DNA distribution pattern and metabolite profile of wild edible lobster mushroom (*Hypomyces lactifluorum*/Russula brevipes)

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DNA distribution pattern and metabolite profile of wild edible lobster mushroom**(*Hypomyces lactifluorum*/*Russula brevipes*)****Genevieve Laperriere^{1,2}, Isabel Desgagné-Penix^{1,2}, and Hugo Germain^{1,2}**

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Abstract

Lobster mushroom is a wild edible mushroom with potential commercial value. It is the product resulting of the infection, most commonly of *Russula brevipes* by *Hypomyces lactifluorum*. This study undertook quantitative polymerase chain reaction analysis of tissues sampled at different infection stages, to investigate *R. brevipes*/*H. lactifluorum* interaction. We followed the colonization of *R. brevipes* sporocarps by *H. lactifluorum* which leads to the edible lobster mushrooms. In parallel, metabolomics analysis was performed to detect differences in metabolite profile among non-infected *R. brevipes* sporocarp and lobster mushroom. The results show that *H. lactifluorum*'s DNA is not restricted to the margin, but is distributed relatively evenly across the sporocarp of the lobster mushroom. DNA of *R. brevipes* was also present throughout the sporocarp, but was less abundant at the margins and increased inwards. *R. brevipes*' DNA also declined as the infection progressed. Metabolomics analysis revealed that the flesh of lobster mushroom, which remains identical in appearance to the flesh of the host, undergoes transformation that alters its metabolite profile, most notably of lipids and terpene compounds. These results define a parasitic relationship between the two species which entails a decline of *R. brevipes*' DNA and a modification of its metabolite profile.

Key words: DNA, lobster mushroom, *Hypomyces lactifluorum*, *Russula brevipes*, parasitism

Introduction

Lobster mushroom is not a species, but the result from an infection of sporocarps of *Russula* spp. [most commonly *Russula brevipes* (Peck)] by *Hypomyces lactifluorum* (Schwein. Fr.) Tul. & C. Tul., an ascomycete from the family *Hypocreaceae* in the order *Hypocreales* (Rogerson and Samuels 1994). This interaction is qualified as parasitism where *Russula* spp is the host and *H. lactifluorum* is the parasite (Beug et al. 2014; Hanlin 1963; Rogerson and Samuels 1994). Its distribution is confined to North America (Rogerson and Samuels 1994). This limited distribution could be explained by the similarly constrained spread of *R. brevipes* and ecological parameters, such as pH, extractable soil phosphorus and stand opening, identified as important predictors of lobster mushroom's presence (Rochon et al. 2009).

There is sparse information on the infection process, life cycle and the colonisation of the host species during the infection such as how each of the two species is distributed throughout the sporocarp during the infection process. Hanlin (1963) suggested that *H. lactifluorum* infects very young *Russula* sporocarp, starting with infection of the gill surfaces, followed by rapid spread of the mycelia over the entire sporocarp. The growth of the host sporocarp continues, but gradually transforms as a result of the influence of the parasite. Rogerson and Samuels (1994) reported that *H. lactifluorum* appeared in consecutive years in the same populations of *Lactarius* or *Russula* species, indicating that infection may occur through *H. lactifluorum*-containing soil or that *H. lactifluorum* somehow grows in parallel with the host mycelium

There are other similar situations in the genus *Hypomyces* where the host sporocarp appearance is changed following *Hypomyces* colonization (Beug et al. 2014;

McNeil 2006; Rogerson and Samuels 1994). *Hypomyces chrysospermus* (Tulasne) can colonize various boletes and covers the host sporocarp with a white layer followed by powdery yellow layer. Similarly, *Amanita* species sporocarp colonized by *Hypomyces hyalinus* (Schweinitz) Tulasne and C. Tulasne develop a fluffy crust with rough surface of various colors. *Hypomyces luteovirens* on *Russula* spp. and *Hypomyces lateritus* on *Lactaria* spp. are other similar cases. Among all these examples, none are considered edible or their edibility is unknown and gives to lobster mushroom a unique character.

The lobster mushroom resulting from the infection is edible and is characterized by an orange to reddish color of the cap surface while the underlying flesh remains white. Its main morphological characteristics are the presence of shallow ridges at the underside of the cap (remnants of the gills of *Russula*), as well as irregularity of the sporocarp and stalk (Hanlin 1963). *R. brevipes*, the most commonly reported host, has a bitter taste, but when associated with *H. lactifluorum*, to become lobster mushroom, is sought and appreciated by mushroom pickers for its meaty texture, distinctive flavor and good taste (McNeil 2006).

