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Acylhomoserine Lactone Production by Bacteria Associated with Cultivated Mushrooms

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ABSTRACT: The main bacterial pathogens of cultivated mushroom as well as mushroom-associated bacteria, which were isolated from *Agaricus bisporus*, *Pleurotus ostreatus* and *Pleurotus eryngii* mushroom niches, were evaluated for the production of *N*-acyl-L-homoserine lactones (AHLs) by using four bioreporters. Furthermore, identification of AHLs by LC-ESI-FTICR MS was performed on culture filtrates of selected pathogens and mushroom-associated bacteria strains, which resulted in inducing at least one of the four bioreporters. Strains of *Burkolderia gladioli* pv. *agariciola*, *Pseudomonas agarici* and *Pseudomonas gingeri*, but not those of *Pseudomonas tolaasii* and *Pseudomonas reactans*, produced an array of AHLs depending on the strain. This is the first report of AHL production by mushroom bacterial pathogens. Forty-four of 236 bacterial isolates obtained from different niches of cultivated mushrooms, in part identified by the Biolog identification system, were demonstrated to produce AHLs. Among them, seven mushroom-associated bacterial species were for the first time demonstrated to produce the above signal molecules. In the culture filtrates of a certain number of isolates/strains the AHL-hydrolyzed forms were also present. The minimal signal inducing concentration (MSIC) of selected pure AHLs was also determined for the four bioreporters used in this study.

KEYWORDS: cultivated mushrooms, quorum sensing, mushroom bacterial pathogens, mushroom associated bacteria

INTRODUCTION

The success of microbes mainly depends on their ability to note and respond quickly to changes in the local niche. Population and growth status of bacteria are important for each individual bacterium cell in the nearby vicinity; this information may affect gene expression and production of diffusible compounds and/or metabolites. More importantly, it enables the bacterium within the population to coordinate metabolic and ecological strategies that would not be successful if attempted by individual bacterial cells. The ability to monitor the local population density is dependent on and mediated by the cell-to-cell communication systems that employ small diffusible signal molecules. The discovery of cell-to-cell communication among bacteria has led to the realization that bacteria are capable of coordinated activity. Quorum sensing (QS) is defined as the coordinate, population-density-dependent regulation of gene expression in individual bacterial cells that is mediated by exchange of extracellular signals and affects a diversity of features in a variety of bacterial species.[1−4]

N-Acylhomoserine lactones (AHLs) are the best investigated intercellular signaling compounds in Gram-negative bacteria (Figure 1).[1,5] QS has been reported to regulate cell metabolism, differentiation, nutrient flux, and other physiological events in bacteria, such as development of cell competence and sporulation, formation of biofilm, induction of virulence factor, induction of bioluminescence, host infection, bacteriocin synthesis and antibiotic synthesis.[1−5] AHL-mediated QS has been addressed as a potential target for an alternative strategy for bacterial disease control based on its inactivation (quorum quenching, QQ).[6−9]

Several pathogenic bacteria are known to be responsible for mushroom diseases. Bacterial diseases of cultivated mushrooms are limiting factors for *Agaricus* and *Pleurotus* spp. cultivation mainly due to the lack of suitable control measures as well as bactericides.[10] Hence, new and alternative control measures appear to be necessary. Bacterial brown blotch diseases of the button (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) mushrooms, caused by several bacteria including *Pseudomonas tolaasii* and *Pseudomonas reactans*, are endemic in mushroom farms and are the cause of significant yield and mushroom quality losses. *P. reactans* is also the cause of the yellowing of the king oyster mushroom (*Pleurotus eryngii*).[11] *Burkholderia gladioli* pv. *agaricicola* is an important pathogen in the mushroom industry; it causes soft rot of *Agaricus bitorquis* and *A. bisporus*. *Pseudomonas gingeri* and *Pseudomonas agarici* are the causes of ginger blotch disease of *A. bisporus* and of brown discoloration in *A. bisporus* and drippy gill in *P. ostreatus*, respectively.[10]

Despite rapidly increasing knowledge of the molecular mechanisms of the QS cascades in various plant-associated bacteria, no information exists about whether AHLs are produced by the mushroom pathogenic as well as mushroom-associated bacteria and on the role of this feature in disease establishment and on host−pathogen interaction.

Therefore, it appeared to be useful to investigate this aspect with the final aim to unravel the possible role of AHL-regulated

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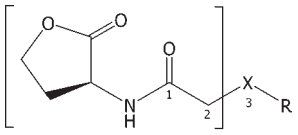

Figure 1. *N*-Acylhomoserine lactones (AHLs): basic chemical structure.

QS in the above bacteria and find out possible QQ-based control measures of the mushroom bacterial diseases.

Here we report on AHL production by representative strains of *P. agarici*, *P. gingeri*, and *B. gladioli* pv. *agaricicola* but not by strains of *P. tolaasii* and *P. reactans*, which appear not to have this gene regulation system. Furthermore, the AHL nature and concentration in culture filtrates of representative strains of pathogens and mushroom-associated bacterial isolates, which were positive in the T-streak assays, were determined by liquid chromatography (LC) coupled to a hybrid quadrupole linear ion trap (LTQ)−Fourier transform ion cyclotron resonance (FTICR) mass spectrometer upon electrospray ionization (ESI) (LC-FTICR MS).[12,13] The minimal signal induction concentration (MSIC) of selected representative AHLs on bioreporters was also determined. The specificity and sensitivity of bioreporters to detect AHLs, also in relation to MSIC of pure AHL analogues as well as the potential use of the findings of this study for QQ-based mushrooms control measure development, are discussed.

MATERIALS AND METHODS

Bacterial Bioreporters and Growth Conditions. Four AHL bioreporters, namely *Chromobacterium violaceum* (CV026),[14] *Escherichia coli* (pSB401), *E. coli* (pSB1075)[15] and *Agrobacterioum tumefaciens* pN7L4 (pDCI41E33),[16] considered in the literature to be useful for the detection of a large variety of structurally different AHLs,[22] were used. *C. violaceum* and *A. tumefaciens* strains and *E. coli* strains were grown at 25 and 37 °C, respectively, on medium B of King (KB).[17] *A. tumefaciens* pN7L4 (pDCI41E33) contains the plasmid pDCI41E33 obtained from *A. tumefaciens* strain harboring *traR* but not *traI*. Furthermore, it contains a *traG*::*lacZ* fusion that, providing exogenous AHL inducer molecules, results in the formation of blue color.[18] *C. violaceum* CV026 is a double-transposon mutant of *C. violaceum* strain ATCC31532 mutated in genes involved in violacein regulation and production, making violacein production dependent upon exogenous AHLs.[14] *E. coli* (pSB401) contains plasmid pSB401 harboring the *luxR* gene and the *lux* operon of *P. fischeri* with a deleted *luxI* region, making light emission dependent on the presence of exogenous AHL.[19] *E. coli* (pSB1075) contains a fusion of lasRIP::luxCDABE on a pUC18 plasmid backbone conferring a bioluminescent phenotype in the presence of AHLs activating the LuxR homologue.[15] For *A. tumefaciens* strain pN7L4 (pDCI41E33), *E. coli* (pSB401) and *E. coli* (pSB1075) antibiotics were incorporated into the media at the following concentrations: gentamicin, 30 μg/mL; tetracycline, 10 μg/mL; and ampicillin, 60 μg/mL, respectively.

