FUNGAL BIOLOGY XXX (2017) 1–11

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio

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

Ana Carolina del V. LEGUINA[a], *Carolina NIETO*[b], *Hipólito M. PAJOT*[a], *Elisa V. BERTINI*[a], *Walter MAC CORMACK*[c], *Lucía I. CASTELLANOS DE FIGUEROA*[a,d], *Carlos G. NIETO-PEÑALVER*[a,d,*]

[a]*PROIMI, CONICET (Planta Piloto de Procesos Industriales Microbiológicos), Av. Belgrano y Pje. Caseros, Tucumán, Argentina*

[b]*Facultad de Ciencias Naturales e IML, Instituto de Biodiversidad Neotropical-CONICET, Tucumán, Argentina*

[c]*Instituto Antártico Argentino, Buenos Aires, Argentina*

[d]*Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina*

ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form
4 September 2017
Accepted 15 October 2017
Corresponding Editor:
Nabla Kennedy

Keywords:
Antarctica
Endophytic
Quorum quenching
Quorum sensing
Soil
Sugarcane

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

* *Corresponding author*. PROIMI (Planta Piloto de Procesos Industriales Microbiológicos), Av. Belgrano y Pje. Caseros, Tucumán T4001MVB, Argentina. Tel.: +54 381 4344888.
E-mail address: cgnieto@proimi.org.ar (C.G. Nieto-Peñalver).

https://doi.org/10.1016/j.funbio.2017.10.006
1878-6146/

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

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals *N*-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

suspensions were vortexed for 2 min and incubated in ice for another 2 min. Eight cycles were repeated for each sample. After DNA precipitation with standard procedures, the D1-D2 domain of the LSU rDNA gene was amplified and sequenced with primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGTGTTTCAAGACGG) (Kurtzman 2011). Isolate identifications were performed by comparison with the GenBank database.

Determination of fungal quorum quenching activity

Yeast isolates were inoculated in wells of microtiter plates containing YM broth diluted 1:10 (YMd) and supplemented with commercial standards of the following AHLs: C6-HSL, C8-HSL, C10-HSL, C12-HSL, 3O-C6-HSL, 3O-C8-HSL, 3O-C10-HSL and 3O-C12-HSL, at a final concentration of 25 μM. YMd was utilized since this medium sustained better the growth of all yeasts, in particular those from Antarctica. Yeasts from Antarctica and sugarcane were cultured statically at 15 °C and 30 °C, respectively, for 48 h. Microtiter plates were then centrifuged and the residual concentrations of AHLs were estimated in cell-free supernatants with bioassays developed with *Chromobacterium violaceum* CV026, *C. violaceum* Vir07 and *Agrobacterium tumefaciens* NT1 (pZLR4). Biosensor strains are characterized by the lack of AHL production but the capacity of responding to AHLs provided exogenously. Once the complex AHL-receptor is formed inside the biosensor cell, a visible and measurable phenotype is produced (Steindler & Venturi 2007). Biosensors were utilized independently to analyze the residual concentrations of AHLs, essentially as described elsewhere with slight modifications (Ravn *et al.* 2001). Briefly, actively growing cultures of *C. violaceum* CV026 and *C. violaceum* Vir07 were inoculated in a soft layer (0.75 % agar) of LB and poured on top of a 1.5 % agar LB layer. Five μl of cell-free yeast supernatants were spotted on top of the soft layer of medium seeded with the corresponding biosensor. Plates were then incubated 18 h at 30 °C and diameters of the violet spots produced by the residual AHL molecules were determined. Unless otherwise stated, *C. violaceum* CV026 was utilized for the analysis of C6-HSL and 3O-C6-HSL, and *C. violaceum* Vir07 was employed for the analysis of C10-HSL, 3O-C10-HSL, C12-HSL and 3O-C12-HSL. The same procedure was followed for the analysis of C8-HSL and 3O-C8-HSL with *A. tumefaciens* NT1 (pZLR4), except that the medium utilized was AT supplemented with 20 μg mL^{-1} X-GAL (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). In addition, for bioassays with *A. tumefaciens* NT1 (pZLR4) samples were first diluted 1:20 and 1:100 in YMd prior to analysis of C8-HSL and 3O-C8-HSL, respectively. Utilizing standard curves, residual concentrations of AHLs in yeast cultures were estimated with mean values of halo diameters obtained from at least two independent determinations. Considering the different sensitivity of each biosensor strain for each AHL, standard curves were constructed independently supplementing sterile YMd with 25 μM, 12.5 μM, 6.25 μM, 3.125 μM, 2.5 μM, 1.56 μM, 0.78 μM and 0.39 μM of each AHL and incubating at 30 °C for 48 h (see Supplementary Table S3). Samples were then analyzed with bioassays utilizing the corresponding biosensor strain as described above. When a residual concentration was below 10 μM, quorum quenching was considered as a fast or rapid activity, while a value above 20 μM was considered as a negative or lack of activity. Values in between were considered as slow quorum quenching activities. Residual concentrations of AHLs (see Supplementary Tables S1 and S2) were hierarchical clusterized with Cluster 3.0 (Stanford University, Palo Alto, CA, USA) with uncentered correlation and complete linkage. Cluster result was visualized with Java TreeView (Saldanha 2004). To test the stability of the molecules, AHLs were incubated in sterile medium under the same conditions, and analyzed with the corresponding biosensor strain.

In addition, *Rhodotorula mucilaginosa* 7Apo1 was also cultured aerobically in YMd broth as described above. At mid- and late-exponential, and stationary phase determined spectrophotometrically, culture samples were centrifuged, the supernatants were filtered with a 0.22 μm filter and stored at −20 °C until further analysis. Cell pellets were washed twice in phosphate buffer 8.3 mM pH 7.2 and resuspended in the same buffer supplemented with protease inhibitor cocktail set IV (Calbiochem). Cells were disrupted with glass beads after 60 cycles of 1 min of vortex and 1 min in ice. Samples were then centrifuged at 8400 g for 20 min at 4 °C and protein extracts were stored at −20 °C. Quorum quenching activities in cell-free supernatants were directly assayed with C6-HSL and C10-HSL as described above. The protein concentrations of intracellular extracts were adjusted to 50 μg mL^{-1} and the quorum quenching activities were assayed with C6-HSL and C10-HSL as described above. Proteins were quantified with Bradford reagent utilizing bovine serum albumin as standard.

Alignments and phylogenetic analysis

The D1-D2 sequences were aligned utilizing the CLUSTAL method with the MEGA6 software (Tamura *et al.* 2013) and manually edited. Phylogenetic analyses were performed by parsimony using the program TNT (Goloboff *et al.* 2008) under equal weights assumption with k value of three. The shortest trees were obtained under Analyze/TraditionalSearch commands; i.e., 500 replicates of random addition sequences followed by tree bisection – reconnection, saving ten trees/replication. The accession numbers (GenBank) concerning each sequence are shown on the tree. Group support was obtained by applying frequency differences (Goloboff *et al.* 2003), which were calculated with 1000 jackknifing replicates (Farris *et al.* 1996) by symmetrical resampling of the original matrix (each character presents the same probability of being positively or negatively reweighted; Goloboff *et al.* 2003). Each jackknifing replicate was calculated by ten random-addition sequences plus tree bisection – reconnection, saving ten trees/replication.