Since knowledge about this mushroom is limited, more information about its formation and structure is necessary to strengthen its utilization and commercialization. Our objectives were to better understand the composition of the lobster mushroom sporocarp during the colonisation of *R. brevipes* by *H. lactifluorum*. We also aimed to assess the metabolite composition profiles to try to understand the flavour changes between non-infected *R. brevipes* (white, bitter taste, non-edible) and lobster mushroom (orange, good taste, edible). To achieve the objectives, we used quantitative real-time polymerase chain reaction (qPCR) technology, to follow the presence of the two species

inside the sporocarp of the lobster mushroom at different infection stages (partially colonized, completely colonized and decaying sporocarp). Finally, in order to evaluate if there were any metabolite changes between the *R. brevipes* and lobster mushroom (infected *R. brevipes*), we used HPLC and UPLC-qTOF-MS to assess the metabolite content.

Materials and Methods

Mushroom collection and tissue sampling

Non-infected *R. brevipes* sporocarps were collected in Parc regional des Grèves (Quebec, CAN) and in Gentilly (Quebec, CAN). Species were identified and confirmed by morphological features as well as Sanger sequencing of the entire ITS region (PCR conditions described hereafter). Infected sporocarps of three infected stages were collected at the Zec Bostonnais (Quebec, CAN) and near the Lake Sabourin (Quebec, CAN). The different infection stages included: i) partially infected sporocarps of *R. brevipes* still having visible gills and white outer flesh, but showing visible reddening of the cap, ii) completely infected sporocarps of *R. brevipes* also called lobster mushroom (sporocarp entirely red, with no visible gills, exhibiting a deformed cap and displayed characteristic orange color and firmer flesh than the *Russula*) and iii) decaying sporocarps of lobster mushrooms characterized by the same characteristics as the completely infected with softened red flesh, indicating that the mushroom has started to decay. Completely infected sporocarps of *R. brevipes* were cut into transversal cross-sections and pieces of an average of 0.04 g were collected, with a new scalpel blade, from the internal portion of the sporocarp. Care was taken not to contaminate each section with tissue/DNA from neighboring sections. Seven pieces from each sporocarp were collected

and these pieces were numbered from the outermost part of the cap inward to the stalk (0 to 6). For the analyses of the proportion of *R. brevipes* and *H. lactifluorum* DNA in different infection stages of the lobster mushrooms sporocarp (non-infected *R. brevipes*, partially, completely and decaying lobster mushroom), tissues were collected at the cap's edge (equivalent of position 0 from Fig. 2), in the middle (equivalent of position 2 from Fig. 2) and in the stalk (equivalent of position 4 or 5 from Fig. 2).

DNA extraction

Fresh fungal tissue was placed in 200µl AP1 buffer and pulverized by a hand drill mounted with a blue pestle, until a homogenous solution was obtained. Fungal DNA was extracted with DNeasy plant kit (Qiagen, Montreal, CAN), according to the manufacturer's instructions. DNA samples were kept frozen at -20°C until analysis. *R. brevipes* DNA was obtained from sporocarps of non-infected *R. brevipes*. For *H. lactifluorum*, DNA was extracted from completely infected sporocarps of *R. brevipes* and isolated by cloning.

PCR amplification and sequencing

We amplified the whole ITS region for *R. brevipes* and *H. lactifluorum* for alignment and specific primers generation (to be used for qPCR analysis). Universal fungal primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (Gardes and Bruns 1993; White et al. 1990) we used. Sequences were compared with those available from the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). PCR was performed with Phusion[®] High-Fidelity DNA Polymerase (New England Biolabs, Whitby, CAN),

according to the manufacturer's instructions under the following conditions: 34 cycles of 10s at 98°C, 30s at 58°C and 30s at 72°C. Reactions were preceded by a 30s denaturation step at 98°C and terminated with a 5 minutes elongation step at 72°C. PCR products were separated by electrophoresis in 1% agarose gel with 1× TAE (Tris-Acetate-EDTA), stained with SYBR[®] Safe DNA Gel (Thermo Fisher Scientific, Ottawa, CAN) and visualized under UV light (ChemiDoc Touch Imaging System, Bio-Rad, Saint-Laurent, CAN). PCR products were purified by QIAquick PCR Purification Kit (Qiagen) and sent to the Québec Genome Innovation Centre of McGill University (Montreal, Quebec, CAN) for Sanger sequencing. Species identities were confirmed using the BLASTn algorithm from the NCBI nucleotide database and BOLDSYSTEMS (<http://www.boldsystems.org>).