Bacterial Mushroom Pathogens. Strains of bacterial mushroom pathogens, their origins, and mushroom host species from which they were isolated are listed in Table 1. Bacteria were maintained as lyophils at 4 °C, and, if not otherwise stated, they were subcultured on Nutrient Agar Glycerol [nutrient broth, 8 g; glycerol, 20 g; and agar, 18 g per 1 L of distilled water (NGA)] for 48 h at 25 °C.

Isolation of Mushroom-Associated Bacteria. A collection of bacterial isolates was obtained from different niches of *A. bisporus*, *P. ostreatus* and *P. eryngii*. Samples of casing soil (in the case of *A. bisporus* and *P. eryngii*), compost and mushroom sporophores were collected from the above mushroom niches. Ten grams of crushed mushroom sporophore tissues, soil and compost was suspended in 100 mL of sterile water containing 0.01% of Tween 20 and kept in agitation at 200 rpm for 2 h. Samples were serially diluted in water and 0.1 mL of the dilutions was spread on the surface of Petri plates containing KB. After 48 h of incubation at 25 °C, individual colonies with different morphologies were picked and streaked on KB, and then single colonies were transferred to NGA slants and used for further studies.

Gram staining, oxidase and catalase, and fluorescent pigment production tests of the mushroom-associated bacteria were performed following the usual procedures.[20]

Identification of Mushroom-Associated Bacteria. The Biolog automated identification system MicroLog 3 Biolog, release 4.20.04 (Biolog, Inc., Hayward, CA), was used for mushroom-associated bacteria identification. Bacterial isolates were grown for 48 h at 25 °C on Biolog Universal Growth Agar (BUG-Agar) and bacterial masses suspended at the manufacturer's recommended density (52% of transmittance at 590 nm) in 20 mL of sterile gelling inoculating fluid (0.40% NaCl, 0.03% Pluronic F-68, 0.01% Gellan Gum). A 150 μL suspension was added to each GN2MicroPlate well. After 24 h of incubation at 30 °C each plate was read automatically with the Micro Station Reader and the data were analyzed using the software for bacterial identification system MicroLog 3 Biolog, release 4.20.04 (Biolog, Inc.). The experiments were performed twice.

Detection of AHL Production with the T-Streak Bioassay. Strains of bacterial mushroom pathogens (Table 1) and mushroom-associated bacterial isolates were evaluated for AHL production in a T-streak assay as previously described using the bioreporters *C. violaceum* (CV026), *E. coli* (pSB401), and *E. coli* (pSB1075) on KB and *A. tumefaciens* pN7L4 (pDCI41E33) on the same medium supplemented with 40 mg of X-Gal/mL.[21] The phenotypic changes associated with the presence of exogenous AHLs were observed as a gradient with the main response observed at the meeting point between the two strains.[22] Induction of bioluminescence at the meeting point of the two strains was detected in the plates by using the Bio-Rad Gel Doc 2000 instrument with the software Quantity one (Chemi doc), version 4.1.

Determination of AHL Production in Liquid Culture. Bacteria that showed positive results in the T-streak assay with at least one of the bioreporters were inoculated in 200 mL of KB broth and incubated at 25 °C under agitation (150 rpm). Every 24 h for 4 days culture aliquots of 5 mL were centrifuged and supernatants were filter sterilized by using the nitrocellulose membrane filter and stored at −20 °C. Also, representative strains of *P. tolaasii* and *P. reactans*, which were negative in the T-streak bioassay, were evaluated for AHL production in the above culture conditions. One hundred microliter sterile culture filtrates were added to wells made in KB plates using sterile cork borer 0.9 mm in diameter. Ten microliter bacterial suspensions (0.2 OD_{590}) of bioreporters were posed at about 0.5 cm distance from the well and incubated at 25 or 37 °C for 48 h. The color/bioluminescence induction associated with the presence of exogenous AHLs in the culture filtrates was detected as reported in the previous paragraph. Culture filtrates of 48 h bacterial cultures were also used for the identification and quantification of AHLs by LC-FTICR MS.

Determination and Quantification of AHL Analogues in Culture Filtrates by Using LCFTICR MS. Selected AHLs, namely, C_4-HSL, C_6-HSL, C_8-HSL, C_{10}-HSL, C_{12}-HSL, C_{14}-HSL, 3-oxo-C_6-HSL, 3-oxo-C_8-HSL and 3-oxo-C_{12}-HSL, all D/L, were purchased from Fluka (Buchs, Switzerland), C_4-HSL-d_5 and C_6-HSL-d_3 were purchased from Cayman Chemical (Ann Arbor, MI). Analytical grade acetonitrile

Table 1. Strains of Bacterial Pathogens of the Cultivated *Agaricus bisporus*, *Pleurotus ostreatus* and *Pleurotus eryngii* Mushrooms Used in This Study