Characterization of quorum quenching activities

After the growth of selected yeast isolates in AHL-supplemented YMd medium, 20 μL of cell-free supernatants were acidified with HCl at a final concentration of 0.1 M, and incubated for 48 h at 4 °C to induce lactone recyclization (Yates *et al.* 2002). Samples were then completely evaporated under vacuum, dissolved in 20 μL of sterile YMd broth and analyzed with the bioassays in plates as described above.

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

Restoration of the AHL activity after acidification, visualized through the induction of violacein production by the biosensor strains, was indicative of lactonase-like activity.

Reverse phase-thin layer chromatography

Rhodotorula mucilaginosa 7Apo1 was cultured statically at 30 °C in YMd supplemented independently with C6-HSL, 3O-C6-HSL, C10-HSL and 3O-C10-HSL at a final concentration of 25 μM. AHLs were also utilized in combinations as follows: C6-HSL + C10-HSL; C6-HSL + 3O-C10-HSL; 3O-C6-HSL + C10-HSL; and 3O-C6-HSL + 3O-C10-HSL at a final concentration of 25 μM for each molecule. After 12, 24 and 36 h samples were withdrawn and residual AHLs were extracted with acidified ethyl acetate (Cha *et al.* 1998). Concentrated extracts were then applied to C18 reversed-phase TLC plates (RP-18 F254S, Merck, Germany), dried with a stream of air and separated by using methanol: water (60:40 v/v) as the solvent. For detection of AHLs, the TLC plates were overlaid with a soft layer of LB seeded independently with *Chromobacterium violaceum* CV026 and *C. violaceum* Vir07 biosensors and incubated for 18–24 h at 30 °C (Cha *et al.* 1998).

Inactivation of quorum sensing molecules in mixed cultures

Mixed cultures of the endophytic *Rhodotorula mucilaginosa* 7Apo1, and *Pseudomonas syringae* pv. *syringae* B728a were prepared. Both strains were precultured aerobically in King B medium at 30 °C for 24 h and cells were utilized to inoculate a fresh medium at initial cell densities of 1.30×10^8 and 7.80×10^5 CFU mL^{-1} for B728a and 7Apo1 strains, respectively. In parallel and as a control, a pure culture of the bacterium was also prepared. All final cultures were performed aerobically at 30 °C. Samples collected after 12, 24 and 36 h were centrifuged and residual AHLs in the cell-free supernatants were estimated with plate bioassays developed with *Agrobacterium tumefaciens* NT1 (pZLR4). To compare the diameters of the halos of X-GAL hydrolysis normalized with respect to the growth of B728a, a comparison of means was performed at each sampling time using Student's *t* test with MINITAB v.17 package. Bacterial growth was measured by serial dilutions and plating on King B media supplemented with cycloheximide (10 μg mL^{-1}). Yeast growth was measured by cell counting with Neubauer chamber.

Results

Presence of quorum quenching activity in yeasts

Antarctic yeasts obtained from different soils, beaches and rocks during the austral summer (Jan.–Mar. 2012), belonged to the genera *Cryptococcus*, *Candida*, *Rhodotorula*, *Leucosporidiella*, *Cylindrobadidium*, *Dioszegia*, *Mrakia*, *Debariomices*, *Bullera*, *Exophiala* and *Sporobolomyces* (Rovati *et al.* 2013). Fungal endophytes could be obtained from leaves, apoplastic fluid and node samples from sugarcane. In comparison to yeasts from Antarctica, the diversity of this group of microorganisms under the utilized conditions was more restricted: only isolates belonging to the genera *Meyerozyma*, *Rhodotorula* and *Sporisorium* were obtained. The bioassays allowed the evaluation of the quorum quenching capacities of the yeasts (see Supplementary Fig S1) revealing a broad spectrum of AHL inactivation profiles. While isolates from both sampling sites could rapidly inactivate some AHLs, others presented a slow or lack of quorum quenching on other AHLs assayed. To analyze how the different isolates were grouped according to their corresponding profiles, residual concentrations, as determined from the haloes in the corresponding bioassays (see Supplementary Tables S1 and S2) were hierarchical clusterized with Cluster 3.0 software. To note, several halo diameters corresponded to concentrations below the lowest values assayed in the standard curves (see Supplementary Table S2). For clustering purposes, the lowest concentration utilized in the corresponding standard curve was utilized. Rapid inactivations are represented in shades of green and slow inactivations are represented in shades of yellow, while lack of activity is in red (Fig 1). The hierarchical clustering showed two main clusters each divided in two subclusters of quorum quenching yeasts, respectively. Subclusters A, B and D were composed of isolates from Antarctic soil and sugarcane, while the subcluster C contained isolates exclusively from Antarctica. Isolates in subcluster A showed the less capacity to inactivate AHLs, being 3OC6-HSL, C12-HSL and 3OC12-HSL most commonly inactivated. This was in contrast with subcluster D, whose members inactivated all the AHLs under the utilized conditions. Subcluster B was mainly characterized by the inactivation of C10-HSL, C12-HSL and 3OC12-HSL, though several isolates could also quench the activity of other AHLs assayed, e. g. C6-HSL and 3OC6-HSL. Finally, no member of subcluster C could quench the activity of 3OC8-HSL, though other AHLs were rapidly inactivated. Noteworthy, no alkalinization could be observed in any yeast culture (data not shown), a condition where AHLs suffer spontaneous hydrolysis of the lactone ring (Yates *et al.* 2002). Analysis of AHLs in sterile medium showed no differences before and after the incubation (data not shown), indicating that the molecules were stable under the conditions utilized.

