Eur J Plant Pathol (2013) 137:119–126
DOI 10.1007/s10658-013-0223-6

Evaluation of oyster mushroom strains for resistance to *Pseudomonas tolaasii* by inoculation in spawned substrates

Rui Ying Zhang · Dan Dan Hu · Jin Gang Gu · Xue Mei Zuo · Qing Xiu Hu · Jin Xia Zhang

Accepted: 25 April 2013 / Published online: 17 May 2013
© KNPV 2013

Abstract Bacterial brown blotch disease, caused by *Pseudomonas tolaasii*, has been one of the most serious bacterial diseases of the oyster mushroom. Resistance to disease has been a priority in breeding programs for the oyster mushroom. In this study, a set of 37 *Pleurotus ostreatus* strains was tested for resistance to the pathogen *P. tolaasii* by two methods, with inoculations on pileus and spawned substrate under controlled environmental conditions. The results showed that the protocol with inoculation on spawned substrate was sensitive and adequate for testing resistance of oyster mushroom to brown blotch disease. According to the disease severity of fruiting bodies with inoculation on spawned substrates, strain ACCC50618 was resistant; ACCC50236 was moderate resistant; and the others were susceptible to the pathogen *P. tolaasii* (ACCC 01267). However, it was difficult to differentiate the resistance of *P. ostreatus* strains to *P. tolaasii* by inoculation on the pileus. This is the first report about resistance evaluation of *P. ostreatus* to brown blotch disease.

R. Y. Zhang · J. G. Gu · X. M. Zuo · Q. X. Hu (✉) · J. X. Zhang (✉)
Key Laboratory of Microbial Resources Collection and Preservation, Ministry of Agriculture, and Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences,
Beijing 100081, China
e-mail: zhangry76@sina.com
e-mail: zhangjinxia@caas.cn

D. D. Hu
Beijing Academy of Science and Technology,
Beijing 100089, China

Keywords *Pleurotus ostreatus* · Bacterial brown blotch disease · *Pseudomonas tolaasii* · Resistance · Evaluation · Inoculation

Introduction

Pleurotus ostreatus (Fr.) Kumm, commonly known as oyster mushroom, has been a commercially important edible mushroom in many countries of the world, with China the major producing and exporting country. The production of the oyster mushroom in China in 2010 reached 4 million tons in fresh weight.

Bacterial brown blotch disease, caused by the bacterium *Pseudomonas tolaasii*, has been one of the most serious bacterial disease for the oyster mushroom (Zhang et al. 2007b; Zhang et al. 2007a). The disease was firstly found on *Agaricus bisporus* nearly 100 years ago (Tolaas 1915). It has been reported that the disease could also affect *Lentinula edodes*, *Flammulina velutipes*, and *Pleurotus eryngii*, and other species (Akihiko et al. 1995; Han et al. 2012; González et al. 2009). The disease often occurred over a large geographical area. The disease incidence differed from year to year. Once the disease occurred in a farm, it was very difficult to control before all of the substrate bags were removed from the farm. The most typical symptom was characterized with brown spots or blotches on the pileus. If moisture conditions favoured the disease, the brown spots and blotches enlarged and coalesced with others; the affected areas were sunken and covered with sticky material. However, the disease affected only the top external layers of the pileus tissues

and was restricted to 2–3 mm below the pileus surface (Zhang et al. 2007b; Soler-Rivas et al. 1999). Tolaasin, an extracellular lipodepsipeptide toxin produced by *P. tolaasii*, has been proved to be the major virulence factor (Rainey et al. 1991; Brodey et al. 1991; Moquet et al. 1996). The pathogen caused blotch symptoms on the pileus by forming membrane pores and disrupting cellular membrane structure (Nutkins et al. 1991; Bassarello et al. 2004; Cho and Kim 2003).