bacterial mushroom pathogen	mushroom host species	geographical origin	strain designation[a]
Pseudomonas tolaasii	*Agaricus bisporus*	Great Britain	NCPPB2192[b]
P. tolaasii	*A. bisporus*	Italy	NCPPB2325
P. tolaasii	*A. bisporus*	Great Britain	ICMP2742
P. tolaasii	*A. bisporus*	The Netherlands	ICMP2838
P. tolaasii	*A. bisporus*	New Zealand	ICMP4227
P. tolaasii	*A. bisporus*	Italy	ICMP6551
P. tolaasii	*A. bisporus*	Canada	ICMP6553
P. tolaasii	*A. bisporus*	Great Britain	ICMP12217
P. tolaasii	*Pleurotus ostreatus*	Italy	USB1 (ICMP13791)[c]
P. tolaasii	*P. ostreatus*	Italy	USB17
P. tolaasii	*P. ostreatus*	Italy	USB22
P. tolaasii	*P. ostreatus*	Italy	USB26
P. tolaasii	*P. ostreatus*	Italy	USB32
P. tolaasii	*P. ostreatus*	Italy	USB42
P. tolaasii	*P. ostreatus*	Italy	USB57
P. tolaasii	*A. bisporus*	Italy	USB66 (ICMP13792)[c]
P. tolaasii	*P. ostreatus*	Italy	USB105
P. tolaasii	*P. ostreatus*	Italy	USB107
P. tolaasii	*A. bisporus*	Italy	USB144
P. tolaasii	*A. bisporus*	Italy	USB151
Pseudomonas reactans	*A. bisporus*	Great Britain	NCPPB1311
P. reactans	*A. bisporus*	Great Britain	ICMP2837
P. reactans	*A. bisporus*	New Zealand	ICMP6953
P. reactans	*A. bisporus*	New Zealand	ICMP6954
P. reactans	*A. bisporus*	Great Britain	ICMP6956
P. reactans	*A. bisporus*	Great Britain	ICMP8873
P. reactans	*P. ostreatus*	Italy	USB6
P. reactans	*P. ostreatus*	Italy	USB16
P. reactans	*P. ostreatus*	Italy	USB20 (ICMP13793)[c]
P. reactans	*P. ostreatus*	Italy	USB49
P. reactans	*Pleurotus eryngii*	Italy	USB63 (ICMP13794)[c]
P. reactans	*A. bisporus*	Italy	USB69 (ICMP13795)[c]
P. reactans	*A. bisporus*	Italy	USB75
P. reactans	*P. eryngii*	Italy	USB91 (ICMP13796)[c]
P. reactans	*P. eryngii*	Italy	USB94
P. reactans	*P. eryngii*	Italy	USB119
P. reactans	*P. eryngii*	Italy	USB125 (ICMP13797)[c]
P. reactans	*A. bisporus*	Italy	USB131
P. reactans	*A. bisporus*	Italy	USB145
P. reactans	*A. bisporus*	Italy	USB157
Burkholderia gladioli pv. *agaricicola*	*Agaricus bitorquis*	Great Britain	ICMP11096*
B. gladioli pv. *agaricicola*	*A. bitorquis*	Great Britain	ICMP11097
B. gladioli pv. *agaricicola*	*A. bitorquis*	Great Britain	ICMP12222
B. gladioli pv. *agaricicola*	*A. bitorquis*	Great Britain	ICMP12220
Pseudomonas gingeri	*A. bisporus*	Great Britain	ICMP8872
Pseudomonas agarici	*A. bisporus*	New Zealand	NCPPB2289
P. agarici	*A. bisporus*	Great Britain	NCPPB2472
P. agarici	*A. bisporus*	Italy	USB78 (ICMP13775)[c]
P. agarici	*A. bisporus*	Italy	USB84
P. agarici	*A. bisporus*	Italy	USB88

[a] NCPPB, National Collection Plant Pathogenic Bacteria, York, U.K.; ICMP, International Collection of Microrganism from Plants, Auckland, New Zealand; USB, Laboratorio di Batteriologia Fitopatologica, Università degli Studi della Basilicata, Italy. [b] Strain type. [c] Strain deposited in International Collection of Microrganism from Plants, Auckland, New Zealand.

(99.9%) and formic acid were purchased from Sigma-Aldrich (Buchs, Switzerland).

Stock solutions were prepared by dissolving the analytes in acetonitrile at a concentration of 1 mg/mL and stored at −20 °C. Standard solutions for LC-MS analyses were prepared by diluting the stock solution to the desired concentration with H_2O/ACN (80:20, v/v) acidified with 0.1% HCOOH. Pure nitrogen (99.996%) was delivered to the LC-MS system as sheath gas. The ion trap pressure was maintained with helium 99.999%, which was used for trapping and for collisional activation of the trapped ions.

All experiments were performed using a Surveyor LC system coupled to a LTQ-FT hybrid linear trap/7-T FTICR mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). A Supelcosil LC-ABZ amide-C16 (5 μm, 250 × 2.1 mm, Supelco Inc., Bellefonte, PA) was used for the separation of AHLs with a guard column of the same packing material (20 × 2 mm, Supelco Inc.). Pure water supplied by a Milli-Q RG unit from Millipore (Bedford, MA) with the addition of 0.1% formic acid was used as eluent A and 0.1% formic acid in acetonitrile as eluent B. The elution gradient was as follows: 0−3 min, 20% B; 3−17 min, solvent B increased linearly to 100%; and 17−27 min at 100% B. The system was re-equilibrated at 80% A for 6 min before the next injection. Separations were performed at ambient temperature of 21 ± 2 °C at a flow rate of 0.3 mL/min, without split before entrance to the ESI source. A total of 20 μL was injected into the chromatographic column. Positive ion ESI-MS was chosen for the detection of *N*-acyl- and *N*-3-oxoacylhomoserine lactones and corresponding hydrolyzed compounds. Mass spectrometric conditions were the same as described previously.[12,13]

To check the response linearity of the method, calibration curves were acquired with known amounts of the selected AHLs reported above in the concentration range of 0.005−15 μM and using C_4-HS-d_5 (3 μM) and C_6-HSL-d_3 (3 μM) as internal standards. ACN/H_2O (80:20, v/v) plus 0.1% HCOOH was used as solvent for all preparations. Six-point calibration curves were plotted as the peak area ratios of analytes to that of the internal standard versus concentration (C_4-HSL-d_5 was used as internal standard for C_4-HSL and C_6-HSL-d_3 for all other compounds); all measurements were done in triplicate. The correlation coefficient (r) was up to 0.9996 for all AHLs under study. Estimated limits of detection (LODs) were 0.5 μM for C_4-HSL, 0.05 μM for 3-oxo-C_6-HSL, 0.01 μM for C_6-HSL, and 0.005 μM for C_8-HSL, C_{10}-HSL, C_{12}-HSL, 3-oxo-C_8-HSL, 3-oxo-C_{12}-HSL and C_{14}-HSL, respectively. The LOD was evaluated as 3.3 times the standard deviation of the intercept divided by the slope.

Determination of Minimal Signal Induction Concentration (MSIC) of AHLs in Bioreporters. To check the MSIC of AHLs to activate a response signal by *C. violaceum* (CV026), *E. coli* (pSB401), and *E. coli* (pSB1075), six standard solution at different concentrations (50, 5, 0.5, 0.05, 0.005, and 0.0005 μM) of C4-HSL, C6-HSL, C8-HSL, C10-HSL, C12-HSL, C14-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL and 3-oxo-C12-HSL were used. For *A. tumefaciens* pN7L4 (pDCI41E33), further dilute solutions were tested.

Aliquots of 50 μL of the AHL stock solutions were added to wells made with a sterile cork borer (diameter = 0.9 mm) in KB media Petri dishes. The corresponding bioreporters were inoculated as 10 μL bacterial suspension (0.2 OD_{590}) at about 0.5 cm distance from the well. After 48 h of incubation at 25 or 37 °C, the occurrence of purple violet color [(*C. violaceum* (CV026)] or dark blue color [(*A. tumefaciens* (pDCI41E33)] was determined. Bioluminescence [(*E. coli* (pSB 401) and (pSB 1075)] was detected by using the Bio-Rad Gel Doc 2000 instrument.