Phylogenetic analysis of quorum quenching yeasts

To analyze the link between the quorum quenching profiles of the isolates and the evolutionary relationships of the isolates, a phylogenetic parsimony tree was constructed. The analysis of the tree showed that the profiles are not always related to the evolutionary relationships of the isolates. Although all the *Meyerozyma guilliermondii* isolates form as expected a monophyletic group (node two, Fig 2), *M. guilliermondii* KX303827 was located in a different cluster (subcluster B) than the isolates *M. guilliermondii* KX303828, KX303829, KX303830, KX303831 and KX303832 (subcluster A) (Fig 1). *M. guilliermondii* All the *Sporisorium* sp. isolates, which presented similar quorum quenching profiles in subcluster B (Fig 1), form as expected a monophyletic group (node three, Fig 2). *Sporisorium Cryptococcus* spp. isolates formed a separated clade (node five, Fig 2) that clearly illustrated the polyphyly of this genus: while most of them were located in subcluster A, a few of them were clustered in subclusters B (*Cryptococcus* sp. KC713866 and *Cryptococcus gilvescens* KC713845) and C (*Cryptococcus* spp. KC713849, KC713840 and KC713850, and

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

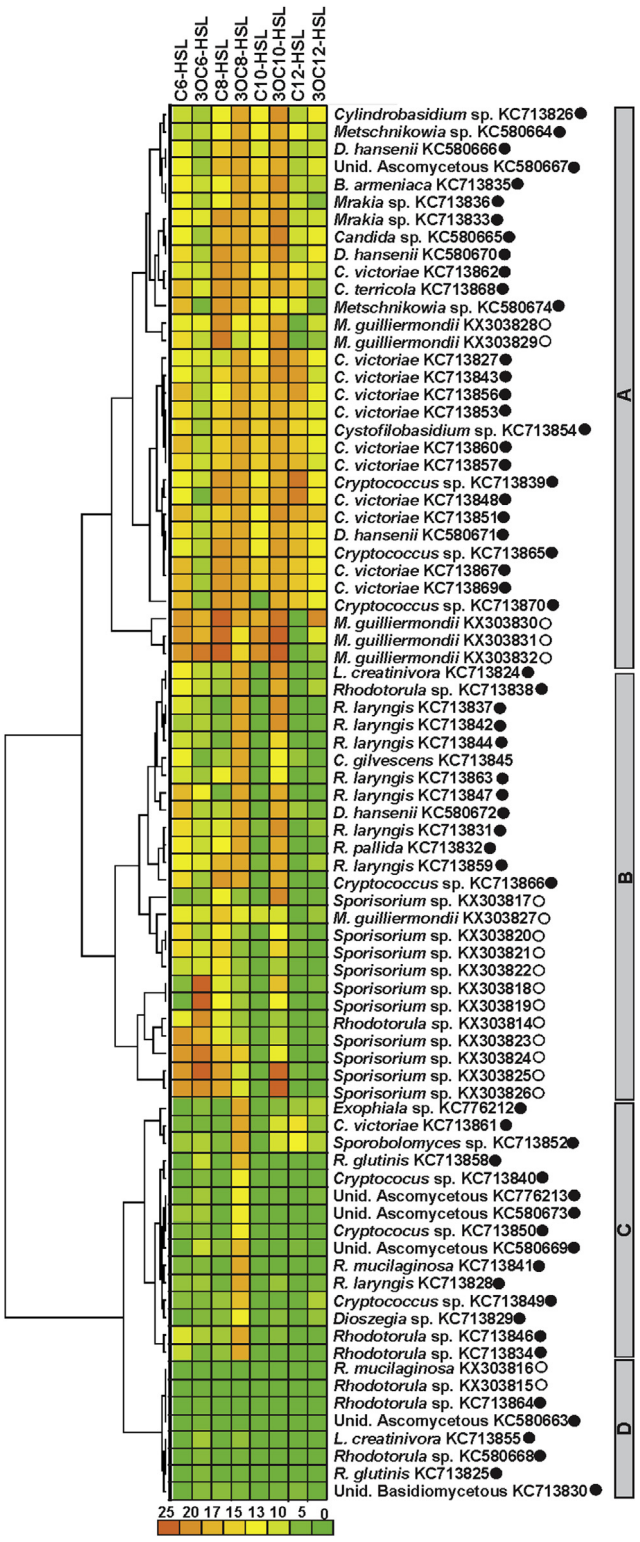

Fig 1 – Clusters of isolates according to their quorum quenching profiles. Remaining concentrations of AHLs were utilized for the hierarchical clusterization. Each isolate is identified according to the corresponding GenBank accession number. White and black circles indicate the endophytic or Antarctic origin of the isolate, respectively. Two main clusters divided in two (A and B) and three (C, D and E) subclusters were identified. Color bar indicates the corresponding AHL concentration expressed in μM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Cryptococcus victoriae KC713861). Other isolates belonged to genera closely related to *Cryptococcus* in node five (i.e., *Mrakia*, *Cystofilobasidium*, *Bullera*, and *Metschnikowia*) were also located in subcluster A (Fig 1). Isolates from the *Rhodotorula* genus and the related genera *Leucosporidiella* and *Sporobolomyces* constituted a distinctive polyphyletic clade in the tree (node four), which was in agreement with their broad diversity of quorum quenching activities (subclusters B, C and D, Fig 1).

Yeasts present reversible and irreversible quorum quenching activities

Fungal isolates from subcluster D, which presented the most prominent quorum quenching activities, were firstly selected for the characterization of their AHL inactivations. As shown in Fig 3, with the exception of *Rhodotorula* sp. KX303815, C6-HSL was mainly inactivated reversibly. Similar behaviors were observed with C8-, 3OC8-, C10 and 3OC10-HSL. In contrast, acidification with HCl only allowed recovering the C12-HSL activity when *Rhodotorula* sp. KC580668 inactivated this molecule. 3OC12-HSL was also mainly inactivated in an irreversible manner. These results suggest that in contrast to the AHLs with a short or a medium acyl chain that are principally inactivated by lactonase-like enzymes, those with a long chain suffer irreversible inactivations through acylase-like activities. To test this hypothesis, the degradation of C12-HSL and 3OC12-HSL by several isolates from the different clusters was characterized. As shown in Fig 4, with a few exceptions the inactivation of these AHLs was irreversible after HCl acidification.

Simultaneous inactivation of short and long chain AHLs by Rhodotorula mucilaginosa *7Apo1*

Rhodotorula mucilaginosa KX303816 obtained from sugarcane could rapidly inactivate all the AHLs analyzed, and showed both lactonase-like and acylase-like activities. This isolate, referred to as *R. mucilaginosa* 7Apo1, was then selected for a deeper characterization. The analysis of cell-free supernatants of *R. mucilaginosa* 7Apo1 cultured in absence of AHLs showed no inactivation activities. However, C6- and C10-HSL could be inactivated by protein extracts obtained from cultures in absence of AHLs at mid- and late-exponential, and stationary phases, indicating that the quorum quenching activity is constitutive and intracellular, at least for this isolate (data not shown). To evaluate the influence of the presence of one AHL on the inactivation of another, *R. mucilaginosa* 7Apo1 was cultured supplementing the medium with combinations of AHL molecules. A visual comparison of all the spots detected after 12 h of incubation with those at the beginning of the assay did not show differences neither in the diameters nor in the intensities of the spots (Fig 5a and 5b, and Fig 5e and 5f). Concentration of C6-HSL decreased after 24 h and was undetectable after 36 h of incubation, independently of the presence or not of C10-HSL or 3OC10-HSL. The inactivation