Many investigations have been carried out to search for an adequate method to prevent or control the disease. Manipulation of the environmental conditions has played an essential role to reduce the disease severity. Calcium chloride and chlorinated compounds are, at the present time, the most commonly utilized chemicals for brown blotch disease control. Several other disinfectants and antibiotics, such as chloramine T and bronopol (Wong and Preece 1985), essential oils (Soković and Van Griensven 2006), and kasugamycin (Geels 1995), have also been tried for their ability to control bacterial blotch disease. Biological control methods with antagonistic microorganisms and/or specific phages have also been investigated (Takanori et al. 1998; Fermor et al. 1991; Tsukamoto et al. 2002; Sahin 2005). However, none has been found fully effective so far. So alternative control strategies were urgently needed for brown blotch disease. Cultivation of resistant varieties is one of the most promising approaches for a sustainable disease control, but suitable varieties are not available in the case of brown blotch disease.

Germplasm evaluation for resistance to brown blotch disease is an essential prerequisite to breed new varieties resistant to the disease. A standardized procedure has been suggested to assess the resistance of *Agaricus bisporus* strains to bacterial brown blotch disease, with inoculation on the pileus surface (Olivier et al. 1997). The results showed that wild strains of *A. agaricus* from France exhibited a wide distribution in disease reaction, ranging from resistant to highly susceptible. However, there have been no reports about resistance evaluation to the bacterial brown blotch disease for oyster mushroom so far. Compared with *Agaricus bisporus*, the genetic diversity of oyster mushroom strains is greater (Alfonso and Pisabarro 2001; Loftus et al. 1988; Bao et al. 2004; Wu et al. 2012). It was possible to evaluate for resistance from a large number of heterogeneous germplasm for oyster mushroom.

The accuracy of the method was very important for resistance evaluation. To improve the precision, the protocol of resistant evaluation should be designed on the basis of dissemination, inoculation, reproduction and infection of *P. tolaasii*. However, the disease cycle of brown blotch disease remained unclear. The results of most studies suggested that the pathogen may have been inoculated on spawned substrate well before the disease occurred (Wong and Preece 1982). The pathogen epiphytically colonized and grew on the vegetative mycelium in the substrate (Preece and Wong 1982; Masaphy et al. 1987; Russo et al. 2003). The epiphytic population of *P. tolaasii* increased rapidly to 10^5–10^7 CFU/g in spawned substrate (Wong and Preece 1982; Wu et al. 1998), but there was no visible infected symptom on vegetative mycelium. During the development and growth of fruiting bodies, the pathogen reached and colonized the pileus, and infected the surface tissue. This could explain in part how severe outbreaks developed so suddenly when the pathogen epiphytically colonized and reproduced on the apparently healthy vegetative mycelium. According to this pathogenic process, inoculation on spawned substrate may be a more comprehensive method to evaluate resistance for oyster mushroom.

The objective of this work was to evaluate the resistance of oyster mushroom strains to *P. tolaasii* by a new method of inoculation on spawned substrates. The resistant strains could be identified and thus used for cultivation, or as source for future breeding programmes.

Material and methods

Pathogen strain and suspension

P. tolaasii strain ACCC01267 (Zhang et al. 2007b) (Agricultural Culture Collection of China, ACCC) isolated from the fruiting bodies of *P. ostreatus* in Beijing was use in this study. An inoculum suspension was fleshly prepared from cultures grown in LB liquid media for 16 h at 28 °C. Cells were harvested, and washed twice with sterile distilled water by centrifugation, and diluted to the concentration of 1×10^9 cfu/ml with sterile distilled water. Bacterial number was determined by plate count for colonies with three repetitions.

Cultivation of the oyster mushroom in controlled environments

A total of 37 *P. ostreatus* strains, collected and maintained in the ACCC, were evaluated for resistance to brown blotch disease in this study (Table 1). These 37 strains were heterogeneous based on a similarity coefficient analysis with SSR markers (Wu et al. 2012). All of the strains were cultivated with the same substrates in a controlled environment. The raw substrate was prepared with the common formula containing 35.5 % cottonseed-hulls, 2 % wheat bran, 0.5 % calcium hydroxide, and 62 % water by weight. The polypropylene bags (17×38 cm) were used as containers to hold 1.5 kg substrate. The bags filled with substrate were autoclaved at 121 °C for 2 h. Twenty bags of one strain were inoculated and incubated at 25 °C. When mycelium colonized completely the substrate, the bags were transferred to a fruiting house. During the fruiting phase, the temperature and relative humidity were adjusted to 16 °C and 90 % respectively. No water was sprayed directly onto the fruiting bodies. Sufficient oxygen and lower carbon dioxide levels were maintained to meet the mushroom development and growth requirements by regular ventilation.