RESULTS

Mushrooms-Associated Bacterial Isolates. From cultivated mushrooms niches 236 bacterial isolates (136 and 100 Gram-positive and -negative, respectively) were obtained. Among them, 183, 22 and 31 isolates were obtained from *P. eryngii*, *P. ostreatus* and *A. bisporus* niches, respectively. Among the Gram-negative isolates 46 belonged to the fluorescent pseudomonads as they were able to produce fluorescent pigments when grown on KB (Table 2).

All of the above bacterial isolates were evaluated for AHL production in the T-streak assay. Only 44 of 236 bacterial isolates, all as expected Gram-negative bacteria, produced AHLs, and among them 38, 1, and 5 were obtained from niches of *P. eryngii*, *P. ostreatus* and *A. bisporus*, respectively. AHL-producing bacteria were isolated from casing soils of *P. eryngii* and *A. bisporus* (24 isolates), compost of *P. eryngii*, *P. ostreatus* and *A. bisporus* (13 isolates) and sporophores of *P. eryngii* (7 isolates). Among the bacterial isolates producing AHLs, only 18 obtained from *P. eryngii* (16 isolates), *P. ostreatus* and *A. bisporus* (1 isolate from each mushroom niche), belonged to the fluorescent pseudomonads.

Thirty-two of 44 AHL-producing bacterial isolates were identified on the basis of nutritional profile obtained with the automated Biolog identification system, by the MicroLog 3 Biolog, release 4.20.04 (Table 2). In particular, they were identified as biotypes of *P. fluorescens* (10 strains), *Rahnella aquatilis* (5 strains), *P. synxantha* (2 strains), *P. putida* (2 strains) and 1 strain of the following bacterial species: *P. aurantiaca*, later reclassified as *P. chlororaphis*; *P. viridilivida*, *P. marginali*, *B. glumae*, *Enterobacter cloacae*, *Acinetobacter calcoaceticus*, *A. baumannii*, *Cytophaga fermentans*, *Achromobacter xylosoxidans* subsp. *denitrificans*, *Brevundimonas vesicularis*, *Chryseobacterium scopthalmum* and *Providencia heimbachae*. Finally, one was identified as belonging to *Pseudomonas* spp. (Table 2). Twelve other isolates were not identified by this identification system (Table 2).

Production of AHLs by Mushroom Bacterial Pathogens. The results on AHL production by bacterial pathogens of cultivated mushrooms, as evaluated with the T-streak assay, are reported in Table 3. In particular, no strains of *P. tolaasii* and *P. reactans* (20 strains of each pathogen) showed positive results with any of the four bioreporters used in this study. On the contrary, all four strains of *B. gldioli* pv. *agaricicola* (type strain ICMP11096, ICMP11097, ICMP12322, and ICMP12220) and one strain of *P. gingeri* (ICMP8872) showed positive results with *C. violaceum* (CV026). Furthermore, three of five strains of *P. agarici* (NCPPB2472, USB78, and USB84) showed positive results with *E. coli* (PSB401) and *A. tumefaciens* pN7L4 (pDCI41E33) (Table 3).

The detection provided by LC-ESI-FTICR MS allowed verification of the occurrence of AHLs produced by bacterial pathogens in liquid cultures (Table 4). In particular, type strain ICMP11096 of *B. gladioli* pv. *agaricicola* produced both the long- and short-chain AHLs ranging from 20 nM for 3-OH-C10-HSL to 2.1 μM for 3-oxo-C4-HSL. On the contrary, strain ICMP12220 of *B. gladioli* pv. *agaricicola* produced only short-chain AHLs, ranging from 60 nM for C6-HSL to 2.4 μM for 3-oxo-C4-HSL. *P. gingeri* ICMP8872 produced the short-chain 3-oxo-C4-HSL at a concentration of 29 μM. Strains of *P. agarici* NCPPB2472 and USB78 produced 3-oxo-C8-HSL and 3-OH-C8-HSL at concentrations of 0.01 and 0.05 μM, respectively. Furthermore, LC-ESI-FTICR MS analysis confirmed the lack of AHLs in the culture filtrates of representative strains of *P. tolaasii* and *P. reactans*.

The presence of chromatographic peaks corresponding to hydrolyzed AHL forms (HSs) for 3-oxo-C4-HSL, C6-HSL, and C8-HSL was observed in the *B. gladioli* pv. *agaricicola*

Table 2. Identification by the Biolog Automated System MicroLog 3 Biolog, Release 4.20.04 (Biolog, Inc., Hayward, CA) and Main Characters of AHL-Producing Bacterial Isolates Associated with *Agaricus bisporus*, *Pleurotus ostreatus* and *Pleurotus eryngii* Cultivated Mushrooms

			biochemical characters			
organism	similarity[a]	designated no.	oxidase	catalase	mushroom source	origin
Pseudomonas fluorescens	0.50	H1-2	+	+	*Pleurotus eryngii*	casing soil
P. fluorescens	0.85	H5-6	+	+	*P. eryngii*	casing soil
P. fluorescens	0.86	J1-4	+	+	*P. eryngii*	sporophore
P. fluorescens	0.79	J2-3	+	+	*P. eryngii*	sporophore
P. fluorescens	0.77	J2-1	+	+	*P. eryngii*	sporophore
P. fluorescens	0.84	J3-2	+	+	*P. eryngii*	sporophore
P. fluorescens	0.65	G4-3	+	+	*P. eryngii*	compost
P. fluorescens biotype F	0.71	D2-1	+	+	*P. eryngii*	compost
P. fluorescens biotype F	0.72	D2-2	+	+	*P. eryngii*	compost
P. fluorescens biotype F	0.61	D2-3	+	+	*P. eryngii*	compost
P. synxantha	0.76	J1-2	+	+	*P. eryngii*	sporophore
P. synxantha	0.77	F7-1	+	+	*P. eryngii*	casing soil
P. putida	0.83	F3-1	+	+	*P. eryngii*	casing soil
P. putida biotype B	0.57	93-2	+	+	*Agaricus bisporus*	compost
P. marginalis	0.81	J1-3	+	+	*P. eryngii*	sporophore
P. viridilivida	0.57	42-1	+	+	*P. ostreatus*	compost
P. aurantiaca	0.66	D3-4	+	+	*P. eryngii*	compost
Pseudomonas spp.	0.36	J1-1	+	+	*P. eryngii*	sporophore
Burkholderia glumae	0.58	D7-4	+	+	*P. eryngii*	compost
Rahnella aquatilis	0.77	D4-5	−	+	*P. eryngii*	compost
R. aquatilis	0.87	H1-4	−	+	*P. eryngii*	casing soil
R. aquatilis	0.69	H1-1	−	+	*P. eryngii*	casing soil
R. aquatilis	0.62	H5-3	−	+	*P. eryngii*	casing soil
R. aquatilis	0.67	G6-1	−	+	*P. eryngii*	compost
Acinetobacter calcoaceticus	0.52	F1-4	−	+	*P. eryngii*	casing soil
Acinetobacter baumannii	0.62	93-1	−	+	*A. bisporus*	compost
Cytophaga fermentans	0.87	D7-3	−	+	*P. eryngii*	compost
Achromobacter xylosoxidans subsp. *denitrificans*	0.57	F1-3	+	+	*P. eryngii*	casing soil
Enterobacter cloacae	0.50	H1-3	+	+	*P. eryngii*	casing soil
Brevundimonas vesicularis	0.90	F7-2	+	+	*P. eryngii*	casing soil
Chryseobacterium scopthalmum	0.59	F2-4	+	+	*P. eryngii*	casing soil
Providencia heimbachae	0.53	81-1	−	+	*A. bisporus*	casing soil
NI[b]		F2-1	+	+	*P. eryngii*	casing soil
NI		H6-2	+	+	*P. eryngii*	casing soil
NI		H9-3	−	+	*P. eryngii*	casing soil
NI		E2-1	+	+	*P. eryngii*	casing soil
NI		E5-4	+	+	*P. eryngii*	casing soil
NI		H4-8	+	+	*P. eryngii*	casing soil
NI		F2-2	+	+	*P. eryngii*	casing soil
NI		D4-3	+	+	*P. eryngii*	compost
NI		E5-3	−	+	*P. eryngii*	casing soil
NI		E9-1	−	+	*P. eryngii*	casing soil
NI		83-2	+	+	*A. bisporus*	casing soil
NI		84-1	+	+	*A. bisporus*	casing soil