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

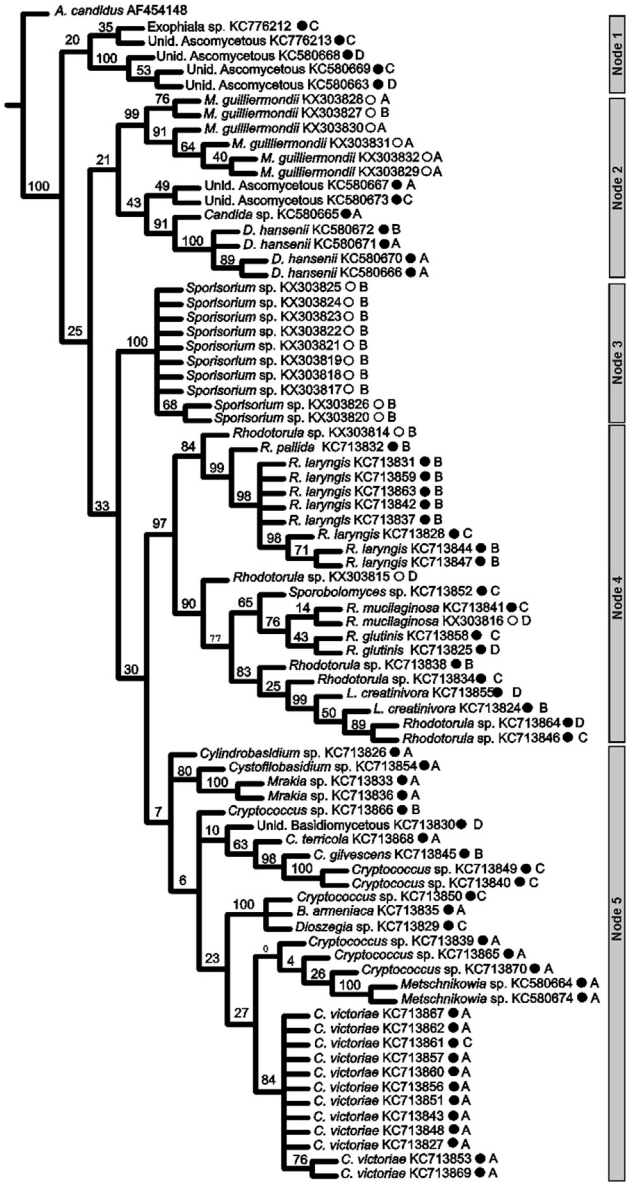

Fig 2 – Phylogenetic tree of the sequence of the D1-D2 domain from the LSU rDNA gene of the isolates. White and black circles indicate the endophytic or Antarctic origin of the isolate, respectively. Localization of each isolate in the hierarchical cluster (Fig 1) is also indicated. Sequence from *Aspergillus candidus* (AF454148) was utilized as outgroup. Symmetrical resampling support values are indicated as numbers above the branches.

of 3OC6-HSL showed to be slower than C6-HSL, evidenced for the remaining quantities of this molecule after 36 h of incubation (Fig 5c and 5d). However, similar to its unsubstituted counterpart, the inactivation was not modified by the concomitant presence of C10-HSL or 3OC10-HSL.

C10-HSL and 3OC10-HSL were almost undetectable after 24 or 36 h (Fig 5g and 5h). Short AHLs, independently of the substitution or not at the third position, did not interfere with the inactivation of C10-HSL or 3OC10-HSL. The chromatographic separation of the AHLs by RP-TLC allowed excluding that the disappearance of the quorum sensing molecule was caused by the production of inhibitory compounds by the yeast, since that molecule would be separated from the AHLs during the RP-TLC.

AHL inactivation in fungal-bacterial mixed cultures

The capacity of signal interruption by *Rhodotorula mucilaginosa* 7Apo1 was analyzed in a mixed culture with an AHL-producer bacterium in King's B medium. For this assay, the plant-associated bacterium *Pseudomonas syringae* pv. *syringae* B728a, a strain which synthesizes 3OC6-HSL (Quiñones *et al.* 2005) was selected. Supernatant samples were analyzed with *Agrobacterium tumefaciens* NT1 (pZLR4) that overexpresses the AHL receptor making it highly sensitive (Cha *et al.* 1998). Fig 6B shows the diameters of the haloes of X-Gal hydrolysis induced by the residual AHL in the cultures normalized to the corresponding growth measured as CFU mL^{-1} (Fig 6A). No differences could be observed after 12 h in mixed cultures, in comparison with the bacterium in pure culture. These results are in concordance with the previous observation in which the quorum quenching activity of *R. mucilaginosa* 7Apo1 was undetectable at the beginning of the yeast growth. However, after 24 of incubation, significantly lower diameters ($P > 0.05$) were measured when *R. mucilaginosa* 7Apo1 was present in the bacterial culture, indicating lower concentrations of the AHL in the supernatants, in comparison to the pure culture of *P. syringae* pv. *syringae* B728a. AHLs present in the bacterial supernatant after 36 h were higher than those observed in mixed cultures, although no significant differences could be measured.

Discussion

In comparison to what is known about bacteria in soil or their interactions with plants, the role and properties of yeasts are largely unknown. Their capacity to degrade bacterial quorum sensing molecules is not an exception. However, two reports showed that yeasts can also present this feature. *Trichosporon loubieri* WW1C isolated from wetland waters from Malaysia is able to degrade the AHLs C4-, C6-, and C8-HSL, and the several 3O-substituted quorum sensing molecules, while C12-HSL and C14-HSL were not degraded (Wong *et al.* 2013). At least for C6-HSL, the degradation is mediated by a lactonase activity in *T. loubieri* WW1C. *Rhodotorula mucilaginosa* B2 and B3, both obtained from water samples also from Malaysia can inactivate C6-HSL, 3OC6-HSL and 3OHC6-HSL through a lactonase (Ghani *et al.* 2014). Results presented in this report show for the first time that quorum quenching activities are widely distributed among yeasts, and that AHLs can be inactivated both reversibly and irreversibly, suggesting the presence of lactonase-like and acylase-like activities. As mentioned above, the dissimilar characteristics of the origin of the isolates and their different evolutionary relationships allow the evaluation of the potential ecological implications of the capacity of fungal AHL quenching. The characterization of the final products after the AHL inactivations, which is of interest

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

Yeast isolate	C6	3OC6	C8	3OC8	C10	3OC10	C12	3OC12
R. glutinis KC713825		ND						
Unid. Basidiomycetous KC580663		ND						
Unid. Ascomycetous KC580668		ND		ND				
Rhodotorula sp. KX303815								
R. mucilaginosa KX303816								

Fig 3 – Characterization of quorum quenching activities of selected isolates from cluster D. After inactivation of the corresponding AHL, supernatant samples were acidified and then analyzed with bioassays as described in the text. Gray and black squares indicate reversible and irreversible quorum quenching activities (i.e., lactonase-like and acylase-like activities), respectively. ND: not determined.

but is beyond the aims of this report, will allow the elucidation of the biochemical nature of these quorum quenching activities. Hierarchical clusterization suggests that common quorum quenching profiles can be found in different niches *Mguilliermondii* *Sporisorium* A contrasting viewpoint was showed by d'Angelo-Picard and col. who suggested that quorum quenching bacteria are not widely present in bulk soil or the rhizosphere of tobacco plants (d'Angelo-Picard *et al.* 2005). Interestingly, degradation capacities in the phylum Actinobacteria, one of the main bacterial groups studied for its quorum quenching potential, have only been found in about 50 % and 35 % of endophytic and rhizospheric isolates, respectively (Chankhamhaengdecha *et al.* 2013).

