2014

Quorum sensing activity and control of yeast-mycelium dimorphism in *Ophiostoma floccosum*

Alexander Berrocal

Instituto Tecnológico de Costa Rica, aberrocal@itcr.ac.cr

Claudia Oviedo

Universidad del Bío Bío

Kenneth W. Nickerson

University of Nebraska-Lincoln, knickerson1@unl.edu

José Navarrete

Universidad del Bío Bío

Follow this and additional works at: <http://digitalcommons.unl.edu/bioscifacpub>

Part of the [Biology Commons](#)

Berrocal, Alexander; Oviedo, Claudia; Nickerson, Kenneth W.; and Navarrete, José, "Quorum sensing activity and control of yeast-mycelium dimorphism in *Ophiostoma floccosum*" (2014). *Faculty Publications in the Biological Sciences*. 560.
<http://digitalcommons.unl.edu/bioscifacpub/560>

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications in the Biological Sciences by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Quorum sensing activity and control of yeast-mycelium dimorphism in *Ophiostoma floccosum*

Alexander Berrocal, Claudia Oviedo, Kenneth W. Nickerson, and José Navarrete

A. Berrocal (*Corresponding author*), Escuela de Ingeniería Forestal, Instituto Tecnológico de Costa Rica, Apartado Postal 159-7050, Cartago 30101, Costa Rica; email: aberrocal@itcr.ac.cr

C. Oviedo, Departamento de Química, Facultad de Ciencias, Universidad del Bío Bío, Avenida Collao 1202, Casilla 5-C, 4051381 Concepción, Chile

K. W. Nickerson, School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, NE 68588-0666, USA

J. Navarrete, Departamento de Ingeniería en Maderas, Facultad de Ingeniería, Universidad del Bío Bío, Avenida Collao 1202, Casilla 5-C, 4051381 Concepción, Chile

Abstract

Quorum sensing (QS) activity in *Ophiostoma* fungi has not been described. We have examined the growth conditions on the control of dimorphism in *Ophiostoma floccosum*, an attractive biocontrol agent against blue-stain fungi, and its relationship with QS activity. In a defined culture medium with L-proline as the N source, a high inoculum size (10^7 c.f.u. ml⁻¹) was the principal factor that promoted yeast-like growth. Inoculum size effect can be explained by the secretion of a QS molecule(s) (QSMs) responsible for inducing yeast morphology. QSM candidates were extracted from spent medium and their structure was determined by GC–MS. Three cyclic sesquiterpenes were found. The most abundant molecule, and therefore the principal candidate to be the QSM responsible for yeast growth of *O. floccosum*, was 1,1,4a-trimethyl-5,6-dimethylene-decalin (C₁₅H₂₄). Other two compounds were also detected.

Keywords: Cyclic sesquiterpenes, Fungal dimorphism, Inoculum size, *Ophiostoma floccosum*, Quorum sensing molecules, Trimethyl-dimethylene-decalin

Introduction

The effect of inoculum size on the control of dimorphism and other vital functions is common among dimorphic fungi (Nickerson et al. 2006) including albino strains from the genus *Ophiostoma* (Berrocal et al. 2012a). Some studies on the growth morphology of *Ceratocystis* (*Ophiostoma*) *ulmi* and *Candida albicans* in liquid culture media, found that their morphology was influenced by the cell density (Kulkarni and Nickerson 1981; Hornby et al. 2001, 2004; Berrocal et al. 2012b).

With the organism that causes Dutch elm disease (*Cer. ulmi*), Hornby et al. (2004) concluded that the effect of inoculum size, initially reported by Kulkarni and Nickerson (1981), was mediated by a quorum sensing molecule (QSM) that induced a change in the morphology of the fungus. This compound was lipophilic (Hornby et al. 2004). Berrocal et al. (2012b) found that the addition of 2-methyl-1-butanol or 3-methylvaleric acid (the fusel alcohol and fusel acid derived from isoleucine, respectively) or 4-hydroxyphenylacetic acid decreased the formation of germ tubes in *O.*

ulmi cultures by more than 50%, thus demonstrating a QSM behavior. A similar effect was obtained using branched-chain amino acids (precursors of fusel alcohols) as N sources (Berrocal et al. 2012b). For *Can. albicans*, Hornby et al. (2001) determined that the QSM secreted at high inoculum density which promoted yeast growth was the sesquiterpene, (*E,E*)-farnesol. On the other hand, tyrosol, a fusel alcohol derived from the amino acid tyrosine, is a QSM that induces mycelia growth of *Can. albicans* (Chen et al. 2004).