Inoculation on the pileus

The first flush of fruiting bodies was used to evaluate resistance to blotch disease with inoculation on the pileus surface in situ according to a modified protocol of Olivier et al. (1997). When the diameter of pileus reached 2–4 cm, a 20 μl aliquot of bacterial suspension (2×10^7 cfu) was dripped onto the pileus surface. For each strain, 10 pileus of five bags (two pileus randomly sampled from a single bag) were treated repetitively. Disease blotches appeared on the pileus after 24 h of inoculation. The first flush of fruiting bodies was removed carefully, and this avoided contamination of the spawned substrate by the pathogen on fruiting bodies. The bags were further incubated to form a second flush of fruiting bodies.

Disease severity for the two flushes of fruiting bodies was assessed according to the affected area based on the modified method of Wong et al. (Wong and Preece 1982). Each of the mushroom fruiting bodies examined in this study was given a score of 0, 1, 2, 3 according to the size of the blotch: 0=no symptom, 1=slight symptom development, with few small spots on the pileus (0.1–1 % area covered by blotch), 2=moderate symptom development, with many small spots on the pileus (1–5 % area covered by blotch), 3=severe symptom development, with many spots or large blotches on the pileus (5–10 % area covered by blotch). The average disease severity of each strain was calculated.

Inoculation on the spawned substrate

When the mycelia had colonized completely the whole substrate in bags, the spawned substrates were inoculated by spraying bacterial suspension (1 ml/bag, 10^9 cfu) on the top surface in quintuplicate for 37 strains. The inoculated bags were incubated at 25 °C for 5 day, and placed in a fruiting house. From the "pinheads" stage of fruiting bodies, the brown spots or blotches began to be visible on the top surface of some primordia. During growth and development, only a few primordia continued to develop and form matured fruitbodies; the others stopped developing, and wilted. When the diameter of pileus reached 2–4 cm, the disease severity of 37 strains was assessed according to the size of the blotches. The first flush of fruiting bodies was removed carefully, and the bags continued in incubation to form the second flush of fruitbodies. The disease severity of the second-flush fruiting bodies was determined according to the size of the blotches as described above.

In order to illuminate the mechanism of resistance of oyster mushroom to *P. tolaasii*, the disease incidence of primordia for four strains (Table 2) with different disease severities was calculated by the formula:

$$\text{Incidence}\,(\%) = \frac{\text{No. of disease primordia}}{\text{No. of total primordia}} \times 100\%$$

The enumeration and identification of *P. tolaasii* in substrate

After the first flush of fruiting bodies was picked up, a 5 g spawned substrate sample was taken from the top surface of bags. The samples were suspended in 45 ml sterile water containing 5 g quartz sand, and shaken for 10 min at 200 rpm. The supernatant was used to prepare a serial tenfold dilution with sterile water. The cell number of *P. tolaasii* was determined by plate count. The colonies of *P. tolaasii* were identified by

Table 1 The disease symptoms and resistance of *P. ostreatus* strains tested with inoculation on substrates