[a] Similarity as calculated by The Biolog automated system MicroLog 3 Biolog, release 4.20.04. [b] NI, not identified on the basis of the nutritional profile as determined with the The Biolog automated system MicroLog 3 Biolog, release 4.20.04 (Biolog, Inc.).

ICMP11096 and ICMP12220 samples; they were not quantified because they were present at too low concentrations.

Production of AHLs by Bacterial Isolates Associated with Mushrooms. Among 236 bacterial isolates associated with mushrooms, 44 produced AHLs as they were able to induce at least one of the bioreporters used in this study. In particular, 24, 22, 15 and 4 of 44 isolates showed positive results with the bioreporters *E. coli* (PSB401), *E. coli* (PSB1075), *A. tumefaciens*

Table 3. Bioreporter Induction by *Agaricus bisporus*, *Pleurotus ostreatus* and *Pleurotus eryngii* Cultivated Mushroom Bacterial Pathogens as Determined in the T-Streak Bioassay

	bioreporters[a]			
bacteria	*Chromobacterium violaceum* (CV026)	*Escherichia coli* (PSB 401)	*Escherichia coli* (PSB 1075)	*Agrobacterium tumefaciens* (pDCI41E33)
Pseudomonas tolaasii	0/20	0/20	0/20	0/20
Pseudomonas reactans	0/20	0/20	0/20	0/20
Pseudomonas gingeri	1/1	0/1	0/1	0/1
Pseudomonas agarici	0/5	3/5	0/5	3/5
Burkholderia gladioli pv. *agaricicola*	4/4	0/4	0/4	0/4

[a] The numerator represents the positive strains, whereas the denominator represents the total number of strains used in the bioassays.

Table 4. AHL Concentration in Culture Filtrates of Bacterial Mushrooms Pathogens as Determined by Using LC-ESI-FTICR MS

	AHL concentration (μM)					
bacteria	3-oxo-C4	C6	C8	3-oxo-C8	3-OH-C8	3-OH-C10
Burkholderia gladioli pv. *agaricicola* ICMP11096[a]	2.1	0.2	2.1		0.1	0.02
B. gladioli pv. *agaricicola* ICMP12220	2.4	0.06	0.1			
P. gingeri ICMP8872	29					
P. agarici NCPPB2472				0.01	0.05	
P. agarici USB78					0.05	

[a] Strain type.

(pDCI41E33) and *C. violaceum* (CV026), respectively (Table 5). The AHL production behavior in liquid culture by the above bacterial isolates is reported in the same table (Table 5). However, not all culture filtrates of the bacterial isolates that were positive in the T-streak assays on *E. coli* (pSB401) and *E. coli* (pSB1075) induced the same bioreporters. On the contrary, the culture filtrates of a further six isolates, negative in the T-streak, induced the bioreporter *C. violaceum* (CV026). All of the culture filtrates of the 44 isolates, independent of the results on the same bioreporter in the T-streak bioassay, activated the bioreporter *A. tumefaciens* (pDCI41E33).

AHLs produced by the mushroom-associated bacteria are reported in Table 6. The bacterial isolates produced 11 structurally different AHLs such as C4-HSL, 3-oxo-C4-HSL, C6-HS, C8-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-OH-C8-HSL, OH-C9-HSL, 3-OH-C10-HSL and 3-OH-C12-HSL. As an example, in Figure 2 are shown the extracted-ion chromatograms (XICs) obtained by LC-ESI-FTICR MS of a cell-free bacterial supernatant of strain H5-6 of *P. fluorescens* associated with mushrooms. As can be seen, only by using accurate mass XIC is it possible to greatly reduce the signal complexity of total ion current trace (data not shown), allowing us to distinguish accurately the AHLs under study. The benefits of using a narrow window of mass-to-charge ratio allows a considerable removal of interference ions along with a very simplified chromatographic profile. Indeed, the combination of exact mass measurement by high-resolution mass spectrometry along with isotopic patterns and chromatographic data (i.e., retention time and peak shape) gives extraordinary insight into the elucidation of the AHLs in the supernatant of the bacterial isolate associated with mushrooms.

As in the case of the bacterial pathogenic strains, the presence of chromatographic peaks corresponding to hydrolyzed lactone for C4-HSL, 3-oxo-C4-HSL, C6-HSL, C8-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL and 3-oxo-C12-HSL was observed in the culture filtrates of several bacterial isolates (Table 6).