Quorum sensing (QS) is a cell concentration-dependent phenomenon (March and Bentley 2004) which, in bacteria and fungi, is mediated by small, diffusible signaling molecules that accumulate in the extracellular environment (Sprague and Winans 2006). These induction molecules are generally, but not always, intraspecific and have a large structural diversity within the group of signaling molecules produced by different organisms. Essentially, what happens is that when one of these molecules accumulates at a high enough concentration, its corresponding response regulator is activated within the cell population and induces gene expression (Hogan 2006). According to Nickerson et al. (2006), for most dimorphic fungi, inoculum size is a major factor associated with their yeast-mycelia choice.

Albino strains of *Ophiostoma* spp. have been proposed as bio-control agents against blue-sap stain in North America, New Zealand, and Chile (Hernández et al. 2012) but the efficiency of commercial production systems is affected by the dimorphic growth exhibited by most *Ophiostoma* albino strains. Production of 100% of the yeast-like form is important for its use as biocontrol agent because the yeast-like form provides reproducible and quantitative dosages without the danger of clogging any spray equipment (Berrocal et al. 2012a).

On the other hand, chemical identity and functionality of sesquiterpenes found in different structures of the plants has been widely reported. However, the reports of those found in fungi cultures are very insufficient, although some of them have biological activities against other fungi and bacteria. In plants the compound, 1,2,4,8-tetramethylbicyclic[6.3.0]undeca-2,4-diene has been detected in: pineapple cores

(Chang-Bin et al. 2011), *Cistanche deserticola* (Sarsenbayev et al. 2013) and *Salvia multicaulis* (Karamian et al. 2013). α -gurjunene occurs in *Achillea tenuifolia* (Shafaghat 2009), tubers of *Cyperus rotundus* (Bisht et al. 2011), *Juniperus communis* (Abbassy and Marei 2013) and *Blumea eriantha* (Pednekar et al. 2013). Specifically in fungi, different cultures of *Muscodor albus* generated a mixture of volatile organic compounds the most abundant being naphthalene (Ezra et al. 2004). Daisy et al. (2002) also reported the production of naphthalene under certain cultural conditions by *Muscodor vitigenus*. Naphthalene generated by this fungus effectively repelled the adult stage of the wheat stem sawfly, *Cephus cinctus*. Naphthalene has also been found as a constituent of other natural biological systems (Daisy et al. 2002). Interestingly, the skeleton of the most abundant compound generated by *O. floccosum* cultures growing with a yeast-like morphology was naphthalene.

The objectives in this study were to determine the culture conditions that promoted yeast-like growth in the albino strain FIF1A55-*Ophiostoma floccosum* Math-Käärík in a defined liquid culture medium, to examine the effect that QS has on yeast-mycelium dimorphism in this strain, and to elucidate the chemical nature of the molecule(s) involved in this phenomenon.

Materials and methods

Microorganisms and growth

The albino strain FIF1A55-*O. floccosum* was from Laboratory of Wood Biodegradation in the Department of Wood Engineering at the University of Bio-Bio (Chile). It was grown on a defined culture medium (GPP) containing (per liter of distilled water): 20 g dextrose, 4 g KH_2PO_4 , 3.2 g Na_2HPO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 μg biotin, 200 μg thiamin HCl, 200 μg pyridoxine HCl, 1 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1 mg FeCl_3 , and 10 mM L-proline as N source. Trace elements were prepared as a 5,000 $\times$ stock solution in 0.1 M HCl. Medium reagents, except C and N sources, were dissolved in distilled water, deposited into their respective culture bottles, and autoclaved at 121 °C

for 15 min. The N and C (dextrose) sources were autoclaved separately and added under sterile conditions to the culture medium (Kulkarni and Nickerson 1981; Hornby et al. 2004).

Inoculum preparation

Erlenmeyer flasks (250 ml) containing 50 ml GPP liquid culture medium were inoculated with cell culture stock and shaken at 150 rpm for 5 days at 25 °C. The cells were collected aseptically, pelleted by centrifugation at 4,750×g for 5 min, and washed three times with equal volumes of 50 mM phosphate buffer (pH 6.5), resuspended in the same buffer and stored at 4 °C.