Strains	Origin names	Pileus color[a]	Inoculation on caps		Inoculation on substrates		
			Disease severity	The color of blotch	Disease severity[b]	The color of blotch	Resistance[c]
ACCC50618	Yefeng 118	Grey	2	Brown	0.45±0.51a	Brown	R
ACCC50236	Tebai 1	White	2	Yellow	1.15±0.75b	Yellow	MS
ACCC50075	1112	Pale grey	2	Brown	2.35±0.81c	Brown	S
ACCC50116	8010	Grey	2	Brown	2.35±0.81c	Brown	S
ACCC50150	Nongda 11	Grey	2	Brown	2.35±0.81c	Brown	S
ACCC50168	Pl-27	Grey	2	Brown	2.35±0.88c	Brown	S
ACCC50495	Jinong 11	Grey	2	Brown	2.35±0.75c	Brown	S
ACCC51550	Jiangdu 5178	Black	2	Brown	2.35±0.88c	Brown	S
ACCC50020	DP02	Pale brown	2	Brown	2.40±0.82c	Brown	S
ACCC50476	Yaguang 1	Brown	2	Brown	2.40±0.82c	Brown	S
ACCC52305	Shiji 3	Brown	2	Brown	2.40±0.88c	Brown	S
ACCC50060	ZM5.23	Grey	2	Brown	2.45±0.83c	Brown	S
ACCC50122	Yunnanbai	Brown	2	Brown	2.45±0.76c	Brown	S
ACCC50123	Ping 2	Brown	2	Brown	2.45±0.83c	Brown	S
ACCC50838	99	Black	2	Brown	2.45±0.83c	Brown	S
ACCC51604	650	Grey	2	Brown	2.45±0.83c	Brown	S
ACCC50165	Zhongshu 10	Pale grey	2	Brown	2.50±0.76c	Brown	S
ACCC50948	Nongke 5	Black	2	Brown	2.50±0.76c	Brown	S
ACCC51123	Qingdaohei	Grey	2	Brown	2.50±0.76c	Brown	S
ACCC51371	Xiuzhengu	Brown	2	Brown	2.50±0.83c	Brown	S
ACCC51557	Jiyin2005	Grey	2	Brown	2.50±0.83c	Brown	S
ACCC51568	Guanping 1	Pale grey	2	Brown	2.50±0.83c	Brown	S
ACCC50121	EA38	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC50596	Xide 89	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC50865	Nanjing 1	Brown	2	Brown	2.55±0.76c	Brown	S
ACCC51372	pg2	Brown	2	Brown	2.55±0.76c	Brown	S
ACCC51652	39	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC51933	Guangdong	Grey	2	Brown	2.55±0.83c	Brown	S
ACCC50050	Ce813	Brown	2	Brown	2.60±0.68c	Brown	S
ACCC51340	Jiangdu2026	Grey	2	Brown	2.60±0.68c	Brown	S
ACCC51553	Kangbing 2	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51570	Daxing	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51601	615	Brown	2	Brown	2.60±0.75c	Brown	S
ACCC51942	Shuangkang	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51423	Heixiuzhegu	Brown	2	Brown	2.65±0.75c	Brown	S
ACCC51556			2	Brown	2.75±0.55c	Brown	S
ACCC51599			2	Brown	2.75±0.55c	Brown	S

[a] The colour of the pileus fruiting and growing at 16 °C. The pileus colour of *P. ostreatus* varied at different temperature. The temperature was lower, the pileus color was darker for the oyster mushroom

[b] Numbers followed by the same letter are not significantly different at $P=0.05$ according to Duncan's multiple range tests

[c] The resistance of strains to bacterial brown blotch disease tested with inoculation on substrate: *R* resistant, *MS* moderately susceptible, *S* susceptible. The resistance of strains were classified according to the disease severity with the testing method of inoculation on spawned substrates

nested PCR methods described by Lee et al. (Lee et al. 2002). Two sets of primers, Pt-1A (5′ATCCCTTCG GCGTTTACCTG3′) and Pt-1D1 (5′CAAAGTAAC CCTGCTTCTGC3′), and Pt-PM (5′TGCCTTACGC GCTGATTGGC3′) and Pt-QM (5′TGATCA AACTCCAGCAATAG3′) were used to amplify DNA only from *P. tolaasii*.

Results

Disease symptoms with inoculation of pileus

All of the 37 strains tested in this study formed fruiting bodies. Based on the colour of the pileus, those 37 strains were divided into six groups with pale brown, brown, grey, pale grey, white and black as listed in Table 1. The blotches appeared after 24 h from inoculation of the pileus. Disease severity, except for blotch colour, was similar for the 37 strains (Table 1). However, the disease did not occur for the second flush of fruiting bodies. Therefore, it was difficult to distinguish the resistance of various strains to the bacterial brown blotch disease by direct inoculation of the pileus.

As showed in Table 1, the colour of botches varied from pale yellow to deep brown for the tested 37 strains. The fruiting bodies of strain ACCC50236 were white, and the blotches were pale yellow. The fruiting bodies of strain ACCC50838 were black, and the blotches were brown. Overhead watering may facilitate the growth and movement of the pathogen, and enlargement of the blotches (Wong and Preece 1982). In this study, no overhead watering was used during the fruiting phase. Each of the locations inoculated by the pathogen suspension became the disease blotches. Most of blotches did not enlarge or coalesces with other spots, across the scale inoculated. There were no disease blotches on the pileus except at inoculated sites. The affected areas were not sunken or covered by evident sticky material.