Cloning of *H. lactifluorum* ITS

ITS PCR products were cloned with pBluescript II KS (+) vector. The vector was digested with the restriction enzyme *EcoRV* (New England Biolabs), and the amplified fragment was inserted by blunt-end ligation with T4 DNA ligase (New England Biolabs), according to the manufacturer's instructions. Ligation reactions were transformed in electro-competent *Escherichia coli* DH10B cells, and positive colonies containing recombinant plasmids were identified by blue/white selection on LB agar plates containing ampicillin 50 µg/ml, X-gal 40 µg/ml and IPTG 0.2 mM. The plasmids were purified with QIAprep Spin Miniprep kit (Qiagen), and amplified fragment integration was confirmed by double digestion with the restriction enzymes *EcoRI* and *XhoI* (New England Biolabs).

Design and specificity testing of primers

H. lactifluorum and *R. brevipes* sequences, obtained by sequencing the ITS region of genomic DNA, and sequences available on NCBI, were aligned with CLC MainWorkbench software 7.6.4 (Qiagen) to identify variable regions for primer design. For qPCR specific primers targeting smaller amplicons were designed for *H. lactifluorum* (H.L-1, ATTACCGAGTCTCCGAACTCCCAA and H.L-2, GATTCGTTTCGCTTGCCCTTTTCG) and for *R. brevipes* (R.B-1, CATCCTCAACCTGCTTCGTTCTATCG and R.B-2, CGAGCGAAACCCAAGACGTAGAAAC-) with a combination of softwares, including Primer3 v. 4.0.0 (<http://primer3.ut.ee/>) and CLC MainWorkbench v. 7.6.4 (Qiagen). The BLASTn algorithm available on NCBI was used to assess the *in silico* specificity of these primers. PCR amplification with novel specific primers was carried out with Hot Start Taq DNA Polymerase (New England Biolabs), according to the manufacturer's standard instructions under the following conditions: 34 cycles of 20s at 95°C, 30s at 56°C and 35s at 68°C. Reactions were preceded by a 30s denaturation step at 95°C and terminated by a 5 minutes elongation step at 68°C. PCR products were visualized as described previously.

qPCR

qPCR assays were performed in 96-well hard-shell PCR plates with the CFX Connect Real-Time system (Bio-Rad) according to the manufacturer's instructions. Each 20µl reaction mixture consisted of 10µl of 2X SsoAdvanced universal SYBR[®] Green Supermix (Bio-Rad), 0.5 µM of each primer and 8 µl of target DNA diluted 1/100. Each

tissue sample underwent two technical replications with three biological replicates for each different infected stage. A negative control sample was included in each assay. The reference gene was the 5.8S ribosomal RNA encoding gene amplified with primers 5.8S-F, CTTTCAACAACGGATCTCTTGGYTC and 5.8S-R, GCAAKGTGCGTTCAAAGATTCG, which were designed to amplify both species. Prior to the experiments, thermal gradient and dilution curves (1, 1/10, 1/100, 1/1,000 and 1/10,000) were generated for each primer pair with pooled DNA samples to determine optimal annealing temperature and reaction efficiency. qPCR products from a few samples for each primer pair were visualized on agarose gel to confirm amplicon size and the absence of non-specific amplification and primer dimers. Amplicon purity was confirmed by melting curve analysis. The optimized thermal cycle protocol included 3 minutes incubation at 98°C followed by 39 cycles of 15s at 98°C, 40s at 60°C or 56.2°C for *H. lactifluorum* and *R. brevipes* respectively and 40s at 72°C followed by melting curves from 65°C to 95°C with 0.5°C increments. Fluorescence threshold level was determined with the default adaptive baseline algorithm of CFX Manager Software 3.1.1517.0823. Relative quantitation assessed the relative amount of *H. lactifluorum* DNA versus *R. brevipes*' DNA at each position in the sporocarp. Normalized expression was calculated by the Livak method ($2^{-\Delta\Delta CT}$) (Livak and Schmittgen 2001). In all cases, the lowest normalized expression served as calibrator, and error propagation was used to calculate standard deviation (Hellemans et al. 2007).