Culture conditions and statistical analysis for evaluation of yeast-like growth

The experimental protocol considered a 2⁴ factorial design of four factors and two levels, without replicates and completely randomized. The factors examined were: **A)** Inoculum size (5 × 10⁵ and 10⁷ c.f.u. ml⁻¹), **B)** Temperature (18 and 26 °C), **C)** Initial pH (5 and 7) and **D)** Shaking speed (100 and 200 rpm). Responses evaluated after 24, 48, and 72 h growth were: yeast/mycelia ratio (%) and cell concentration (c.f.u. ml⁻¹). The experimental design, variance analysis, and subsequent statistical analysis of the data for a 95% confidence level were done using Design Expert 8.0.1 software (Stat Ease Inc., Minneapolis, Minnesota, USA). Other growth conditions were evaluated previously

Table 1. Variables evaluated that did not control by themselves fungal dimorphism or promoted yeast-like morphology in *O. floccosum* liquid cultures.

Variable	Range evaluated
Complex liquid culture medium (yeast-malt extract-antibiotics) (g l ⁻¹)	4–15
Glucose concentration (%)	1–4
Temperature (°C)	18–26
pH	5–7
Phosphate concentration (g l ⁻¹)	1.5–6
L-arginine, ammonium sulfate, L-asparagine, L-cysteine or L-methionine (mM) ^a	5–20
Shaking speed (rpm)	100–200

a. Other N sources were also evaluated

(Table 1); however they did not have any effect in promoting the yeast-like morphology in *O. floccosum* after 24 h of growth.

Preparation of spent culture medium

Spent culture medium, defined as the supernatant (sterile and cell-free) of a fungus culture, was obtained by inoculating 1 l GPP medium in an Erlenmeyer flask (5 l) with blastospores (2 × 10⁷ c.f.u. ml⁻¹) from the albino strain *O. floccosum* FIF1A55. The flask was shaken 100 rpm for 72 h. The initial pH was 5 ± 0.2.

Extraction of the quorum sensing molecules (QSM)

The spent culture medium of the albino strain *O. floccosum* FIF1A55 was extracted with ethyl acetate (5:1 v/v). The extract was collected and the solvent removed under a continuous flow of N₂ at room temperature. The residue was resuspended in methanol and bioassayed to measure the QSM activity.

Growth evaluation

For the evaluation of QSM activity arising by using different concentrations of extract of GPP-spent medium (See Table 2 below), the cell number and morphology

Table 2. Dose response of QSM activity of different concentrations of extract of GPP spent medium (resuspended in methanol), as mechanism of control of the morphology of cultures of *O. floccosum* growing in GPR medium at 24 h of culture.

Volume of extract added (µl)	Culture yeast proportion (%)
15	65
20	70
30	78
45	86
50	90
Control ^a	11
Methanol ^b	11

a. Cultures established in fresh GPR medium and no spent medium extract was added

b. Cultures established in fresh GPR medium and 50 µl methanol were added

Figure 1. Microphotography of mycelial growth in a culture of the albino strain FlF1A55-*O. floccosum* in GPR defined liquid culture medium. Scale bar = 50 μm

were determined with a hemacytometer with a fluorescence microscope. Five fields of view of the hemacytometer were counted per sample and five samples were taken per treatment. All experiments were done in triplicate. Only differentiated cells were quantified and normalized to 100% (yeast/filamentous cells). Cells with buds were counted as yeasts if they had a visible constriction at the bud site and cells forming germ tubes were classified as germinated if the length the germ tube was greater than half of the diameter of the spore (Hornby et al. 2004). A one-way ANOVA was performed. For morphological evaluation at least 10 microphotographs were taken per sample.