The presence of an AHL acylase in the isolates analyzed in this report is, at least, debatable due to the fact that the traditional separation between this activity and a lactonolysis is only based in the recovery or not of the lactone ring after acidification with HCl (Yates *et al.* 2002). As mentioned above, microorganisms can utilize these bacterial signals as a source of nutrients and energy. For instance, *T. loubieri* WW1C can utilize 3OC6-HSL as an energy and nitrogen source (Wong *et al.* 2013). It is then expected that the corresponding AHL will not be recoverable by acidification after being metabolized. As discussed above, the determination of the final products after AHL inactivation could shed light on this subject. Nevertheless, degradation of C12-HSL and 3OC12-HSL irreversibly mainly through acylase-like activities could be of ecological significance. Although these molecules are usually present at concentrations as low as 5 μM (Puskas *et al.* 1997), under oligotrophic conditions as those found in Antarctic soils, yeasts could utilize AHL molecules to sustain their growth. Results presented in this report also suggest a hidden role for endophytic yeasts, whose effects in the inner tissues of host plants is largely unknown. Our results show that quorum quenching is present in yeast genera associated with beneficial (*Meyerozyma* and *Rhodotorula*) and deleterious (*Sporisorium*) effects on plants (Deligios *et al.* 2015; Elsharkawy *et al.* 2015; Taniguti *et al.* 2015). Noteworthy, plants respond actively to AHLs with either a short or a long acyl chain, and this response requires the cleavage of the acyl chain by means of a plant-derived fatty acid amide hydrolase (Palmer *et al.* 2014). It is then plausible that endophytic yeasts play a role in the plant response to bacterial signals through the hydrolysis of the AHLs.

The long of and the substitution in the acyl chain of an AHL has an influence on the facility of these molecules to be

Yeast isolate	SC	C12	3OC12
M. guilliermondii KX303828	A		ND
M. guilliermondii KX303831	A		ND
Rhodotorula sp. KX303814	B		ND
Sporisorium sp. KX303818	B	ND	
Sporisorium sp. KX303817	B	ND	
Sporisorium sp. KX303820	B	ND	
Sporisorium sp. KX303821	B	ND	
Sporisorium sp. KX303822	B	ND	
Sporisorium sp. KX303823	B		
Sporisorium sp. KX303824	B	ND	
Sporisorium sp. KX303825	B	ND	
Sporisorium sp. KX303819	B		
Sporisorium sp. KX303826	B		
R. laryngis KC713831	B		
R. pallida KC713832	B		
R. laryngis KC713837	B		
Rhodotorula sp. KC713838	B		ND
R. laryngis KC713844	B		
C. gilvescens KC713845	B		
R. laryngis KC713847	B		
R. laryngis KC713859	B		
R. laryngis KC713863	B		ND
Cryptococcus sp. KC713866	B		
R. laryngis KC713828	C		
Dioszegia sp. KC713829	C		
Rhodotorula sp. KC713834	C		
Unid. Ascomycetous KC580669	C		
Cryptococcus sp. KC713840	C		
R. mucilaginosa KC713841	C		
Rhodotorula sp. KC713846	C		
Cryptococcus sp. KC713849	C		
Cryptococcus sp. KC713850	C		
R. glutinis KC713858	C		
Rhodotorula sp. KC713864	D		

Fig 4 – Characterization of quorum quenching activities on C12-HSL and 3OC12-HSL of selected isolates from different clusters. After inactivation of the corresponding AHL, supernatant samples were acidified and then analyzed with bioassays as described in the text. Gray and black squares indicate reversible and irreversible quorum quenching activities (i.e., lactonase-like and acylase-like activities), respectively. ND: not determined.

inactivated by some groups of yeasts. For instance, while *Cryptococcus* spp. are mainly characterized by the inactivation of 3OC6- and C8-HSL, the endophytic *Meyerozyma guilliermondii* and *Sporisorium* spp. present as common features the inactivation of C8-, C12- and 3OC12-HSL. On the other side, 3OC8-HSL could not be degraded by several isolates. The crystallographic characterization of the bacterial acylase PvdQ shows that its higher affinity for AHLs with a long acyl chain is explained by a hydrophobic substrate-binding pocket (Bokhove *et al.* 2009). By contrast, the AiiA lactonase from *Bacillus thuringiensis* that possesses a wider range of AHL substrates, present

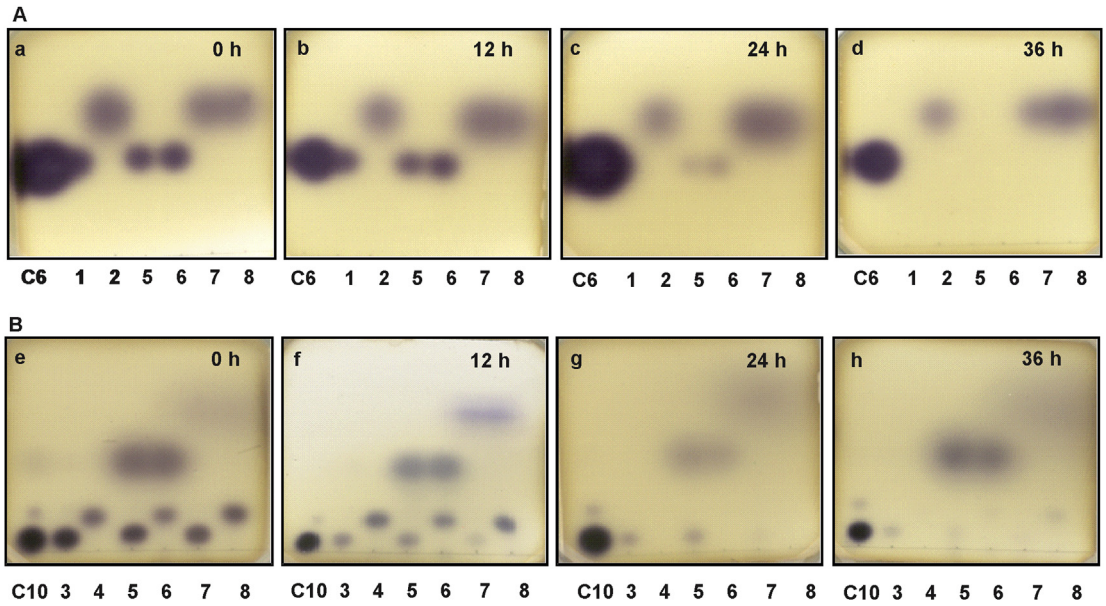

Fig 5 – Analysis of organic extracts from supernatants of *R. mucilaginosa* 7Apo1 cultures. RP-TLC were developed with (A) *C. violaceum* CV026 and (B) *C. violaceum* Vir07. Yeast cultures were supplemented with (1) C6-HSL, (2) 3OC6-HSL, (3) C10-HSL or (4) 3OC10-HSL alone or in the combinations (5) C6-HSL + C10-HSL; (6) C6-HSL + 3OC10-HSL; (7) 3OC6-HSL + C10-HSL; (8) 3OC6-HSL + 3OC10-HSL. Organic extracts from yeast cultures were obtained after (a and e) 0, (b and f) 12, (c and g) 24, and (d and h) 36 h of incubation. Commercial standards of C6-HSL (a–d) and C10-HSL (e–h) were also included.