Metabolites analysis

Lobster mushrooms (completely infected) and non-infected *R. brevipes* sporocarp were used for HPLC-DAD (high performance liquid chromatography-diode array

detector) analysis. Each mushroom sample (1 g) was ground in 10 ml of 80% methanol and agitated for 24 h at room temperature. Extracts were filtered through Whatman paper No. 4. The filtered methanol/water solution was extracted twice with 10 ml of 100% hexane. The aqueous phase was collected and evaporated at room temperature under a chemical hood. After evaporation, metabolites were re-suspended in 0.1 ml of 20% methanol. The extracts were analysed with a Prominence-I liquid chromatograph HPLC (Shimadzu, Maryland, USA). Separation was achieved in an analytical reverse phase C₁₈ column (Kinetex 5µm x 150mm x 4.6mm, Phenomenex, California, USA) at 30°C. The mobile phases were constituted of (A) 2.5% acetic acid in water and (B) 100% acetonitrile. 20µl of extract was injected and separated by the gradient method: 100% A for 5 min, 98% A and 2% B for 2 min, 80% A and 20% B for 2 min, 60% A and 40% B for 15 min, 40% A and 60% B for 10 min, 20% A and 80% B for 10 min, 2% A and 98% B for 6 min and, finally, 98% A and 2% B for 4 min at a flow rate of 0.5 ml/min. DAD in full scan mode detected absorption at wavelengths between 190 to 800 nm. To better understand the metabolites composition and their identity, one sample of completely infected and non-infected *R. brevipes* were analyzed using UPLC-MS (Ultra Performance Liquid Chromatography-Mass spectrometry). The UPLC-MS analysis was performed using a Waters Acquity Ultra-Performance LC system (Waters), equipped with a binary pump system (Waters). An Acquity Ethylene Bridged Hybrid (BEH) C₁₈ column (100mm x 2.1mm id x, 1.7 mm particle size) from Waters was used. The molecules were separated with a mobile phase that consisted of 0.2% acetic acid (eluent A) and acetonitrile (eluent B), the flow-rate was 0.2 mL/min. The initial elution gradient was 2% B ; 0-1 min, 2–100% B; 1-30 min, isocratic 100% B; 30-33 min, 100-2% B ;33-33.5

min, isocratic 2% B; 33-40 min. The MS analyses were carried out on a QTOF Micro mass spectrometer (Waters) equipped with a Z-spray electrospray interface. The analysis was performed in both positive and negative mode and the data were acquired through a masses scan from 100 to 1250 m/z without collision. The ionization source parameters were; source temperature 120°C; cone gas flow rate 50 L/h and desolvation gas flow rate 350 L/h; desolvation temperature, 200°C. Nitrogen (99% purity) was used as nebulizing gas. Data acquisition was carried out with the MassLynx 4.1 software. Masses extraction, deconvolution, isotopes and library search was performed using MZMine 2 (Pluskal et al. 2010).

Results

***H. lactifluorum* and *R. brevipes* primers produce specific amplicons**

Both primer pairs amplified genomic DNA extracted from a lobster mushroom (completely infected sporocarps of *R. brevipes*), which should contain both DNAs (Fig. 1, lane 2 and 6). PCR products generated with specific primers for *H. lactifluorum* (H.L-1 and H.L-2) and *R. brevipes* (R.B-1 and R.B-2) were detected at expected sizes, 207 bp for *H. lactifluorum* (Fig. 1, lane 1 and 2) and 164 bp for *R. brevipes* (Fig. 1, lane 6 and 7). No amplifications were detected with *H. lactifluorum* primers for *R. brevipes* genomic DNA (Fig. 1, lane 3) and with *R. brevipes* primers combined with plasmid containing the *H. lactifluorum* ITS fragment (Fig. 1, lane 5). We conclude that the primers designed yielded specific amplicons suitable for qPCR experimentation.

Colonization of *R. brevipes* by *H. lactifluorum* in lobster mushroom sporocarp

To assess the composition of the lobster mushroom sporocarp and to investigate the colonization of *R. brevipes* by *H. lactifluorum*, we conducted qPCR amplification.