Bioassay to measure the activity of the QSM

Activity of the QSM of different concentrations of extract of GPP-spent medium was evaluated by means of a central composite design (response surface methodology). Thirteen flasks (25 ml) with 5 ml defined culture medium with L-arginine as N source (medium defined as GPR) were inoculated and incubated at 25 °C with shaking at 150 rpm. GPR was chosen because by itself it promotes mycelial growth (Figure 1). After 24 h inoculation, the morphology of cultures was evaluated using phase contrast microscopy. A total of eight controls were used for this experiment, the first two corresponded to fresh GPR medium with two inoculum

sizes (3.5×10^5 and 1.2×10^7 c.f.u. ml^{-1}) designated C1 and C2, respectively. The other two controls were cultures of low and high inocula size growing into fresh GPR medium to which 50 μl methanol was added; they were designated C3 and C4, respectively. With these controls, the effect of methanol in fungi morphology was evaluated. In addition, a control was used to evaluate the loss of QSM activity in the spent medium by using 76% of fresh GPR (enriched culture medium 2 $\times$) and 24% of spent medium extracted with ethyl acetate at both inoculum size (3.5×10^5 and 1.2×10^7 c.f.u. ml^{-1}), designated C5 and C6, respectively. The aqueous phase of the spent media extracted with ethyl acetate was previously re-extracted with hexane (to remove remnant ethyl acetate) because trace levels of ethyl acetate inhibit fungal growth. Finally, the positive control was 76% of fresh GPR (enriched culture medium 2 $\times$) and 24% of fresh spent medium (with QSM activity), at the respective low and high concentrations (C7 and C8).

Analysis of spent medium using gas chromatography-mass spectrometry (GC/MS)

GC/MS analyses were done on GPP fresh filtered spent medium and on the fraction of GPP spent medium extracted with ethyl acetate, since both types of media demonstrated QS activity. Fresh spent medium was analyzed using headspace analysis that allows the extraction, concentration and analysis of volatile compounds in liquid or solid samples placed in standard vials. The extracted spent medium was analyzed by direct injection into the GC/MS. Both analyses were done in duplicate. The GC/MS equipment used was a GC 7,890 A and a MS Agilent MS 5975C coupled with headspace analysis, it had an electron capture detector (ECD) and a flame ionization detector (FID). The column used was a HP5 MS column (30 m $\times$ 0.25 mm, 0.25 μm film. The operative conditions were: 40 °C for 3 min followed by a gradient of 10 °C min^{-1} until 230 °C and maintaining this temperature for 8 min: Split ratio 8:1, 7,9 ml min^{-1} ; injector 200 °C; MS source: 230 °C; MS quad: 150 °C; Aux 2: 280 °C. Total run was 30 min and compounds were identified with the Electron Ionization (EI) mass spectral library (NIST 05) from the National Institute of Standard and Technology, Ringoes, NJ, which has the spectra for 163,198 compounds.

Figure 2. Effect of inoculum size on the morphology of albino strain FIF1A55 *O. floccosum* in GPP defined liquid culture medium. Fluorescence microscopy; Scale bars = 10 μ m. Inoculum condition was 100% yeasts-like morphology

Results

Conditions for yeast-like growth

O. floccosum F1F1A55 grew as mycelia in defined liquid media with L-arginine (GPR), L-asparagine (GPAs), L-methionine (GPM), or ammonium sulfate (GPA) as the N source. Figure 1 shows that the cells were almost exclusively mycelial in GPR. However, the combination of a high inoculum size ($\geq 10^7$ c.f.u. ml⁻¹) and L-proline as the N source promoted the greatest percentage of yeasts, as well as a higher concentration of spores for the albino strain (Figure 2). The proline-containing GPP medium was chosen because it has previously been shown to promote yeast-like development in the related fungus *Cer. ulmi* (Kulkarni and Nickerson 1981). To maximize the percentage yeasts, we varied the inoculum size, temperature, pH, and agitation speed (Table 1). Of these, the agitation speed had no effect on morphology while the acidic initial pH (5) and growth temperature (26 °C) had only slight contributions. A high inoculum size and L-proline as the N source were dominant factors. Other N sources as well as varying the phosphate, N, and glucose levels did not promote yeast-like morphology in *O. floccosum* cultures (Table 1). The conditions that promote yeast like morphology in GPP were: high inoculum size (10⁷/ml), low pH (pH5), and a temperature of 26 °C. Once reproducible growth conditions had been identified for this strain, the importance of the inoculum size effect suggested the next step was to elucidate the QSMs that promoted the yeast-like morphology. It is much easier to identify QSMs when fungi are grown in a defined medium such as GPP (Hornby et al. 2001).