Table 2 Bacterial brown blotch disease incidence of primordia tested by inoculation on spawned substrate

Strains	The disease incidence of primordia (%)[a]
ACCC50618	7.51±2.59a
ACCC50236	29.37±6.06b
ACCC51652	45.64±5.74c
ACCC50838	48.51±7.13c

[a] Numbers followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range tests

Disease symptoms with inoculation on spawned substrates

The disease spots or blotches appeared on the primordia. The disease incidence of primordial for the strains ACCC50236, ACCC50618, ACCC50838, and ACCC51652 were as listed in Table 2.

As the fruiting bodies grew, the spots and blotches increased and enlarged. Disease severity was determined according to the size of the blotches (Table 1). The results showed that strain ACCC50618 produced the lowest symptoms. Most of the pileus of ACCC 50618 were healthy, with only a few producing small spots. Strain ACCC 50236 produced moderate symptoms, with many small spots, which did not enlarge or coalesce, appearing on the pileus. The other 35 strains produced more severe symptoms, with many spots or large blotches on the pileus (5–10 % area covered by blotch). Disease symptoms and severity of second flush fruiting bodies were similar with those of the first flush for the 37 strains. According to the disease severity of the two flushes of fruiting bodies, strain ACCC 50618 was resistant; strain ACCC 50236 was moderately susceptible, while the other 35 strains were susceptible to the brown blotch disease.

The pathogen inoculated in spawned substrates colonized and caused infection of two flushes of fruiting bodies. This phenomenon is similar to that which occurs on farms. Combining the reports (Wong and Preece 1982; Preece and Wong 1982; Masaphy et al. 1987; Russo et al. 2003), it is inferred that the pathogen colonized in spawned substrates comprised the main inoculum source. This could explain in part why the disease has been difficult to control by spraying disinfectants or antibiotics onto fruiting bodies, It did not eradicate or reduce the inoculum in the spawned substrate contained in bags.

The number of *P. tolaasii* in spawned substrate

After the first flush of fruiting bodies was picked up, the *P. tolaasii* in the substrate was recovered and enumerated by plate counting for colonies. Colonies on plates which produced two bands of 449 bp and

249 bp by nested PCR (Fig. 1) were considered to be *P. tolaasii*. The number of *P. tolaasii* colonies recovered from substrate of four strains was not significantly different regardless of treatment (Table 3). It was inferred that the resistance of those four strains tested with inoculation in spawned substrate may not correlate with the bacterial populations in substrate.

Table 3 *P. tolaasii* population recovered from 1 g substrate sampled after picking of the first flush of fruitbodies.

Strains	Bacteria population ($\times 10^6$)[a]
ACCC50838	1.48±0.41a
ACCC50618	1.52±0.15a
ACCC50236	1.58±0.28a
ACCC51652	1.68±0.23a

[a] Numbers followed by the same letter are not significantly different at $P=0.05$ according to Duncan's multiple range tests

Discussion

Resistance to disease has been a priority in breeding programs of the oyster mushroom. In this study, two methods were used to test the resistance of 37 strains to *P. tolaasii*. The results showed that the protocol with inoculation on spawned substrate was sensitive and adequate for testing resistance of oyster mushroom to brown blotch disease. Strain ACCC50618 was resistant; ACCC50236 was moderate resistant; and the other strains were susceptible to the pathogen *P. tolaasii* (ACCC 01267). This is the first report about resistance evaluation of *P. ostreatus* to brown blotch disease.

It was reported that the resistance of *A. bisporus* strains could be assessed by a procedure involved incubation of first flush pileus with a droplet of suspension of *P. tolaasii* and incubation for 48 h in a moist chamber at 16 °C (Olivier et al. 1997). However, the results in this study suggested that it was difficult to differentiate the resistance to *P. tolaasii* for *P. ostreatus* strains by inoculation on pileus. Even the resistant strains ACCC50618 and ACCC50236 were susceptible to the pathogen when inoculation was made on the pileus. So there may be mechanisms other than susceptibility of the pileus to the pathogen or tolaasin, responsible for the resistance found for strains ACCC50618 and ACCC50236.