a solvent-exposed groove that binds molecules with variable acyl chains (Liu *et al.* 2008). Recently, a report showed that the fungus *Coprinopsis cinerea* hydrolyze intracellularly AHLs by means of two lactonases with sequence homology to AiiA lactonase from *B. thuringiensis* (Stöckli *et al.* 2016). Up to date, the enzymes involved in AHL degradation by yeasts have not been characterized. The genome sequence of *R. mucilaginosa* C2.5t1 recently published (Deligios *et al.* 2015) will undoubtedly facilitate the identification of the enzymes related to AHL degradation in this genus. However, a tblastn search with the AiiA sequence as query did not retrieve any significant result from the *R. mucilaginosa* C2.5t1 sequence (data not shown), suggesting that novel enzymes could be involved in quorum quenching by yeasts.

Phylogenetic analysis of quorum quenching yeasts shows remarkable aspects of this feature. *Cryptococcus* is a polyphyletic genus related to *Bullera* and *Dioszegia* (Fig 2). The analyzed isolates were mainly located in subcluster A. The prominent exceptions located in subclusters B and C could be a manifestation of its polyphyly. It is also plausible that this particular biochemical property may have been acquired through horizontal gene transfer, a phenomenon already described in this genus (Kavanaugh *et al.* 2006). Similarly, Antarctic and endophytic isolates from the *Rhodotorula* genus show a broad range of quorum quenching profiles and are all located in a polyphyletic branch of the tree.

The lack of interference of one molecule with the inactivation of a second AHL by *R. mucilaginosa* 7Apo1 suggests that more than one enzyme or biochemical pathway could be involved in the inactivation of these bacterial signals. Noteworthy, under the conditions utilized for this report, degradation of C6-HSL by this isolate is reversible, as shown by the recovery of the quorum sensing activity after acidification mediated, suggesting the activity of a lactonase enzyme. In contrast, 3OC6-HSL, C10-HSL and 3OC10-HSL follow an irreversible inactivation. These observations support the hypothesis of not a common reaction in the inactivation of all AHLs by yeasts.

Quorum sensing regulated phenotypes are directly related to the concentration of the corresponding quorum sensing signals, which in turns are dependent on the cell density of the producing microorganism. The co-culture of the AHL-degrading *R. mucilaginosa* 7Apo1 with the 3OC6-HSL-producing *Pseudomonas syringae* pv. *syringae*, a condition of constant supply of molecules to the reaction media, showed that the AHLs are always detectable in the supernatant (Fig 6). These findings allow the supposition that the co-occurrence in a niche of quorum quenching microorganisms together with an AHL producer could not completely eliminate the quorum sensing signals but to modulate the bacterial density required for triggering a particular phenotype. In this regard, it is interesting to speculate about the ecological consequences of the reversible or irreversible quenching, i. e. interference, of bacterial quorum sensing systems by yeasts in different niches. This activity could have *in planta* a beneficial effect through the interference of quorum sensing systems of pathogenic bacteria; or a detrimental effect when the system of a plant growth-promoting bacterium is interfered. On the other side, this activity could also have other effects, considering that it has been suggested that quorum sensing is of importance in the regulation of the production of enzymes involved in nitrogen cycling in soil (DeAngelis *et al.* 2008).