Chemical nature and activity of the QSMs in spent GPP medium

The organic phase of the spent medium from GPP yeast-like cultures (extracted with ethyl acetate and subsequently resuspended in 100% methanol) demonstrated QSM activity in that when added to fresh GPR with L-arginine as the N source, it promoted cell budding rather than germination. With unsupplemented GPR, during the first 24 h growth the albino strain F1F1A55-*O. floccosum* grew as mycelia independent of the initial cell concentration. However, the addition of increasing concentrations from the spent

medium (15, 20, 30, 45, and 50 µl) reversed the mycelial growth morphology while generating 65, 70, 78, 86 and 90% yeast cultures, respectively (Table 2). Statistical difference was found. Neither the methanol solvent control nor the aqueous phase of the spent media prevented mycelial growth (Figure 3).

Analysis of spent medium using GC/MS

GC/MS analysis using both the headspace technique with fresh filtered spent medium (Figure 4a) and direct injection of the lipid soluble extract from the spent medium (Figure 4b) demonstrated the presence of three chemicals in the spent media from the albino strain of *O. floccosum*. The three peaks detected in the spent medium, all at a confidence index $\geq 90\%$, were cyclic sesquiterpenes. The compounds were α -Gurjunene (peak area 11%), 1,2,4,8-tetramethylbicyclic[6.3.0] undeca-2,4-diene (peak area 21%), and 1,1,4a-trimethyl-5,6-dimethylene-decalin (peak area 68%) (Figure 4). None of these compounds has previously been identified as a volatile organic compound (VOC) from *Ceratocystis/Ophiostoma* (Hansen 1993) or any other fungus (Kramer and Abraham 2012; Morath et al. 2012). (These structures must be considered as preliminary until they are confirmed by more rigorous methods of natural product identification.) Also, due to the chemical nature of these three compounds (cyclic sesquiterpenes), any one of them could be the QSM responsible for signaling and yeast growth in the *O. floccosum* albino strain.

Discussion

Our study defined the growth conditions needed for reproducible control of morphology and production of 99–100% yeasts by a promising biocontrol agent against blue-sap stain, the albino strain F1F1A55 of *O. floccosum*. GPP, a defined glucose-proline-salts medium (Kulkarni and Nickerson 1981) was used as a starting point and temperature, pH, aeration, inoculum size, glucose concentration, phosphate concentration, and the N source were varied. L-proline was the best N source for yeast production, with the dominant variable being an inoculum size $\geq 10^7$ cells ml⁻¹. As expected from previous studies on related fungi (Hornby et al. 2004; Berrocal et al. 2012b), the high cell density which promoted

Figure 3. Yeast/mycelium ratio observed in a bioassay for QSM activity in the albino strain F1F1A55-*O. floccosum*. Symbols: GPR = glucose phosphate arginine medium, GPP = glucose phosphate proline, low inoculum size = 3.5×10^5 c.f.u. ml⁻¹, high inoculum size = 1.2×10^7 c.f.u. ml⁻¹, methanol = 50 μ l

yeast production was mediated by an extracellular lipophilic molecule or group of molecules termed QSMs.

The ethyl acetate-extractable supernatants from the albino strain of *O. floccosum* contained both the biological activity to block mycelial growth and three cyclic sesquiterpenes (Figure 4) as identified by GC/MS in comparison with the NIST 05 library. Hansen (1993) reviewed the VOCs produced by *Ceratocystis* and *Ophiostoma* sp: 27 volatile molecules, including 13 sesquiterpenes of which 10 were cyclic sesquiterpenes, were identified. α -amorphene, γ -amorphene, δ -cadinol, 6-protoilludene, cerapicol and leptographiol are worth mentioning due to their similar structures to the molecules found in the spent medium from the albino strain F1F1A55-*O. floccosum*. The three cyclic sesquiterpenes which we found (Figure 4) are distinct from these 10 and thus, if further chemical characterization confirms the GC/MS identifications, we will have added three more cyclic sesquiterpenes to the repertoire produced by *Ophiostoma* sp. Additionally, Hansen (1993) concluded that *Ceratocystis* sp. produced non-cyclic sesquiterpenes whereas *Ophiostoma* sp. produced cyclic sesquiterpenes. Our findings agree with that conclusion. Finally, there are probably many more VOCs yet to be discovered since the secondary metabolites produced are very strain dependent as well as media dependent and culture dependent (Hansen 1993; Kramer and Abraham 2012) and often a single strain can produce

several related sesquiterpenes (Zeringue et al. 1993; Morath et al. 2012).