MSIC of AHLs in Bioreporters. Bioreporter *A. tumefaciens* pN7L4 (pDCI41E33) responded to all nine selected AHLs (C4-HSL, C6-HSL, C8-HSL, C10-HSL, C12-HSL, C14-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL and 3-oxo-C12-HSL) used in this study. On the contrary, *C. violaceum* (CV026), *E. coli* (PSB401), and *E. coli* (PSB1075) responded to five (C4-HSL, C6-HSL, C8-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL), four (C6-HSL, 3-oxo-C8-HSL, 3-oxo-C12-HSL, 3-oxo-C6-HSL) and two (3-oxo-C12-HSL, C12-HSL,) AHLs, respectively (Table 7). However, the different AHLs showed different MSICs also in relation to the bioreporter used (Table 7). In particular, C4-HSL did not induce a response by *E. coli* (PSB401) and *E. coli* (PSB1075) even when 50 μM solutions were used. C6-HSL, 3-oxo-C6-HSL and 3-oxo-C8-HSL induced *C. violaceum* (CV026), *E. coli* (PSB401) and *A. tumefaciens* pN7L4 (pDCI41E33) but not *E. coli* (PSB1075) even when 50 μM solutions were used. C8-HSL induced *C. violaceum* (CV026) and *A. tumefaciens* pN7L4 (pDCI41E33) but not *E. coli* (PSB401) and *E. coli* (PSB1075) even when 50 μM solutions were used. C10-HSL induced *A. tumefaciens* pN7L4 (pDCI41E33) but did not induce *C. violaceum* (CV026), *E. coli* (PSB401) and *E. coli* (PSB1075) even at 50 μM. Similarly, C12-HSL and C14-HSL induced *E. coli* (PSB1075) but not *C. violaceum* (CV026) and *E. coli* (PSB401) even at 50 μM. 3-Oxo-C12-HSL induced signal in *E. coli* (PSB401), *A. tumefaciens* pN7L4 (pDCI41E33) and *E. coli* (PSB1075) but not in *C. violaceum* (CV026).

DISCUSSION

Many plant-associated bacteria have been reported to produce AHLs. For example, Cha et al.[23] reported AHL production by plant-associated bacteria, and Steidle et al.[24] documented AHL

Table 5. AHL Production by Bacteria Associated with *Agaricus bisporus*, *Pleurotus ostreatus*, and *Pleurotus eryngii* Cultivated Mushrooms as Determined with T-Streak with Bioreporters

	bioreporters[a]							
	Chromobacterium violaceum (CV026)		*Escherichia coli* (PSB 401)		*Escherichia coli* (PSB 1075)		*Agrobacterium tumefaciens* (pDCI41E33)	
bacteria	T-streak	culture filtrate	T-streak	culture filtrate	T-streak	culture filtrate	T-streak	culture filtrate
H1-2	−	+	+	−	−	−	−	+
H5-6	−	+	+	−	−	−	−	+
J1-4	−	−	+	−	−	−	−	+
J2-3	−	−	+	−	+	−	−	+
J2-1	−	−	+	−	−	−	−	+
J3-2	−	−	−	−	+	−	−	+
G4-3	−	−	−	−	+	−	−	+
D2-1	+	+	+	−	+	−	+	+
D2-2	+	+	+	−	+	−	+	+
D2-3	+	+	+	−	+	−	+	+
J1-2	−	−	+	−	−	−	−	+
F7-1	−	−	−	−	+	−	−	+
F3-1	−	−	−	−	+	−	−	+
93-2	−	−	+	−	−	−	+	+
J1-3	−	−	+	−	−	−	−	+
42-1	−	−	−	−	+	−	−	+
D3-4	−	+	+	−	−	−	+	+
J1-1	−	−	+	−	−	−	−	+
D7-4	−	−	−	−	−	−	+	+
D4-5	+	+	+	−	+	−	+	+
H1-4	−	−	+	−	−	−	−	+
H1-1	−	−	+	−	−	−	−	+
H5-3	−	−	−	−	+	−	−	+
G6-1	−	−	−	−	+	−	−	+
F1-4	−	−	−	−	+	−	+	+
93-1	−	−	+	−	−	−	+	+
D7-3	−	+	−	−	−	−	+	+
F1-3	−	−	−	−	−	−	+	+
H1-3	−	−	−	−	+	−	−	+
F7-2	−	−	−	−	+	−	−	+
F2-4	−	−	−	−	+	−	−	+
81-1	−	−	−	−	−	−	+	+
F2-1	−	−	+	−	+	−	−	+
H6-2	−	−	+	−	−	−	−	+
H9-3	−	−	+	−	−	−	−	+
E2-1	−	+	+	−	−	−	−	+
E5-4	−	−	+	−	−	−	−	+
H4-8	−	−	+	−	+	−	−	+
F2-2	−	−	+	−	+	−	−	+
D4-3	−	+	−	−	+	−	+	+
E5-3	−	−	−	−	+	−	−	+
E9-1	−	−	−	−	+	−	−	+
83-2	−	−	−	−	−	−	+	+
84-1	−	−	−	−	−	−	+	+

[a] +, induction of bioreporters; −, no induction of bioreporters.

production by rhizosphere bacteria. Elasri et al.[18] made a systematic study and showed that AHL production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. To our knowledge, however, no data on AHL production by mushroom-associated bacteria are available. In this context, we screened for AHL production the main mushroom bacterial pathogens as well as bacteria associated with *A. bisporus*, *P. ostreatus* and *P. eryngii* niches. None of the

Table 6. AHL Concentration in Culture Filtrates of Bacteria Associated with Mushrooms by Using LC-FTICR MS[a]

	AHL concentration (μM)																	
	C4-		3-oxo-C4-		C6-		C8-		3-oxo-C8-		3-oxo-C10-		3-oxo-C12-					
bacteria	HSL	HS	HSL	HS	HSL	HS	HSL	HS	HSL	HS	HSL	HS	HSL	HS	3-OH-C8- HSL	3-OH-C9- HSL	3-OH-C10- HSL	3-OH-C12- HSL
H1-2					1.4	1.8	0.006											
H5-6					1.2	1.6	0.5	0.8							0.03	0.03	0.0048	
J1-4			1.4	1.4														
J2-3				2.6	2.5													
J2-1				9.3	9.3													
J3-2	5.0	7.0	0.1	0.6	0.05	0.3	0.0005	0.002			0.01	0.05	0.03	0.1				
G4-3	8.0	15.3	0.07	1.1	0.1	0.6	0.001	0.009	0.001	0.01	0.04	0.2	0.05	0.2				
D2-1					0.7													
D2-2					2.1	0.3	0.01		0.01									
D2-3					1.7	1.5		0.3	0.005									
J1-2	8.3	14.0	0.9	1.2	0.07	0.5	0.0006	0.004										
F7-1					0.1	0.07	1.9	2.2							0.1	0.1	0.031	
F3-1					0.7	7.1	0.02	0.06										
93-2	10.5	17.4		0.2	0.1	0.6	0.001	0.005		0.008	0.02	0.2	0.05	0.2				
J1-3			1.7	1.8														
42-1	7.8	10.6	4.8	4.9	0.08	0.3				0.001	0.009	0.06	0.02	0.1				
D3-4					1.5	1.2	0.003	0.1	0.01									
J1-1	3.5	6.9	2.2	2.7	0.02	0.2	0.0004	0.001										
D7-4					0.7	2.0	0.007	0.02	0.004									
D4-5	26.0				0.04													
H1-4					1.3	9.0	0.03	0.03										
H1-1																		
H5-3							0.6	0.7							0.02	0.02	0.002	
G6-1	8.6	11.2			0.1	0.5	0.003	0.005			0.03	0.09	0.06	0.2				
F1-4					0.3	1.4	1.1	8.5							0.2	0.1	0.06	0.07
93-1	7.3	15.0		0.9	0.08	0.4	0.002	0.006		0.007	0.02	0.2	0.04	0.1				
D7-3					1.2	2.3		0.4	0.01									
F1-3					0.4	1.1	1.4	7.4							0.2	0.2	0.06	0.06
H1-3					0.2	3.9												
F7-2					0.08	0.06	1.6	2.1							0.09	0.1	0.02	
F2-4					0.3	4.8	0.002	0.04										
81-1	3.0	5.5	2.1	2.4	0.04	0.2	0.1	0.1		0.003	0.01	0.08	0.01	0.09				
F2-1					0.2	0.6	1.6	6.7							0.2	0.2	0.07	0.03
H6-2					1.7	5.1	0.7	4.1							0.1	0.08	0.03	0.01
H9-3					0.02		0.3	0.4							0.01	0.01	0.003	
E2-1					1.7	2.4	0.0006	0.2	0.01									
E5-4					0.01													
H4-8					0.7	1.8	0.1	0.5							0.01	0.005		
F2-2					0.2	0.3	1.3	3.9							0.1	0.1	0.04	0.01
D4-3	12.6				0.01				0.007									
E5-3					0.7	1.6	0.003	0.01	0.002									
E9-1					0.2	2.1		0.01										
83-2			4.4	3.9	0.09	0.2	0.1	0.3										
84-1	3.3	5.0	0.4	1.0	0.2	0.5	0.1	0.1			0.01	0.04	0.03	0.1				

