carbonyl group or a hydroxyl group (Fuqua et al. 1996).

The inactivation of the signal communication, mainly through the degradation of quorum sensing molecules, a phenomenon called quorum quenching, could play a role in the interactions among AHL-producers, non-producers and the hosts (Grandclément et al. 2016). Degradation of AHLs by lactonase enzymes that reversibly hydrolyze the AHL lactone ring, has been described in diverse bacterial genera, including *Bacillus*, *Agrobacterium*, and *Arthrobacter*, among others. Acylases, which catalyze the irreversible AHL degradation by cleavage of the acyl chain, have been reported in genera such as *Streptomyces*, *Comamonas*, and *Shewanella*. Other enzymes involved in quorum quenching are the oxidases/reductases that produce a modification in the signal molecule altering its quorum sensing activity (Koul & Kalia 2017; Grandclément et al. 2016). Degradation of AHLs by filamentous fungi has only been reported in a few isolates (Stöckli et al. 2016; Uroz & Heinonsalo 2008). Up to date very little is known about this biochemical feature in yeasts. It has been shown that the yeasts *Trichosporon loubieri* and *Rhodotorula mucilaginosa* can also inactivate AHLs (Ghani et al. 2014; Wong et al. 2013). Phylogenetic unrelatedness between these two basidiomycetes suggests that AHL degradation could be broader present that previously thought.

The aim of this work is the evaluation of the capacity of AHL inactivation by yeasts through a wide survey of quorum quenching activities in isolates obtained from Antarctic soil and as endophytes of sugarcane plants. The geographic characteristics of the sampling sites and phylogenetic relationships of the analyzed yeasts allow the evaluation of the potential ecological implications of the capacity of fungal AHL inactivation.

Materials and methods

Yeast isolates and culture media

Antarctic yeasts were obtained from 25 de Mayo/King George Island. Isolation and description of isolates, including latitude and longitude locations of sampling sites, were described in a previous report (Rovati et al. 2013). Soil temperatures were always between 3 and 10 °C at each sampling site (J. I Rovati, personal communication). For the isolation of endophytic yeasts, sugarcane plants (cultivar LCP 85-384) at different growth stage without symptoms of being unhealthy were obtained from two sugarcane plantations in Tucumán, Argentina (26°47′17.09″S–65°11′53.08″O; and 26°47′18.64″S–65°12′8.81″O, respectively) in July, 2013. Plants were cultured in a slightly acid Argiudol soil, and samples were obtained with an environment temperature of 30 °C. Roots, leaves, nodes and internode sections were surface sterilized in 70 % ethanol for 5 min followed by 10 min in sodium hypochlorite (6.25 %) and five washes with distilled water (Lalande et al. 1989). Surface sterilization was controlled by rolling the samples on the same media utilized for the isolation (see below) (Lalande et al. 1989). Samples were macerated in phosphate buffer 10 mM pH 7 and serial dilutions were plated in YM pH 6.8 (Rovati et al. 2013) and PDA (potatoe dextrose agar, Britania, Argentina) pH 5.2, media supplemented with tetracycline (15 μg mL^{-1}) and ampicillin (100 μg mL^{-1}) to inhibit bacterial growth. Internode sections were also centrifuged for 15 min (5000 g) for the obtainment of the apoplastic fluids, which were plated as described before. Plates were incubated at 30 °C for 48–72 h. Isolated colonies were restreaked on the same media until pure cultures were obtained.

Bacterial strains

Chromobacterium violaceum CV026 (McClean et al. 1997) and *C. violaceum* Vir07 (Morohoshi et al. 2008) were cultured in LB broth supplemented with 50 μg mL^{-1} kanamycin at 30 °C. *Agrobacterium tumefaciens* NT1 (pZLR4) (Cha et al. 1998) was cultured in AT medium (Tempé et al. 1978) supplemented with 40 μg mL^{-1} gentamycin. For *Pseudomonas syringae* pv. *syringae* B728a (Quiñones et al. 2004), King B medium was utilized (King et al. 1954).

Molecular identification of endophytic yeasts

For DNA extraction, endophytic yeasts were cultured in YM agar at 30 °C, harvested and resuspended in 300 μL of extraction buffer (Tris–HCl 100 mM, SDS 1 %, Triton X100 2 %, EDTA 10 mM, NaCl 100 mM). After addition of isoamyl alcohol and saturated phenol, cryovials containing the cell

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