Melt curve was done for each reaction and in all cases only one dissociation curve was present. Samples were placed on a gel to visualize size and specificity of amplicons (Fig. S1). DNA from *H. lactifluorum* was detected throughout the sporocarp (Fig. 2b). Same results were obtained with other sporocarp (Figs. S2a and S2c). Interestingly, DNA from *R. brevipes* was less abundant at the cap's edge (position 0 and 6) than towards the interior of the sporocarp (position 3-5) (Fig. 2c). Its levels varied between the sporocarp tested, but it was always less abundant at the margin (Figs. S2b and S2d).

Proportion of *H. lactifluorum* and *R. brevipes* in different infection stages of the lobster mushroom sporocarp

A melt curve was performed and samples were placed on a gel as previously described. With *R. brevipes* primers no non-specific bands were detected (Fig. S3a). However, when using *H. lactifluorum* primers minor non-specific bands were detected in some of the stages, but *H. lactifluorum* specific band was always present (Fig. S3b), however these likely appeared very late in the PCR since they do not appear to affect quantitation; as seen in figure 2b, *H. lactifluorum* is undetected in *R. brevipes* compared to infected mushroom. *H. lactifluorum* DNA was found to be increasingly present as the infection progressed (Fig. 3b). *R. brevipes*' DNA was also present in all stages, but was only detected at a very low level in decaying lobster mushroom sporocarp (Fig. 3c). Taken together, the data suggest that, as infection progresses, the *R. brevipes*' DNA declines in the sporocarp concurrently with the increasing *H. lactifluorum* DNA amount. Same results were obtained with other biological replicates with both primer pairs (Fig. S4).

Metabolites analysis of *R. brevipes* and lobster mushroom sporocarps

Although orange coloration of the lobster mushroom sporocarp (exterior) is much different from the white exterior of sporocarps of *R. brevipes*, the color and appearance of their interior flesh is identical. Various metabolites may be responsible for the differences in phenotype, taste and edibility of the two mushrooms. The HPLC results were identical respectively for all sporocarps and only one representative chromatogram is shown. A striking difference between chromatograms at 254 nm was seen between 32 and 38 min for extracts of *R. brevipes* (Fig. 4a) versus those of lobster mushroom (Fig. 4b). 3-D absorption plots, which allowed easier visualization of peaks throughout the light spectrum, showed similar striking differences including the presence of a peak between 30-35 minutes at 450nm in lobster mushroom (Figs. 4c and 4d). These results clearly demonstrate that *H. lactifluorum* alters the metabolite profile (composition and level) of *R. brevipes* flesh and contains metabolites absent from *R. brevipes*. UPLC-qTOF-MS detected a total of 183 masses in positive and negative mode, 38 of which were specific to the lobster mushroom and 78 were specific to *R. brevipes* (Table S1). In order to simplify the output of the mass spectrometry data, we grouped the metabolites into large families: lipids, alkaloids, terpenoids, phenolics, amino acids and others. The most striking difference between the general composition is the large diversity of terpenoids present in uninfected sporocarps of *R. brevipes* which are completely absent from the infected *R. brevipes* sporocarps (Fig. 5a and c). Conversely, the lobster mushroom contained a much larger diversity of lipid compounds (Fig. 5b) and amount of lipids (Fig. 5d) when compared with *R. brevipes* (Fig. 5c). Table S1 lists the detail of all the metabolites found in lobster mushroom and *R. brevipes*.

Discussion

The complex biology of lobster mushroom is far from being fully understood and the *R. brevipes* and *H. lactifluorum* species distribution throughout the sporocarp and during the course of the infection process has not been examined previously. Results of our qPCR analysis show that *H. lactifluorum*'s and *R. brevipes*' DNAs are both present in every infection stage analyzed and are distributed throughout the sporocarp. However, as the mushroom ages, the proportion of *R. brevipes*' DNA relative to *H. lactifluorum* DNA decreases sharply, suggesting that *R. brevipes* is being degraded. We also show that the metabolite profile of *R. brevipes* is modified by the presence of *H. lactifluorum*.

In this study, we designed specific primer pairs and confirmed their specificity for amplifying *H. lactifluorum* and *R. brevipes* ITS regions (Fig. 1). Our results showed that *H. lactifluorum*'s DNA is distributed throughout the sporocarp and is not limited to the margin, as hinted by the orange color present only at the sporocarp surface, in fact the parasite penetrates the sporocarp (Fig. 2b). As for *R. brevipes*, its DNA is also present throughout the whole sporocarp. It is, however, less abundant at the edge than in the middle of the cap (Fig. 2c). Hanlin (1963) observed infection of the gills on very young carpophore and he suggested that the *H. lactifluorum* mycelium, after infecting the gills, spreads to the entire cap surface. The surface of the *R. brevipes* sporocarp gets completely covered by a thick orange layer, as a result of skyrin production originating from the parasite (Helfer 1991; Poldmaa 2011). However, neither our results nor Hanlin can demonstrate whether the infection process is initiated from the outside of the sporocarp or if the *H. lactifluorum* mycelium is also present inside when the red color appears at the surface.