[a] HSL, homoserine lactone; HS, hydrolyzed homoserine.

P. tolaasii or *P. reactans* strains showed positive results with the four AHL bioreporters nor did they accumulate AHLs in liquid culture as confirmed by LC-ESI-FTICR MS analysis. Apparently these two pathogens lack the QS regulatory system based on the AHL as signal molecules. The production of tolaasins, the main virulence factor of *P. tolaasii*, is, in fact, regulated by the GacS/GacA two-component regulatory system,[25] which is thought to respond to environmental stimuli. However, so far nothing is known of the production regulation of the virulence factor White Line Inducing Principle (WLIP) in *P. reactans*.[11] On the contrary, the four strains of *B. gladioli* pv. *agaricicola*, three of five strains of *P. agarici* and the strain of *P. gingeri* used in this study

produced AHLs. This is the first report of AHL production by strains of *B. gladioli* pv. *agaricicola*, which is considered as a potential threat to the mushroom industry.[10] However, culture filtrates of two strains of *B. gladioli* pv. *agaricicola* showed a different AHL profile because the type strain ICMP11096 produced either short- or long-chain AHLs, whereas strain ICMP12220 produced only short-chain AHL ones. Not clear is the biological meaning, if any, of the above different AHL profiles, but differences in secondary metabolite production among strains of bacterial species are a common feature. The AHL production is common among pathogens belonging to the genus *Burkholderia*, and this feature in some of the species has been demonstrated to regulate important traits including virulence.[26] For example, in *B. glumae*, the causal agent of rice grain rot, the production of two major virulence factors such as toxoflavin and lipase has been demonstrated to be regulated by AHLs.[27,28] Similarly, in *B. plantarii* the production of the virulence factor polygaluctaronase is also regulated by AHLs.[29] Hence, it is not excluded that the QS mediated by AHLs may have a role in the production of antimicrobial substances and/or hydrolytic enzymes of *B. gladioli* pv. *agaricicola*, which may be important in the biology and virulence of this pathogen.[10,30−32]

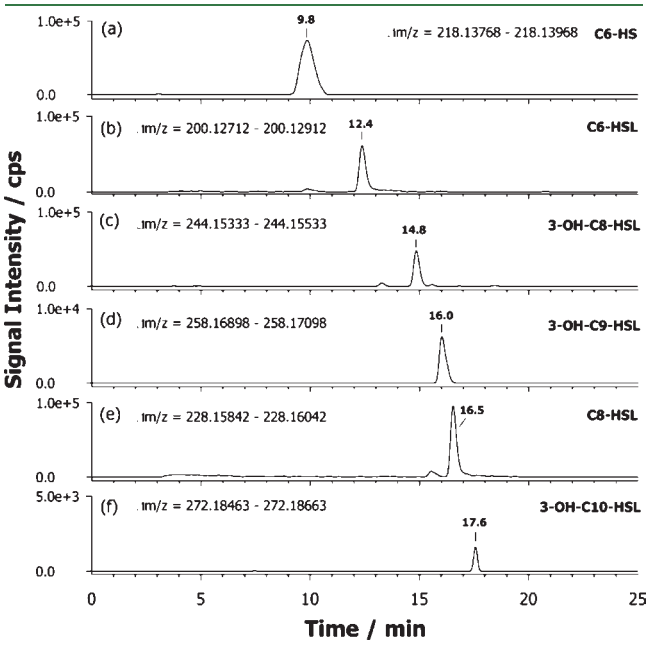

Figure 2. LC-ESI-FTICR-MS extracted-ion chromatograms under optimized working conditions of AHLs occurring in a associated bacterial isolate H5-6. The ions monitored are displayed in each trace and correspond to the most abundant protonated molecules, $[M + H]^+$, using a ± 0.0010 m/z unit centered around each selected ion. LC column and linear gradient were as described under Materials and Methods. Data acquisition was made on a LTQ-FT hybrid linear trap/ICR mass spectrometer equipped with a 7 T magnet using software Excalibur 2.0 (Thermo). Note the occurrence of peaks C6-HSL (plot a) and the corresponding hydrolyzed molecule C6-HS (plot b), 3-OH-C8-HSL isomer (plot c), 3-OH-C9-HSL isomer (plot d), C8-HSL isomer (plot e), and 3-OH-C10-HSL isomer (plot f).