The yeast-like morphology obtained in cultures of *O. floccosum* with L-proline as the N source, is consistent with studies done in the species *O. ulmi* (Muthukumar and Nickerson 1984; Hornby et al. 2004) and would be explained by the effect the N source has on the production of QSMs. For instance, in both *S. cerevisiae* (Chen and Fink 2006) and *Can. albicans* (Ghosh et al. 2008) ammonium salts restrict the production of fusel alcohols (signaling compounds for these fungal species) due to the inhibition of their biosynthetic pathway, whereas poor N sources such as L-proline, γ -amino butyric acid (GABA), urea, purines, and polyamines activate it.

The secretion of secondary metabolites by fungal cultures is very common, especially by the *Ophiostomatoide*s. Hansen and Abraham (1988) found that formation of 6-protoilludene, a volatile metabolite from cultures of *O. piceae* thought to be an intermediate in the formation of various sesquiterpenes, depends on the chemical nature of the N source. After testing several nitrates (ammonium nitrate and calcium nitrate) and amino acids (asparagine, glutamine, alanine, leucine, isoleucine and phenylalanine), Hansen et al. (1986) found that higher yields of 6-protoilludene were only obtained in the presence of phenylalanine and isoleucine (fusel alcohol precursors). This is another specific example of the effect that the N source can have on the formation of secondary metabolites in cultures *Ophiostoma*.

Figure 4. GC-MS analysis for the albino strain F1F1A55-*O. floccosum* spent medium. **a)** Spent medium filtered and analyzed by headspace technique. **b)** Spent medium extract analyzed by direct injection into the GC/MS. In both cases MS fragmentation patterns of peak 3 were included. The principal identified compounds (in parentheses the retention time in min) were: **1)** α -Gurjunene (16.64), **2)** 1,2,4,8-tetramethylbicyclic[6.3.0]undeca-2,4-diene (16.88), **3)** 1,1,4a-trimethyl-5,6-dimethylene-decalin (16.97)

Figure 4. Continued

Hornby et al. (2004) suggested that the biological importance of some of the volatile terpenoid compounds, including cyclic sesquiterpenes, was to mediate the control of inoculum size-dependent

dimorphism in *O. ulmi*. Therefore, due to the presence of cyclic sesquiterpenes in the spent medium from the albino strain F1F1A55-*O. floccosum*, where the culture grew exclusively in the form of yeast, we can suggest

that one or more of these compounds were the QSMs responsible for inhibiting the mycelia growth in this species. Nevertheless, further research is necessary in order to prove this hypothesis.

In nature, more than 23,000 isoprenoids have been characterized having very diverse structures and functions (Sacchetti and Poulter 1997). One of the most studied isoprenoid is farnesol, a non-cyclic sesquiterpene that has been identified as the QSM for *Can. albicans* (Hornby et al. 2001).

Some VOCs, such as naphthalene and its derivatives, have very rarely been described as a product from biological sources; in fungi the first case was the endophytic fungus *Muscodor vitigenus* (Daisy et al. 2002). In other organisms, only recently has naphthalene been found as a constituent of natural biological systems, having been reported from *Magnolia* flowers and in the nest material of Formosan subterranean termites (Azuma et al. 1996; Chen et al. 1998). The presence of the chemical in both of these places may function as protection from insects. Ezra et al. (2004) found that *Muscodor albus* make other VOCs such as naphthalene and an alcohol, an acid, and/or other naphthalene/azulene derivatives that possess antibiotic activity both in vivo and in artificial mixture.

The production of 1,1,4a-trimethyl-5,6-dimethylenedecalin (a naphthalene-related compound), by an albino strain of *O. floccosum* with yeast-like morphology is intriguing for three reasons: (1) naphthalene and its derivatives have rarely been described in fungi, (2) the presence of naphthalene smell in cultures of this fungus with almost 100% yeast like morphology, and (3) the absence of this smell in cultures with mycelial-morphology growth. These arguments lead to the idea that this compound would have quorum sensitivity activity in an albino strain of *O. floccosum*.