R. brevipes' DNA was present in all infected stages, but only at a very low level in completely infected sporocarp and decaying lobster mushroom (Fig. 3c). Moreover its presence in completely infected sporocarp reached its maximum level in the stipe although we see from figure 3 that the amount of *R. brevipes* DNA is very low at this stage, relative to that of *H. lactifluorum* DNA (Fig.3c). *R. brevipes*' DNA appeared to be degrading during the evolution of the infection and it was not the case for *H. lactifluorum*'s DNA, which was still abundant (Fig. 3b). Our findings support parasitism between the two species, as proposed by other authors (Beug et al. 2014; Hanlin 1963; Rogerson and Samuels 1994). A parasitic system is an ecological relationship where the parasite benefits from its host either by feeding, hiding or breeding at its expense (Zelmer 1998). Parasitic fungi utilize nutrients from the host and have an entirely win-lose relationship (Petersen 2012). The decline of *R. brevipes*' DNA could be explained by the fact that traffic of the major nutriment is essentially one way from host to parasite (Lewis 1973). *H. lactifluorum* could steal available nutrients from *R. brevipes* and then expand through the sporocarp. Lacking proper nutrition, *R. brevipes* would deteriorate and die. We cannot rule out the possibility that *H. lactifluorum* could secrete a toxin or an enzyme to kill or digest *R. brevipes* to sustain its own growth.

Difference in metabolite contents between *R. brevipes* and lobster mushroom

We wanted to investigate a potential explanation for the gustative difference between *R. brevipes*, which does not have a good taste, and lobster mushroom, which presents a very distinct flavor really appreciated by consumers.

Major change between non-infected *R. brevipes* sporocarp and lobster mushrooms is the difference of diversity and amount of fatty acids compounds (Fig.5). Fatty acids

were detected in much higher proportion in lobster mushrooms than non-infected *R. brevipes* (Fig. 5). Analyses of fatty acid composition revealed that several edible mushrooms showed a wide diversity of fatty acid (Pedneault et al. 2006; Ribeiro et al. 2009). Some of these species such as *Russula cyanoxantha* (Shaeffer) Fries, *Suillus granulatus* (Fries) Kuntze and *Amanita caesarea* Pomerleau are appreciated for their good taste (McNeil 2006). In most of the cases mushrooms shown a higher amount of unsaturated fatty acid versus saturated fatty acids (Dimitrijevic et al. 2017; León-Guzmán et al. 1997; Pedneault et al. 2006; Ribeiro et al. 2009). It was also the case of lobster mushroom (Table S1). However, unsaturated fatty acid could be precursors of flavor compounds and it was reported in tomato plant that a change in the level of fatty acid could change its flavor profile and consequently its taste (Wang et al. 1996).

Free amino acid was another compound more abundant in the lobster mushrooms than non-infected *R. brevipes* and some compounds as monosodium glutamate-like components (MSG-like) and 5' nucleotides were only detected in lobster mushroom (Fig. 5) (Table S1). Aspartic and glutamic acids were described as monosodium glutamate-like components (MSG-like) (Yamaguchi 1979). These components are found to be taste-active and give flavor to mushrooms (Chen 1986). It also associated to the umami and palatable taste (Yamaguchi 1979). MSG-like are found in high proportion in some wild edible mushroom species as for example *Amanita rubescens*, *Pleurotus ostreatus* and *Lentinula edodes* (León-Guzmán et al. 1997; Yang et al. 2001). MSG-like and 5' nucleotides have a synergistic effect on flavour and increase the umami taste (Yamaguchi et al. 1971). Aspartic acid (M+H 146,0574) and pseudouridine (M+H 243,0846) detected in lobster mushrooms could play a role in it tasty flavor (Table S1).