Strain ICMP8872 of *P. gingeri* showed positive results only with *C. violaceum* (CV026) and accumulated in liquid cultures a high amount (29 μM) of 3-oxo-C4-HSL, and hence it is difficult to understand why a so relatively huge amount of 3-oxo-C4-HSL present in the culture filtrate of *P. gingeri* did not induce the bioreporters *E. coli* (PSB401) and *A. tumefaciens* pN7L4 (pDCI41E33), which, on the contrary, were demonstrated to be induced by the near structural analogue 3-oxo-C6-HSL with a relatively low MSIC. Furthermore, three of the five strains of *P. agarici* induced *E. coli* (PSB401) and *A. tumefaciens* pN7L4 (pDCI41E33) but not the other bioreporters. Although it has been demonstrated that several plant pathogenic *Pseudomonas* spp. are able to produce AHLs,[18] this is the first report of the ability of the mushroom pathogens *P. gingeri* and *P. agarici* strains to produce AHLs. Of interest is the determination of the potential role of the AHL-based QS system in these bacterial species and, in particular, in *P. gingeri*, which is known to produce toxic substances that may be potentially involved in the bacterium biology and/or virulence.[10]

Forty-four Gram-negative of the 236 bacterial isolates associated with mushroom obtained in this study were demonstrated to produce AHLs. Whereas strains of *P. fluorescens*, *P. synxantha*, *P. putida*, *P. aurantiaca* (synonym *P. chlororaphis*),[33] *R. aquatilis*, *B. glumae*, *E. cloacae*, *C. scopthalmum*, and *A. baumannii* are already known to produce AHLs,[18,24,27,34−37] the ability of strains of *P. marginalis*, *P. viridilivida*, *C. fermentans*, *A. xylosoxidans* subsp. *denitrificans*, *A. calcoaceticus*, *B. vesiculari*, and *P. heimbachae* to produce AHLs, to our knowledge, is a new finding.

Table 7. Minimal Signal Induction Concentration of AHL Compounds on *Chromobacterium violaceum* (CV026), *Escherichia coli* (PSB 401), *Escherichia coli* (PSB 1075) and *Agrobacterium tumefaciens* (pDCI41E33) Bioreporters

	MSIC (μM)			
AHL	*C. violaceum* (CV026)[a]	*E. coli* (PSB 401)[a]	*E. coli* (PSB 1075)[a]	*A. tumefaciens* (pDCI41E33)[a]
C4-HSL	25.0	NI[b]	NI	3.05×10^{-13}
C6-HSL	0.25	12.5	NI	1.52×10^{-13}
C8-HSL	1.25	NI	NI	3.05×10^{-10}
C10-HSL	NI	NI	NI	1.52×10^{-10}
C12-HSL	NI	NI	25.0	3.05×10^{-10}
C14-HSL	NI	NI	NI	7.81×10^{-6}
3-oxo-C6-HSL	1.25	0.5	NI	1.56×10^{-5}
3-oxo-C8-HSL	1.25	0.25	NI	1.56×10^{-5}
3-oxo-C12-HSL	NI	25.0	12.5	7.81×10^{-6}

[a] Amount of a given AHLs added to a well cut in the KB Petri plate needed to induce bioreporter response. [b] NI, not induced also when 50 μM solutions of the different AHLs were used.

Interestingly, AHLs bearing a 3-hydroxyalkanoyl group are common among *Proteobacteria* [38].

However, discrepancies were observed between results obtained in the induction of bioreporter in the different bioassays by some bacterial isolates associated with mushroom. For example, six bacteria assayed with the bioreporter *C. violaceum* (CV026) gave negative response in the T-streak assay but showed positive results when culture filtrates were used in the same assay. This finding may be explained by the fact that the AHL production in liquid culture was higher than in the T-streak assay conditions. Moreover, a synergic effect of the different AHLs may also be involved. However, these speculations need to be ascertained. Furthermore, some bacterial strains accumulated in liquid cultures quantities of AHLs higher than MSICs on the bioreporter [i.e., *C. violaceum* (CV026)], but either the T-streak assays or the assays with culture filtrates were negative on the bioreporter. Also in these cases, no immediate explanations are available. The latter observed contradictory results may be due to the interference of metabolites or proteins of AHL-producing bacteria that could inhibit the signal induction in the particular bioreporter. In *A. tumefaciens* pN7L4 (pDCI41E33) the activity of the QS regulator protein TraR is antagonized by several QS antiactivator proteins. TraM is one such protein, encoded by the Ti plasmid in *A. tumefaciens* and also found in other members of Rhizobiaceae.[39] Furthermore, Geske et al.[40] identified two non-native AHLs, a bromophenyl AHL (4-bromo-PHL) and an indole AHL, that showed significant inhibition of the signal by *P. aeruginosa* (LasR) circuit using a fluorescent PAO-JP2 reporter strain. No indications on the possible interfering factors in the bacterial cultures in this study are so far available. The sensitivity of the bioreporters is a useful and important requirement for AHL detection, but only a few papers dealing with the quantitative aspects and detection limits of bioreporters are so far available,[14,34,41,42]. However, the above findings appear to be fragmentary because they refer to only some AHLs, and specific data on the bioreporters used in this study, which are considered to be useful for AHL evaluation, are not yet available.[22] This and the observed discrepancies between results of the T-streak assays and those obtained with LC-ESI-FTICR MS analysis prompt us to determine the response of the bioreporters to nine selected synthetic commercially available AHLs. *A. tumefaciens* pN7L4 (pDCI41E33) was the less specific bioreporter because it was induced by AHLs with C4−C14 acyl side chains showing oxo and OH functional groups in position 3; furthermore, it was the most sensitive one. More specific and less sensitive were the other bioreporters. The observed specificity of the four bioreporters confirmed data already available in the literature,[22] whereas their sensitivity is to be considered a new finding. On the basis of previous data[22] and findings reported in this paper, the use of *A. tumefaciens* pN7L4 (pDCI41E33) may be very useful for the evaluation of AHL bacterial production in liquid culture. Less useful, because of the false-negative results, may be the same bioreporter in the T-streak assay. There is a widespread occurrence of cell-to-cell signaling among different bacterial species and, furthermore, many organisms produce the same AHL signal molecules to regulate different phenotypes.[43,44] Thus, one would predict that some form of interspecies communication would be likely in environments where different autoinducer-producing bacterial species inhabit a common niche. It seems likely that in the environment one bacterial species may produce AHLs that may interfere positively or negatively in the QS system of other bacteria inhabiting the same niche. For example, it was demonstrated that phenazine biosynthesis can be stimulated in one population of *P. aureofaciens* by AHLs produced by a distinct population of the same organism.[45] Similarly, TraR of *A. tumefaciens* respond to signals (cognate and noncognate) produced by other microorganisms in the same niche.[23] Interspecies signaling between bacterial species using AHL molecules has also been suggested to occur between *P. aeruginosa* and *B. cepacia* in people with cystic fibrosis characterized by high morbidity and mortality.[46,47] To avoid such interference and crosstalk with neighboring bacteria it has been suggested that bacteria such as *Xanthomonas* spp. may use non-AHL-based QS systems with consequent competitive advantage in that niche. Thus, the development of specific signaling molecules used to sense not only con-specific bacteria but also certain non-con-specific ones present in certain specific niches is a clever mechanism for community level regulation of gene expression. In conclusion, the hypothesis that coexisting bacterial populations associated with mushroom could interact with pathogen bacterial strains, also unable to produce AHLs, at the level of gene expression with the exchange of AHLs should be considered.[48] Future studies in this regard are highly desirable because they would potentially permit the development of innovative and ecofriendly control measures.

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