The physiological and ecological functions of fungal VOCs have recently been reviewed (Morath et al. 2012; Kramer and Abraham 2012). Clearly the volatiles should contribute to an organism's fitness to survive but in general their biological significance is largely unknown. In some cases the VOCs acted as autoinducers and one or more of the cyclic sesquiterpenes we have found in *O. floccosum* (Figure 4) acts as a QSM to promote yeast growth or to block mycelial growth. Interactions with bacteria, other fungi, insects, and plants have also been proposed. Among these are

to attract insect pollinators and nematodes, presumably in order to spread the fungus, participate in bio-control (*Trichoderma*) and sick building syndrome, and repel or kill potential competing fungi (Nickserson et al. 2006). Interestingly, very similar considerations and ideas were presented by Waksman (1941) in his classic review on antagonistic relations among microorganisms. The task to elucidate ecological functions of secondary metabolites in nature is not trivial (Kramer and Abraham 2012).

Conclusions

A high inoculum size of the albino strain of *O. floccosum* (FIF1A55) was associated to the secretion of at least one QSM. This compound could inhibit mycelial growth and promote yeast-like growth. Analysis by GC-MS revealed that three signaling molecules (possible QSMs), soluble in non-polar organic solvents, were cyclic sesquiterpenes. Due to the abundance of 1,1,4a-trimethyl-5,6-dimethylenedecalin in the spent medium, this compound could be the principal QSM candidate responsible for signaling the yeast-like morphology in the albino strain FIF1A55-*O. floccosum*. These candidate compounds are not commercially available and for that reason it would be necessary to isolate and test them as QSM, to confirm or reject the hypothesis.

Acknowledgments — The principal author would like to thank the personnel of the Laboratory of Biodegradation in the Department of Wood Engineering at the University of Bío-Bío (Chile) for all support. To the Graduate School and Department of Wood Engineering at the University of Bío-Bío, the OEA Scholarship Program, and the Scholarship Program of the Technology Institute of Costa Rica for providing the funds to carry out this research. We also thank Ph.D. Juanita Freer and Susana Casas from the Laboratory of Renewable Resources at the University of Concepción for providing the facilities for GC-MS analyses.

References

- Abbassy MA, Marei GI (2013) Antifungal and chemical composition of essential oils of *Juniperus communis* L. and *Thymus vulgaris* L. against two phytopathogenic fungi. *J Appl Sci Res* 9:4584–4588