In addition, *R. brevipes* showed a higher abundance of terpene and phenolic compounds (Fig. 5a) while terpenes were not detected in the lobster mushroom (Fig. 5b). These secondary metabolites are more specific to plant, bacteria and fungi and are produced, in part, for protection and defense (Hopkins 2003). *R. brevipes* could produce a high diversity of these metabolites as a protectant against insects or fungivores. A group of sesquiterpenes were isolated in mushrooms of the *Russulaceae* family and some of these molecules had a pungent taste for human (Magnusson et al. 1973; Magnusson et al. 1972). A bioassay using a natural fungivore, the opossum *Didelphis virginiana*, identified five species of mushroom members of the *Russulaceae* family, previously reported as having a taste pungent to human, which were not eaten and rejected by the opossum (Camazine et al. 1983). These mushrooms may contain similar sesquiterpenoids as those identified in *R. brevipes* (Mizuno 1995). Indeed, several sesquiterpenoid compounds were specifically detected in *R. brevipes* including lactarorufin (M+H 265.1672) and turmerone (M+H 265.1672) which may contribute to unliked aroma and taste (Table S1). Terpenoid compounds were not detected in the lobster mushroom (Fig. 5b and d). Given that several terpenoid compounds are known to have a pungent and bitter taste, it is tempting to speculate that the absence to terpenoids in the lobster mushroom is a factor which makes it more palatable than *R. brevipes* (Figs. 5a and b).

A striking difference between figures 5a and 5b is the ‘disappearance’ of the terpenoid compounds, in particular sesquiterpene (Table S1) that seem to be converted into lipids when the *R. brevipes* is being infected. In fact, it is highly unlikely that sesquiterpenes or any terpenoids would be converted to fatty acids. To our knowledge no known enzyme can catalyze the direct conversion of sesquiterpenes or terpenoids into

fatty acids. However, *H. lactifluorum* may have the capacity to catabolize terpenoids into simpler carbene-based molecules from which they could build lipids. A common element in the terpene and fatty acid biosynthesis pathway is acetyl coenzyme A (acetyl-CoA) (Hopkins 2003). When *R. brevipes* is uninfected acetyl-CoA would be used largely for the production of terpenes and more precisely sesquiterpenes (Table S1). When *H. lactifluorum* infects sporocarp of *R. brevipes*, the same enzyme could be used to the benefit of the fatty acid metabolic pathway.

Conclusions

To our knowledge, this is the first study using PCR technologies to analyse *H. lactifluorum*'s and *R. brevipes*' relationship and that investigated the causes of the taste differences between the two mushrooms. The main findings described is that at the stage that lobster mushroom is eaten it contains only traces amount of *R. brevipes* in its sporocarp. However, our metabolite analyses demonstrated that the metabolite profile specific to *R. brevipes* has been completely altered and that several molecules of the terpenoid family, that may be responsible for the bad taste of *R. brevipes*, have disappeared and that fatty acids are very abundant in the lobster mushroom.

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Figure Legends

Fig. 1 PCR amplification products obtained with specific primers for *H. lactifluorum* (H.L-1, H.L-2) and *R. brevipes* (R.B-1, R.B-2). Plasmid *H.l* corresponds to plasmid DNA containing *H. lactifluorum* ITS fragment, gDNA L.M corresponds to genomic DNA extracted from a lobster mushroom, gDNA *R.b.* corresponds to genomic DNA extracted from a non-infected *R. brevipes* sporocarp. Negative = negative control.

Fig. 2 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA inside sporocarp of lobster mushroom. (A) Sporocarp transversal cross-section and localization of sampled tissues. Samples 0 to 6 indicate sampled tissue localization. (B) qPCR results of *H. lactifluorum*'s and (C) of *R. brevipes*' DNA inside lobster mushroom.

Fig. 3 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA at different infection stages and in non-infected *R. brevipes*. (A) Pictures of different infected sporocarps: non-infected *R. brevipes*, partially infected, completely infected (mature lobster mushroom) and decaying lobster mushroom sporocarp. (B) qPCR results of *H. lactifluorum*'s DNA and (C) of *R. brevipes*' DNA in a series of samples at different infection stages. Tissues were collected at the cap's edge (equivalent of position 0 from Fig. 2), in the middle (equivalent of position 2 from Fig. 2) and in the stalk (equivalent of position 4 or 5 from Fig. 2).

Fig. 4 HPLC chromatogram of extracts from non-infected *R. brevipes* and lobster mushroom sporocarps. (A) HPLC chromatogram at 254 nm of non-infected *R. brevipes* and (B) lobster mushroom extracts. (C) 3-D HPLC plot showing the 15-40 min range of *R. brevipes* and (D) lobster mushroom.