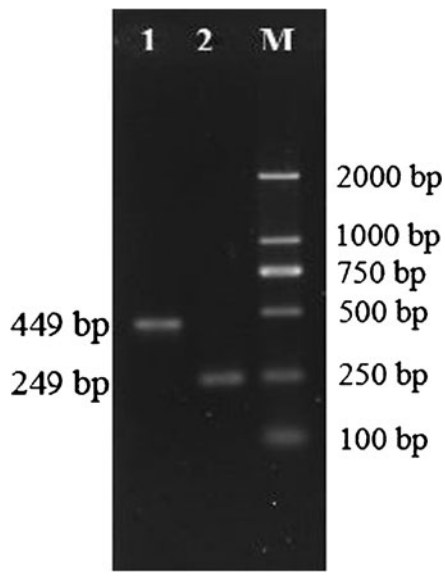

Fig. 1 The profile of nested PCR products for *P. tolaasii* colony. Lane 1, the DNA fragments of 449 bp amplified with primers Pt-1A and Pt-1D1 using the DNA template isolated from *P. tolaasii* colony; Lane 2, the DNA fragments of 249 bp amplified by nested PCR with primers Pt-PM and Pt-QM from the products of the last round PCR; Lane M, DL2,000™ DNA marker (TaKaRa)

The resistance of *P. ostreatus* may be the result of a serial of interactions between host and pathogen. The antagonistic action of vegetative mycelium to *P. tolaasii* may reduce the pathogen population in spawned substrate. The results showed that ACCC50618 was resistant; ACCC50236 was moderate resistant; ACCC51652 and ACCC50838 were susceptible to the pathogen *P. tolaasii* (ACCC 01267). However, the epiphytic populations of *P. tolaasii* for these four strains were not significantly different as shown in Table 3. These results suggested that the resistance of strains ACCC50618 and ACCC50236 was not necessarily related to the pathogen population in the substrate.

The bacteria may reach and colonize on the surface of the pileus during the early stage of fruiting body development while the young pileus is still in contact with the substrate. The greater populations of bacteria inoculated from spawned substrates may result in more severe infection. A similar phenomenon has been reported in fire blight occurring on apple and pear (Thomson 1986; Farkas et al. 2012). Epiphytic bacterial populations developed on the stigma, from where the pathogen colonized the hypanthium and invaded the tissues through the stomata of the nectary under favourable conditions (Thomson 1986; Farkas et al.

2012). The disease incidence of primordia may result directly from the colonization of bacteria from the substrate. The disease severity of the pileus was consistent with disease incidence of primordia for tested strains with inoculation on spawned substrates. So resistance evaluation with inoculation on spawned substrate could exhibit integrated resistance of strains, including movement of the pathogen from spawned substrate to primordia. In addition, it is important to control the transfer of pathogen from the spawned substrate to the pileus during the the early stages of fruiting body development for the management of this disease.

It was reported that the brown strains were less susceptible than white strains for *A. bisporus* (Olivier et al. 1997; Moquet et al. 1998; Moquet et al. 1999). However, the results in this study suggested that the susceptibility of *P. ostreatus* was not related to pileus colour (Table 1). In this study, the results showed that the most of strains were susceptible to the disease, including the important cultivars ACCC50838 and ACCC51652, cultivated widely in China for their desirable agronomic traits. Strain ACCC50236 is also planted widely in some districts for their white fruiting bodies, which is popular in supermarkets. The strain ACCC50236 was rarely found diseased in farms, even during the disease-prone period of production. The results in this study indicated that the strain ACCC50618 was resistant to brown blotch disease. However, it has rarely been cultivated by growers, because the fruiting bodies are very fragile and easily broken during harvest and transport. It could be used as a germplasm for breeding programs to develop new resistant cultivars.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 31101588), and the China agriculture research system (No.CARS-24).

References

Akihiko, T., Kazuo, S., Shigeyuki, M., & Ikuo, O. (1995). Occurrence of *Pseudomonas tolaasii* on fruiting bodies of *Lentinula edodes* formed on quercus logs. *Mycoscience, 36*(3), 283–288.