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

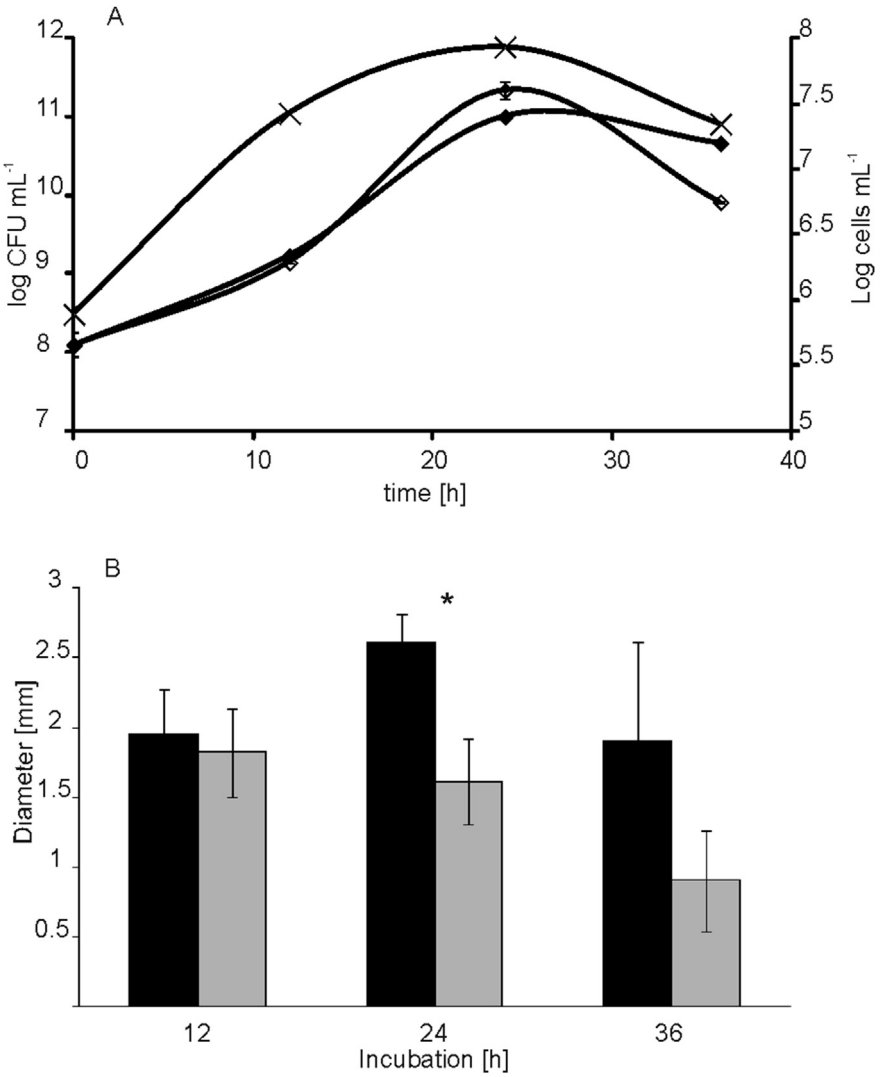

Fig 6 – *R. mucilaginosa* 7Apo1 inactivation of AHLs produced by *P. syringae* pv. *syringae* B728a. (A) Yeast and bacterial growth as determined by cell counting in Neubauer chamber, and plating in selective medium, respectively. ×, *R. mucilaginosa* 7Apo1 in mixed culture with *P. syringae* pv. *syringae* B728a; ◆ *P. syringae* pv. *syringae* B728a in pure culture; ◇ *P. syringae* pv. *syringae* B728a in mixed culture with *R. mucilaginosa* 7Apo1. (B) Diameters of the halos obtained in bioassays with *A. tumefaciens* NT1 (pZLR4) were normalized to the corresponding bacterial growth. Black and gray bars correspond to AHLs produced by *P. syringae* pv. *syringae* B728a in pure and mixed culture, respectively. The symbol * identifies significant differences between pure and mixed cultures based on Student's *t* test ($P < 0.05$). Error bars represent standard deviations.

It is possible that the quorum quenching capacity could confer ecological advantages in a niche through the protection from the effects of AHLs on yeasts. To note, AHLs modify the filament formation in the opportunistic pathogen *Candida albicans* (Hogan *et al.* 2004). These molecules also induce the budding, reduce cell size and increase the ethanol tolerance in *Saccharomyces cerevisiae* (Ren *et al.* 2016). The effect of AHLs on other yeasts has not been reported. As shown in this report, the analysis of phylogenetically unrelated yeasts from Antarctic soil and sugarcane, and the characterizations performed demonstrates for the first time that quorum quenching is widely present in this group of fungi and that this feature could have ecological implications. Beyond the ecological significance of the quorum quenching activities of yeasts, it can be speculate about novel biotechnological applications for this fungal enzymes or microorganisms. For instance, the inhibition of biofilm formation by pathogenic bacteria through the enzymatic inactivation of AHLs is a promissory field of research (Kalia & Kumar 2015). In this way, the restricted quorum quenching activities of some isolates, like *Cryptococcus* spp. that mainly degrade 3OC6-HSL and C8-HSL, or the broad activities of others, such as *Rhodotorula* spp. that inactivate all the AHLs assayed, could find specific biotechnological applications.

Conflict of interest

No conflict of interest to declare.

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006

Acknowledgments

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 946), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT), and Secretaría de Ciencia, Arte e Innovación Tecnológica de la Universidad Nacional de Tucumán (SCAIT). We thank Dirección Nacional del Antártico – Instituto Antártico Argentino (DNA – IAA) for providing access to sampling sites at 25 de Mayo/King George Island, and Dr. Laura Tórtora (EEAOC, Tucumán, Argentina) for providing sugarcane plants.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funbio.2017.10.006.

REFERENCES

Bokhove M, Nadal P, Quax WJ, Dijkstra BW, 2009. The quorum-quenching *N*-acyl homoserine lactone acylase PvdQ is an Ntn-hydrolase with an unusual substrate-binding pocket. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0911839107.

Cha C, Gao P, Chen YC, Shaw PD, Farrand SK, 1998. Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. *Molecular Plant-Microbe Interactions* **11**: 1119–1129. https://doi.org/10.1094/MPMI.1998.11.11.1119.

Chankhamhaengdecha S, Hongvijit S, Srichaisupakit A, Charnchai P, Panbangred W, 2013. Endophytic actinomycetes: a novel source of potential acyl homoserine lactone degrading enzymes. *BioMed Research International* **2013**: 782847. https://doi.org/10.1155/2013/782847.

d'Angelo-Picard C, Faure ZD, Penot I, Dessaux Y, 2005. Diversity of *N*-acyl homoserine lactone-producing and -degrading bacteria in soil and tobacco rhizosphere. *Environmental Microbiology* **7**: 1796–1808. https://doi.org/10.1111/j.1462-2920.2005.00886.x.

DeAngelis KM, Lindow SE, Firestone MK, 2008. Bacterial quorum sensing and nitrogen cycling in rhizosphere soil. *FEMS Microbiology Ecology* **66**: 197–207.

Deligios M, Fraumene C, Abbondio M, Mannazzu I, Tanca A, Addis MF, Uzzau S, 2015. Draft genome sequence of *Rhodotorula mucilaginosa*, an emergent opportunistic pathogen. *Genome Announcements* **3**: 00201–00215.

Elsharkawy MM, Nakatani M, Nishimura M, Arakawa T, Shimizu M, Hyakumachi M, 2015. Suppression of rice blast, cabbage black leaf spot, and tomato bacterial wilt diseases by *Meyerozyma guilliermondii* TA-2 and the nature of protection. *Acta Agriculturae Scandinavica, Section B* **65**: 629–636.

Farris JS, Albert VA, Källersjö M, Lipscomb D, G KA, 1996. Parsimony jackknifing outperforms neighborjoining. *Cladistics* **12**: 99–124.

Fuqua C, Winans S, Greenberg E, 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annual Reviews in Microbiology* **50**: 727–751.

Ghani NA, Sulaiman J, Ismail Z, Chan X-Y, Yin W-F, Chan K-G, 2014. *Rhodotorula mucilaginosa*, a quorum quenching yeast exhibiting lactonase activity isolated from a tropical shoreline. *Sensors (Basel, Switzerland)* **14**: 6463–6473. https://doi.org/10.3390/s140406463.

Goloboff PA, Farris JS, Källersjö M, Oxelman B, Ramírez MJ, Szumick C, 2003. Improvements to resampling measures of group support. *Cladistics* **19**: 324–332.

Goloboff PA, Farris JS, Nixon K, 2008. TNT, a free program for phylogenetic analysis. *Cladistics* **24**: 774–786.

Grandclément C, Tannières M, Moréra S, Dessaux Y, Faure D, 2016. Quorum quenching: role in nature and applied developments. *FEMS Microbiology Reviews* **40**: 86–116. https://doi.org/10.1093/femsre/fuv038.

Hassan N, Rafiq M, Hayat M, Shah AA, Hasan F, 2016. Psychrophilic and psychrotrophic fungi: a comprehensive review. *Reviews in Environmental Science and Bio/Technology* **15**: 147–172. https://doi.org/10.1007/s11157-016-9395-9.

Hogan DA, Vik A, Kolter R, 2004. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Molecular Microbiology* **54**: 1212–1223. https://doi.org/10.1111/j.1365-2958.2004.04349.x.

Kalia VC, Kumar P, 2015. Potential applications of quorum sensing inhibitors in diverse fields. In: Kalia VC (ed.), *Quorum Sensing vs Quorum Quenching: a battle with no end in sight*. Springer-Verlag, pp. 359–370.