Fig. 5 Pie chart indicating some compounds groups detected by UPLC-qTOF-MS analysis. Percentage of diversity of the selected families of compounds for (A) non-infected *R. brevipes* and (B) lobster mushroom and the relative amount of each compound group calculated with the total area under the curve for (C) non-infected *R. brevipes* and (D) lobster mushroom.

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Supplementary Data

gen-2017-0168.R1Suppla: Fig. S1 Amplicons place on agarose gel after qPCR reaction with both specific primers on mature lobster mushroom sporocarp for one biological replicate. (A) *R. brevipes* primers (R.B-1, R.B-2) and (B) *H. lactifluorum* primers (H.L-1, H.L-2). Numbers 0 to 6 indicate sampled tissue localization in the sporocarp (lobster mushroom). Negative = negative control.

gen-2017-0168.R1Supplb: Fig. S2 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA inside sporocarp of lobster mushroom in two other biological replicates. (A) and (C) qPCR results for *H. lactifluorum*'s DNA and (B) and (D) for *R. brevipes*'s DNA. Samples 0 to 6 indicate sampled tissue localization.

gen-2017-0168.R1Supplc: Fig. S3 Amplicons placed on agarose gel after qPCR reaction with both primers pairs on different infection stages and in non-infected *R. brevipes*. (A) *R. brevipes* primers (R.B-1, R.B-2) and (B) *H. lactifluorum* primers (H.L-1, H.L-2). All infection stages are represented: non-infected sporocarp of *R. brevipes*, partially infected, completely infected and decaying lobster mushroom sporocarp. M (margin), C (center) and S (stalk) indicate sample tissue localization.

gen-2017-0168.R1 Suppld: Fig. S4 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA at different morphological infection stages and in non-infected *R. brevipes* in two other biological replicates. (A) and (C) qPCR results for *H. lactifluorum*'s DNA and (B) and (D) for *R. brevipes*' DNA.

gen-2017-0168.R1Supple: Table S1. Raw metabolomics data's obtained in non-infected *R. brevipes* (RU_NEG= Russula negative mode and RU_POS= Russula positive mode)

and lobster mushroom sporocarp (DRS_NEG= Lobster mushroom negative mode and DRS_POS= Lobster mushroom positive mode) by UPLC-qTOF-MS analyses.

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Fig. 1 PCR amplification products obtained with specific primers for *H. lactifluorum* (H.L-1, H.L-2) and *R. brevipes* (R.B-1, R.B-2). Plasmid H.l corresponds to plasmid DNA containing *H. lactifluorum* ITS fragment, gDNA L.M corresponds to genomic DNA extracted from a lobster mushroom, gDNA R.b. corresponds to genomic DNA extracted from a non-infected *R. brevipes* specimen. Negative = negative control.

31x15mm (600 x 600 DPI)

Fig. 2 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA inside sporocarp of lobster mushroom. (A) Sporocarp transversal cross-section and localization of sampled tissues. Samples 0 to 6 indicate sampled tissue localization. (B) qPCR results of *H. lactifluorum*'s and (C) of *R. brevipes*' DNA inside lobster mushroom.

46x27mm (600 x 600 DPI)

Fig. 3 qPCR results of *H. lactifluorum*'s and *R. brevipes*' DNA at different infection stages and in *R. brevipes*. (A) Pictures of different infected specimens: non-infected *R. brevipes*, partially infected specimen, completely infected specimen (mature lobster mushroom) and decaying specimen. (B) qPCR results of *H. lactifluorum*'s DNA and (C) of *R. brevipes*' DNA in a series of samples at different infection stages.

70x58mm (600 x 600 DPI)

Fig. 4 HPLC chromatogram of extracts from *R. brevipes* and lobster mushroom specimens. (A) HPLC chromatogram at 254 nm of *R. brevipes* and (B) lobster mushroom extracts. (C) 3-D HPLC plot showing the 15-40 min range of *R. brevipes* and (D) lobster mushroom.

49x29mm (600 x 600 DPI)

Fig. 5 Pie chart indicating some compounds groups detected by UPLC-qTOF-MS analysis. Percentage of diversity of the selected families of compounds for (A) *R. brevipes* and (B) lobster mushroom and the relative amount of each compound group calculated with the total area under the curve for (C) *R. brevipes* and (D) lobster mushroom.

67x59mm (600 x 600 DPI)