Alfonso, L., & Pisabarro, A. G. (2001). Use of molecular markers to differentiate between commercial strains of the button mushroom *Agaricus bisporus*. *FEMS Microbiology Letters, 198*, 45–48.

Bao, D., Kinugasa, S., & Kitamoto, Y. (2004). The biological species of oyster mushrooms (*Pleurotus* spp.) from Asia based on mating compatibility tests. *Journal of Wood Science, 50*(2), 162–168.

Bassarello, C., Lazzaroni, S., Bifulco, G., LoCantore, P., Iacobellis, N. S., Riccio, R., et al. (2004). Tolaasins A-E, five new lipodepsipeptides produced by *Pseudomonas tolaasii*. *Journal of Natural Products, 67*(5), 811–816.

Brodey, C. L., Rainey, P. B., Tester, M., & Johnstone, K. (1991). Bacterial blotch disease of the cultivated mushroom is caused by an ion channel forming lipodepsipeptide toxin. *Molecular Plant-Microbe Interactions, 4*, 407–411.

Cho, K. H., & Kim, Y. K. (2003). Two types of ion channel formation of tolaasin, a *Pseudomonas peptide* toxin. *FEMS Microbiology Letters, 221*(2), 221–226.

Farkas, Á., Mihalik, E., Dorgai, L., & Bubán, T. (2012). Floral traits affecting fire blight infection and management. *Trees - Structure and Function, 26*(1), 47–66.

Fermor, T. R., Henry, M. B., Fenlon, J. S., Glenister, M. J., Lincoln, S. P., & Lynch, J. M. (1991). Development and application of a biocontrol system for bacterial blotch of the cultivated mushroom. *Crop Protection, 10*(4), 271–278.

Geels, F. P. (1995). *Pseudomonas tolaasii* control by kasugamycin in cultivated mushrooms (*Agaricus bisporus*). *Journal of Applied Bacteriology, 79*(1), 38–42.

González, A. J., González-Varela, G., & Gea, F. J. (2009). Brown blotch caused by *Pseudomonas tolaasii* on cultivated *Pleurotus eryngii* in Spain. *Plant Disease, 93*(6), 667.

Han, H. S., Jhune, C. S., Cheong, J. C., Oh, J. A., Kong, W. S., Cha, J. S., et al. (2012). Occurrence of black rot of cultivated mushrooms (*Flammulina velutipes*) caused by *Pseudomonas tolaasii* in Korea. *European Journal of Plant Pathology*, 1–9.

Lee, H. I., Jeong, K. S., & Cha, J. S. (2002). PCR assays for specific and sensitive detection of *Pseudomonas tolaasii*, the cause of brown blotch disease of mushrooms. *Letters in Applied Microbiology, 35*(4), 276–280.

Loftus, M. G., Moore, D., & Elliott, T. J. (1988). DNA polymorphisms in commercial and wild strains of the cultivated mushroom, *Agaricus bisporus*. *Theoretical and Applied Genetics, 76*(5), 712–718.

Masaphy, S., Levanon, D., Tchelet, R., & Henis, Y. (1987). Scanning electron microscope studies of interactions between *Agaricus bisporus* (Lang) Sing hyphae and bacteria in casing soil. *Applied and Environmental Microbiology, 53*(5), 1132–1137.

Moquet, F., Mamoun, M., & Olivier, J. M. (1996). *Pseudomonas tolaasii* and tolaasin: comparison of symptom induction on a wide range of *Agaricus bisporus* strains. *FEMS Microbiology Letters, 142*(1), 99–103.

Moquet, F., Mamoun, M., RamosGuedes-Lafargue, M., Olivier, J. M., & Savoie, J. M. (1998). Differences in susceptibility of *Agaricus bisporus* strains to bacterial blotch and in natural cap colour related to compost composition. *Plant Breeding, 117*(4), 385–388.

Moquet, F., Desmerger, C., Mamoun, M., Ramos-Guedes-Lafargue, M., & Olivier, J.-M. (1999). A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color. *Fungal Genetics and Biology, 28*(1), 34–42.

Nutkins, J. C., Mortishire-Smith, R. J., Packman, L. C., Brodey, C. L., Rainey, P. B., Johnstone, K., et al. (1991). Structure determination of tolaasin, an extracellular lipodepsipeptide produced by the mushroom pathogen, *Pseudomonas tolaasii* Paine. *Journal of the American Chemical Society, 113*(7), 2621–2627.