Kavanaugh L, Fraser J, Dietrich F, 2006. Recent evolution of the human pathogen *Cryptococcus neoformans* by intervarietal transfer of a 14-gene fragment. *Molecular Biology and Evolution* **23**: 1879–1890.

King EO, Ward MK, Raney DE, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *The Journal of Laboratory and Clinical Medicine* **44**: 301–307.

Koul S, Kalia VC, 2017. Multiplicity of quorum quenching enzymes: a potential mechanism to limit quorum sensing bacterial population. *Indian Journal of Microbiology* **57**: 100–108.

Kurtzman C, 2011. *The Yeasts: a taxonomic study*. Elsevier Science, Amsterdam.

Lalande R, Bissonnette N, Coutlée D, Antoun H, 1989. Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. *Plant and Soil* **115**: 7–11.

Lawley B, Ripley S, Bridge P, Convey P, 2004. Molecular analysis of geographic patterns of eukaryotic diversity in Antarctic soils. *Applied and Environmental Microbiology* **70**: 5963–5972.

Liu D, Momb J, Thomas PW, Moulin A, Petsko GA, Fast W, Ringe D, 2008. Mechanism of the quorum-quenching lactonase (AiiA) from *Bacillus thuringiensis*. 1. Product-bound structures. *Biochemistry* **47**: 7706–7714.

McClean K, Winson M, Fish L, Taylor A, Chhabra SMC, Daykin M, Lamb J, Swift S, Bycroft B, Stewart G, Williams P, 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiology* **143**: 3703–3711.

Morohoshi T, Kato M, Fukamachi K, Kato N, Ikeda T, 2008. *N*-acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. *FEMS Microbiology Letters* **279**: 124–130. https://doi.org/10.1111/j.1574-6968.2007.01016.x.

Palmer AG, Senechal AC, Mukherjee A, Ané J, Blackwell HE, 2014. Plant responses to bacterial *N*-acyl-L-homoserine lactones are dependent on enzymatic degradation to L-homoserine. *ACS Chemical Biology* **9**: 1834–1845.

Puskas A, Greenberg EP, Kaplan S, Schaefer AMYL, 1997. A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. *Journal of Bacteriology* **179**: 7530–7537.

Quiñones B, Dulla G, Lindow SE, 2005. Exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **18**: 682–693.

Quiñones B, Pujol CJ, Lindow SE, 2004. Regulation of AHL production and Iis contribution to epiphytic fitness in *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **17**: 521–531.

Ravn L, Christensen AB, Molin S, Givskov M, Gram L, 2001. Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. *Journal of Microbiological Methods* **44**: 239–251. https://doi.org/10.1016/S0167-7012(01)00217-2.

Ren G, Ma A, Liu W, Zhuang X, Zhuang G, 2016. Bacterial signals N-acyl homoserine lactones induce the changes of morphology and ethanol tolerance in Saccharomyces cerevisiae. *AMB Express* **6**: 117.

Romero ER, Scandaliaris J, Zigonzelli PA, Leggio Neme MF, Giardina JA, Fernández de Ulivarri J, Casen SD, Tonatto MJ, Alonso LGP, 2009. La caña de azúcar: características y ecofisiología. In: Romero ER, Digonzelli PA, Scandaliaris J (eds), *Manual del cañero*. Estación Experimental Agroindustrial Obispo Colombres, Las Talitas, pp. 13–22.

Rovati JI, Pajot HF, Ruberto L, Mac Cormack W, Figueroa LIC, 2013. Polyphenolic substrates and dyes degradation by yeasts from 25 de Mayo/King George Island (Antarctica). *Yeast* 459–470. https://doi.org/10.1002/yea.2982.

Saldanha AJ, 2004. Java Treeview–extensible visualization of microarray data. *Bioinformatics (Oxford, England)* **20**: 3246–3248. https://doi.org/10.1093/bioinformatics/bth349.

De Sordi L, Mühlschlegel FA, 2009. Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communicative network regulating microbial coexistence and virulence. *FEMS Yeast Research* **9**: 990–999. https://doi.org/10.1111/j.1567-1364.2009.00573.x.

De Souza RS, Okura VK, Armanhi JS, Jorrín B, Lozano N, da Silva MJ, González-Guerrero M, Aráujo LM, Verza NC, Bagheri HC, Imperial J, 2016. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports* **6**: 28774.

Steindler L, Venturi V, 2007. Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. *FEMS Microbiology Letters* **266**: 1–9. https://doi.org/10.1111/j.1574-6968.2006.00501.x.

Stöckli M, Lin C, Sieber R, Plaza DF, Ohm RA, Künzler M, 2017. *Coprinopsis cinerea* intracellular lactonases hydrolyze quorum sensing molecules of Gram-negative bacteria. *Fungal Genetics and Biology* **102**: 49–62 https://doi.org/10.1016/j.fgb.2016.07.009.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Taniguti LM, Schaker PDC, Benevenuto J, Peters LP, Carvalho G, Palhares A, Quecine MC, Nunes FRS, Kmit MCP, Wai A, Hausner G, Aitken KS, Berkman PJ, Fraser JA, Moolhuijzen PM, Coutinho LL, Creste S, Vieira MLC, Kitajima JP, Monteiro-Vitorello CB, 2015. Complete genome sequence of *Sporisorium scitamineum* and biotrophic interaction transcriptome with sugarcane. *PloS One* **10**: e0129318. https://doi.org/10.1371/journal.pone.0129318.

Tempé J, Petit A, Holsters M, van Montagu M, Schell J, 1978. Thermosensitive step associated with transfer of the Ti plasmid during conjugation: possible relation to transformation in crown gall. *Proceedings of the National Academy of Sciences of the United States of America* **74**: 2848–2849.

Uroz S, Heinonsalo J, 2008. Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. *FEMS Microbiology Ecology* **65**: 271–278. https://doi.org/10.1111/j.1574-6941.2008.00477.x.

Vishniac HS, 2006. Yeast biodiversity in the Antarctic. In: Rosa C, Péter G (eds), *Biodiversity and Ecophysiology of Yeasts*. Springer-Verlag, Berlin, pp. 419–440.

Wong C-S, Koh C-L, Sam C-K, Chen JW, Chong YM, Yin W-F, Chan K-G, 2013. Degradation of bacterial quorum sensing signaling molecules by the microscopic yeast *Trichosporon loubieri* isolated from tropical wetland waters. *Sensors (Basel, Switzerland)* **13**: 12943–12957. https://doi.org/10.3390/s131012943.

Yates EA, Philipp B, Buckley C, Atkinson S, Chhabra SR, Sockett RE, Goldner M, Dessaux Y, 2002. N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infection and Immunity* **70**: 5635–5646. https://doi.org/10.1128/IAI.70.10.5635.

Please cite this article in press as: Leguina Ana Carolina del V, et al., Inactivation of bacterial quorum sensing signals N-acyl homoserine lactones is widespread in yeasts, Fungal Biology (2017), https://doi.org/10.1016/j.funbio.2017.10.006