- Azuma H, Toyota M, Asakawa Y, Kawano S (1996) Naphthalene—Constituent of magnolia flowers. *Phytochemistry* 42:999–1004
- Berrocal A, Navarrete J, Oviedo C (2012a) Efecto de diferentes condiciones de crecimiento en el dimorfismo levadura-micelio de la cepa albina PcF2A29 de la especie *Ophiostoma piceae*. *Maderas-Cienc Tecnol* 14:91–102
- Berrocal A, Navarrete J, Oviedo C, Nickerson KW (2012b) Quorum sensing activity in *Ophiostoma ulmi*: Effects of fusel oils and branched chain amino acids on yeast-mycelial dimorphism. *J Appl Microbiol* 113:126–134
- Bisht A, Bisht GRS, Singh M, Gupta R, Singh V (2011) Chemical composition and antimicrobial activity of essential oil of tubers of *Cyperus rotundus* Linn. collected from Dehradun (Uttarakhand). *J Pharm Biomed Sci* 2:661–665
- Chang-Bin W, Sheng-Hui L, Yu-Ge L, Ling-Ling L, Wen-Xiu Y, Guang-Ming S (2011) Characteristic aroma compounds from different pineapple parts. *Molecules* 16:5104–5112
- Chen H, Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohols. *Gene Dev* 20:1150–1161
- Chen J, Henderson G, Grimm CC, Lloyd SW, Laine RA (1998) Termites fumigate their nests with naphthalene. *Nature* 392:558–559
- Chen H, Fujita M, Feng Q, Clardy J, Fink GR (2004) Tyrosol is a quorum sensing molecule in *Candida albicans*. *Proc Natl Acad Sci USA* 101:5048–5052
- Daisy BH, Strobel GA, Castillo U, Ezra D, Sears J, Weaver DK, Runyon JB (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology* 148:3737–3741
- Ezra D, Hess WM, Strobel GA (2004) New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. *Microbiology* 150:4023–4031
- Ghosh S, Kebaara BW, Atkin AL, Nickerson KW (2008) Regulation of aromatic alcohol production in *Candida albicans*. *Appl Environ Microb* 74:7211–7218
- Hanssen HP (1993) Volatile metabolites produced by species of *Ophiostoma* and *Ceratocystis*. In: Wingfield MJ, Seifert KA, Webster JF (eds.) *Ceratocystis and ophiostoma: Taxonomy ecology and pathogenicity*. APS Press, St. Paul, pp 117–125
- Hanssen HP, Abraham WR (1988) Sesquiterpene alcohols with novel skeletons from the fungus *Ceratocystis piceae* (Ascomycotina). *Tetrahedron* 44:2175–2180
- Hanssen HP, Sprecher E, Abraham WR (1986) 6-protoilludene, the major volatile metabolite from *Ceratocystis piceae* liquid cultures. *Phytochemistry* 25:1979–1980
- Hernández V, Avramidis S, Navarrete J (2012) Albino strains of *Ophiostoma* spp. fungi effect on radiata pine permeability. *Eur J Wood Prod* 70:551–556
- Hogan DA (2006) Talking to themselves: Autoregulation and quorum sensing in fungi. *Eukaryot Cell* 5:613–619
- Hornby JM, Jensen E, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl Environ Microb* 67:2982–2992
- Hornby JM, Jacobitz-Kizzier SM, McNeel DJ, Jensen EC, Treves DS, Nickerson KW (2004) Inoculum size effect in dimorphic fungi: Extracellular control of yeast-mycelium dimorphism in *Ceratocystis ulmi*. *Appl Environ Microb* 70:1356–1359
- Karamian R, Asadbeg M, Pakzad R (2013) Essential oil compositions and in vitro antioxidant and antibacterial activities of the methanol extracts of two *Salvia* species (*Lamiaceae*) from Iran. *Intl J Agri Crop Sci* 5:1171–1182
- Kramer R, Abraham WR (2012) Volatile sesquiterpenes from fungi: What are they good for? *Phytochem Rev* 11:15–37
- Kulkarni RK, Nickerson KW (1981) Nutritional control of dimorphism in *Ceratocystis ulmi*. *Exp Mycol* 5:148–154
- March JC, Bentley WE (2004) Quorum sensing and bacterial cross-talk in biotechnology. *Curr Opin Biotechnol* 15:495–502
- Morath SU, Hung R, Bennett JW (2012) Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. *Fungal Biol Rev* 26:73–83
- Muthukumar G, Nickerson KW (1984) Ca(II)-Calmodulin regulation of fungal dimorphism in *Ceratocystis ulmi*. *J Bacteriol* 159:390–392
- Nickerson KW, Atkin AL, Hornby JM (2006) Quorum sensing in dimorphic fungi: farnesol and beyond. *Appl Environ Microb* 72:3805–3813
- Pednekar PP, Vakil BV, Sane RT, Datar AG (2013) Phytochemical profile and antioxidant activity of the essential oil from *Blumea eriantha* DC. *Int J Pharm Sci* 5:404–413
- Sacchettini JC, Poulter CD (1997) Creating isoprenoid diversity. *Science* 277:1788–1789
- Sarsenbayev KN, Sarsenbayeva AB, Balmukanov KU (2013) chemical composition of low-molecular weight organic compounds (LMWOC) of water extracts from *Cistanche Deserticola* stolones depending on treatment. *World Appl Sci J* 25:28–35
- Shafaghat A (2009) Composition and antibacterial activity of the volatile oils from different parts of *Achillea tenuifolia* Lam. from Iran. *J Med Plants Res* 8:93–98
- Sprague GF Jr, Winans SC (2006) Eukaryotes learn how to count: Quorum sensing by yeast. *Gene Dev* 20:1045–1049
- Waksman SA (1941) Antagonistic relations of microorganisms. *Bacteriol Rev* 5:231–291
- Zeringue HJ Jr, Bhatnagar D, Cleveland TE (1993) C₁₅H₂₄ volatile compounds unique to aflatoxigenic strains of *Aspergillus flavus*. *Appl Environ Microb* 59:2264–2270