Olivier, J. M., Mamoun, M., & Munsch, P. (1997). Standardization of a method to assess mushroom blotch resistance in cultivated and wild Agaricus bisporus strains. *Canadian Journal of Plant Pathology, 19*(1), 36–42.

Preece, T. F., & Wong, W. C. (1982). Quantitative and scanning electron microscope observations on the attachment of *Pseudomonas tolaasii* and other bacteria to the surface of *Agaricus bisporus*. *Physiological Plant Pathology, 21*(2), 251–257.

Rainey, P. B., Brodey, C. L., & Johnstone, K. (1991). Biological properties and spectrum of activity of tolaasin, a lipodepsipeptide toxin produced by the mushroom pathogen *Pseudomonas tolaasii*. *Physiological and Molecular Plant Pathology, 39*(1), 57–70.

Russo, A., Filippi, C., Tombolini, R., Toffanin, A., Bedini, S., Agnolucci, M., et al. (2003). Interaction between gfp-tagged *Pseudomonas tolaasii* P12 and *Pleurotus eryngii*. *Microbiological Research, 158*(3), 265–270.

Sahin, N. (2005). Antimicrobial activity of *Streptomyces* species against mushroom blotch disease pathogen. *Journal of Basic Microbiology, 45*(1), 64–71.

Soković, M., & Van Griensven, L. J. L. D. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal of Plant Pathology, 116*(3), 211–224.

Soler-Rivas, C., Jolivet, S., Arpin, N., Olivier, J. M., & Wichers, H. J. (1999). Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. *FEMS Microbiology Reviews, 23*(5), 591–614.

Takanori, T., Akira, S., & Hitoshi, M. (1998). Isolation of a gram-positive bacterium effective in suppression of brown blotch disease of cultivated mushrooms, *Pleurotus ostreatus* and *Agaricus bisporus*, caused by *Pseudomonas tolaasii*. *Mycoscience, 39*(3), 273–278.

Thomson, S. V. (1986). The role of the stigma in fire blight infections. *Phytopathology, 76*(5), 476–482.

Tolaas, A. G. (1915). A bacterial disease of cultivated mushroom. *Phytopathology, 5*, 51–54.

Tsukamoto, T., Murata, H., & Shirata, A. (2002). Identification of non-Pseudomonad bacteria from fruit bodies of wild Agaricales fungi that detoxify tolaasin produced by *Pseudomonas tolaasii*. *Bioscience, Biotechnology, and Biochemistry, 66*(10), 2201–2208.

Wong, W. C., & Preece, T. F. (1982). *Pseudomonas tolaasii* in cultivated mushroom *Agaricus bisporus* crops: numbers of the bacterium and symptom development on mushrooms grown in various environments after artificial inoculation. *Journal of Applied Microbiology, 53*(1), 87–96.

Wong, W. C., & Preece, T. F. (1985). *Pseudomonas tolaasii* on mushroom (*Agaricus bisporus*) crops: bactericidal effects of six disinfectants and their toxicity to mushrooms. *Journal of Applied Bacteriology, 58*(3), 269–273.

Wu, R. M., Palmer, B., & Cole, A. (1998). Phenotypic variation and survival of genetically marked *Pseudomonas tolaasii* in mushroom compost. *Canadian Journal of Microbiology, 44*(4), 373–377.

Wu, D. D., Zhang, Z., Zhang, R. Y., Hu, D. D., Gu, J. G., Hu, Q. X., et al. (2012). Characterization of 18 novel polymorphic SSR markers and evaluation of genetic diversity within oyster mushroom strains cultivated in China. *Acta Edulis Fungi, 19*(2), 15–25.

Zhang, R. Y., Hu, D. D., Zuo, X. M., Wang, H. X., & Jiang, R. B. (2007a). Research advancement on brown blotch disease of oyster mushroom and button mushroom. *Acta Phytophylacica Sinica, 34*(5), 549–554.

Zhang, R. Y., Zuo, X. M., & Jiang, R. B. (2007b). Isolation and identification of the pathogenic bacteria causing brown blotch disease of oyster mushroom. *Edible Fungi of China, 26*(5), 58–60.